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ANIMAL GENETICS AND BREEDING

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SCIENTIFIC NEWS

GENETIC DIVERSITY ANALYSIS OF SIX VIETNAMESE INDIGENOUS CHICKEN VARIETIES USING MTDNA D-LOOP REGION

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ABSTRACT

This study was aimed to analyze partial mtDNA D-loop sequences of six Vietnamese indigenous chicken varieties, including Dong Tao, Ho, Mong Tien Phong, To, Mia and Sau Ngon to access genetic diversity and the maternal lineages of origin of them. A 525bp fragment of the mtDNA D-loop region was sequenced from a total of 129 chicken of the six varieties. A neighbor-joining phylogenetic tree was assembled from the haplotypes obtained and reference sequences of mtDNA D-loop sequences of Red Junglefowl and domestic chickens from National Center for Biotechnology Information database. Evaluation of genetic relationships between the six varieties was carried out with pairwise fixation index (F_{ST}). In total, 27 haplotypes were identified in the chickens studied. These haplotypes were classified in three haplogroups (A, B and E) with the majority grouped in haplogroup B and haplogroup A. All six chicken breeds studied were distributed into two to three haplogroups and all three haplogroups found in this study are also represented by red junglefowls. The genetic information of this study provided further evidence to prove that these six Vietnamese indigenous chicken varieties have likely originated from multiple maternal lineages and potentially descended from the red junglefowl.

Keywords: *Mitochondrial DNA D-loop, Vietnamese Indigenous Chicken, Genetic Diversity, Maternal Lineage.*

1. INTRODUCTION

Being the most extensively distributed of the poultries, the domestic chicken (*Gallus gallus domesticus*) provides humans with a stable sources of protein, including both meat and eggs (FAO, 2007). A study on earliest archaeological chicken bones from China (Xiang *et al*., 2014), dating back to around 10,000 B.P, also suggested that northern China represents one region of the earliest chicken domestication, possibly dating as early as 10,000 B.P. These early domesticated chickens contributed to the gene pool of modern chicken populations. Several studies suggested that the genetic diversity of domestic chickens have been contributed by human migration, which enables genetic material exchanges among chickens of different origins and locations and also the wide distribution of common maternal lineages in different defined geographic areas (Liu *et al*., 2006; Cuc *et al*., 2011).

Domestic chickens are commonly believed to be descendants of the red jungle fowl as Darwin firstly indicated in the comparisons of morphology and production among *Gallus* species (Darwin, 1868). Genetic resources of chicken consist of a diverse range of breeds and populations comprising red jungle fowl, native and fancy breeds, middle-level food producers, industrial stocks, and specialized lines. Vietnam possesses many indigenous breeds with exotic traits and high performance (Cuc *et al*., 2011).

Mitochondrial DNA (mtDNA) has been used to gain molecular information to identify the origin of breeds. Proteins are not encoded in the D-loop region and this region evolves much faster than other regions of the mtDNA genome. Mitochondrial DNA and especially D-loop sequences have been used in phylogenetic analysis for the past 20 years

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(Moore, 1995). Regarding the use of mtDNA D-loop in analyzing chicken genetic material, Indonesian indigenous chickens have been reported to distribute in five clusters, being associated to reference chicken sequences from India, China, and Indonesia (Sulandari *et al*., 2008). Thai indigenous chickens were grouped into 6 haplogroups and likely to be descended from a common ancestor, the Red Jungle Fowl, with multiple maternal lineages (Teinlek *et al*., 2017). Some Vietnamese indigenous chickens breeds/varieties were classified into 8 clusters which related to reference chicken and red jungle fowl sequences of Indian, China and Southeast Asian origins (Cuc *et al*., 2011). These studies all suggested that Southeast Asian indigenous chickens originated from multiple maternal lineages.

Vietnamese local chicken breeds are specific for particular regions and they are assumed showing specific adaptation to climate, disease, local low input and low output production system (Vang, 2003). Thus, they might be used as a large natural gene pool for future breeding to meet specific objectives. Six varieties of Vietnamese chickens in this study are Dong Tao chicken, Ho chicken, Mong Tien Phong chicken, To chicken, Mia chicken and Sau Ngon chicken, which are only cultivated locally and in small areas but still have large market demand. Therefore, the aim of this study is to analyses the mtDNA D-loop sequences of six Vietnamese indigenous chicken varieties, including Dong Tao chicken, Ho chicken, Mong Tien Phong chicken, To chicken, Mia chicken and Sau Ngon chicken and access their mtDNA haplotypes, and to furthermore reveal maternal lineages of origin of them.

2. MATERIALS AND METHODS

A total number of 129 blood sample were collected randomly from six Vietnamese indigenous chicken varieties (Table 1). Genomic DNAs were extracted from blood samples after a lysis step with Protease K with a standard Phenol-Chloroform protocol (Natalia *et al*., 2001).

Table 1. Distribute areas and collect areas of the six Vietnamese indigenous chicken breeds

Chicken breeds	Distribute area	Collect area	Number of samples
Dong Tao	Khoai Chau, Hung Yen	Faculty of Animal Science, VNUA	24
Mong Tien Phong	Duy Tien, Ha Nam	Duy Tien, Ha Nam	22
Ho	Thuan Thanh, Bac Ninh	Faculty of Animal Science, VNUA	22
To	Quynh Phu, Thai Binh	Lien Ninh Experimental Farm, NIAS	21
Mia	Son Tay, Hanoi	Faculty of Animal Science, VNUA	22
Sau Ngon	Tan Son, Phu Tho	CARBTA, VNUA	18

The partial mtDNA D-loop region was amplified using specific primers (Table 2) based on the partial chicken mitochondrial genome GenBank accession number AB098668.1 (Komiyama *et al*., 2003), and complete chicken mitochondrial genome GenBank accession number NC_001323.1 (Desjardins and Morais, 1990). PCR was performed in 50µl reactions containing 1X ThermoPol® Reaction Buffer, 2.5mM of each dNTPs, 1.25IU Taq DNA polymerase (New England Biolabs, USA), 1µM of each primer, and 10ng/nL of genomic DNA. PCR amplification was carried out on a Thermal Cycler GeneAtlas S System (ASTEC, Japan). PCR conditions were as follows: initial denaturation at 95^{0}C for 5min, followed by 35 cycles of denaturation at 95^{0}C for 30s, annealing at 60^{0}C for 30s and extension at 72^{0}C for 1min, the last cycle was followed by 72^{0}C for 5min. Samples were then sent to 1st BASE (Singapore) for DNA Sequencing+ PLUS service.

All 129 mtDNA nucleotide sequences obtained in this study were viewed with Chromas 2.6.4, edited with BioEdit Sequence Alignment Editor version 5.0.9 and aligned by using the ClustalX 2.1 program (Thompson *et al*., 1997). Identical sequences were considered as the same haplotype using DnaSP 5.10. The mtDNA

D-loop partial sequences diversity indices (nucleotides diversity, haplotypes diversity (Nei, 1987), the position and number of polymorphic sites and corresponding haplotypes analysis was conducted using DnaSP 5.10. Pairwise fixation index (F_{ST}) were computed to quantify the maternal genetic differentiation by using Arlequin version 3.5 (Excoffier and Lischer, 2010).

Table 2. Primers used for PCR amplification of HV1 region from the D-loop

Primer types	Primer name	5′ to 3′ sequence
Forward	L16750	AGGACTACGGCTTGAAAAGC
Reverse	H522	ATGTGCCTGACCGAGGAACCAG
	CR1b	CCATACACGCAAACCGTCTC

A total number of 24 reference red jungle fowl and domestic chicken mtDNA sequences (Teinlek *et al.*, 2017) of 13 haplogroups (Miao *et al.*, 2012) were used to classify the haplotypes from this study.

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).

3. RESULTS AND DISCUSSION

3.1. MtDNA D-loop sequence variability and population diversity

The sequences from nucleotide 10 to 535 were used for analysis. Alignment of 129 Vietnamese indigenous chicken partial mtDNA D-loop sequences was done to a reference sequence from GenBank (accession number AB098668.1). A number of 27 haplotypes were identified in 129 mtDNA D-loop partial sequences with a total of 27 variable sites (Figure 1).

	011	012	026	167	190	199	207	212	217	220	225	228	233	243	246	256	261	296	310	315	342	355	362	374	443	447	477	487
Ref	T	T	C	T	T	T	A	A	T	T	C	C	C	T	T	T	C	T	C	T	A	T	C	C	C	C	C	C
VHB01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*	.	.	.
VHB02	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*	.	.	.
VHB03	.	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*	.	.	.
VHB04	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	*	.	.	.
VHB05	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	*	.	.	.
VHB06	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	*	.	.	.
VHB07	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.	*	.	.	.
VHB08	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	*	.	.	.
VHB09	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	*	.	.	.
VHB10	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	*	.	.	.
VHB11	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	*	.	.	.
VHB12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	*	.	.	.
VHB13	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	*	.	.	.
VHB14	.	A	A	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	*	.	.	.
VHB15	.	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	*	.	.	.
VHB16	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	*	.	.	.
VHB17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	*	.	.	A
VHB18	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	C	.	.	.	.	.	A	*	.	A	.
VHA01	.	.	.	C	.	.	.	G	.	.	T	.	.	.	C	.	.	C	.	C	.	.	.	.	*	.	.	.
VHA02	.	.	A	C	.	.	.	G	.	.	T	.	.	.	C	.	.	C	.	C	.	C	T	.	*	.	.	.
VHA03	.	.	.	C	.	.	.	G	.	.	T	.	.	.	C	.	.	C	.	C	.	C	T	.	*	.	.	.
VHA04	.	.	.	C	.	.	.	G	.	.	T	.	.	.	C	.	.	C	.	C	.	.	T	.	*	.	.	.
VHA05	.	.	.	C	C	.	.	G	.	.	T	.	.	.	C	.	.	C	.	C	.	.	.	.	*	.	.	.
VHA06	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	C	.	C	.	.	.	.	*	.	.	.
VHE01	.	.	.	.	.	.	.	G	C	.	.	.	.	C	C	C	T	C	T	C	.	.	.	.	*	T	.	.
VHE02	.	.	.	.	.	C	.	G	C	.	.	.	.	C	C	C	T	C	T	C	.	.	.	.	*	T	.	.
VHE03	.	.	.	.	.	.	.	G	C	.	.	.	.	C	C	C	T	C	T	C	G	.	.	.	*	T	.	.

Figure 1. Sequence variation and number of polymorphic sites observed among haplotypes based on 129 chicken D-loop sequences of six Vietnamese indigenious chickens with the partial chicken mitochondrial genome GenBank accession number AB098668.1.

Asterisk marks (*) mean nucleotide deletion, dots (.) indicate identity with the refference sequence and different base letters denote subsitution. Vertically oriented numbers indicate the site position on the refference sequence and the sequences shown are only the variable sites.

All observed haplotypes have a nucleotide deletion at position 443 compared to the reference sequence. The pattern of variability displayed a high level of variation between nucleotide 167 and 487. The nucleotide substitutions found in 27 haplotypes comprised two T/A, and four C/A trans-versions and the rest were three A/G, seven C/T and ten T/C transitions (Table 3). This displays a strong tendency of substitution toward transition. In addition, one three-variants site (T/C/A) was observed and located.

Table 3. Nucleotide subsitution in partial D-loop

Subsitution	Variable sites
T/C/A	11
T/A	12, 220
A/G	207, 212, 342
C/A	26, 374, 477, 487
C/T	225, 228, 233, 261, 310, 362, 447
T/C	167, 190, 199, 217, 243, 246, 256, 296, 315, 355

Out of 27 haplotypes observed, only five (18.75%) were shared between populations and 22 (81.25%) were singletons (Table 4). The representative population of Mong Tien Phong chicken held the highest number of haplotypes (10 haplotypes) and the highest level of haplotype diversity (Hd=0.8139) while Dong Tao chicken and Sau Ngon chicken were slightly lower (8 and 6 haplotypes; Hd=0.7246 and Hd=0.7190, respectively). The average nucleotide diversity (π) of all 129 mtDNA D-loop HV1 region sequences was approximately 0.00766. The highest nucleotide diversity (0.00558) was observed in the Mong Tien Phong chicken representative population while the lowest was observed in Ho chicken population (0.0220). The other four populations were around 0.00467 and 0.00349 (Table 4).

Table 4. Diversity indices of 6 chicken breeds

Item	n	H	Hd	P	π
Dong Tao	24	8	0.7246	11	0.00467
Mong Tien Phong	22	10	0.8139	17	0.00558
Ho	22	6	0.5931	10	0.00220
To	21	4	0.6143	11	0.00426
Mia	22	5	0.5801	7	0.00349
Sau Ngon	18	6	0.7190	15	0.00396
Total	129	27	0.7936	27	0.00766

n, number of sequences; H, number of haplotypes; Hd, haplotype diversity; P, number of polymorphic sites; π, nucleotides diversity

3.2. Genetic differentiation among the six Vietnamese indigenous chicken populations

In table 5, the estimated values of pairwise differentiation between the six Vietnamese indigenous chicken populations were displayed.

Table 5. Pairwise F_{ST} between 6 chicken breeds

Item	D	M	H	T	I	S
D	-	-	-	-	-	-
M	0.00646	-	-	-	-	-
H	0.08328	0.01105	-	-	-	-
T	0.75078	0.70801	0.80818	-	-	-
I	0.02598	0.01621	0.02317	0.76551	-	-
S	0.16519	0.06867	0.10064	0.73725	0.11550	-

D, Dong Tao; M, Mong Tien Phong; H, Ho; T, To; I, Mia; S, Sau Ngon.

The two populations with the biggest difference were Ho chicken and To chicken (F_{ST}=0.80818) and two populations with the smallest difference were Dong Tao chicken and Mong Tien Phong chicken (F_{ST}=0.00646). Dong Tao chicken, Mong Tien Phong chicken, Ho chicken and Mia chicken were fairly closely related to each other (average F_{ST}=0.02769). Sau Ngon chicken were also fairly close to the group of four. To representative population were quite different from the rest (average F_{ST}=0.75395).

3.3. Phylogenetic analysis and distribution of haplotypes

The phylogenetic tree (Figure 2) and haplotype distribution (Table 6) demonstrated the distribution of all 27 haplotypes of Vietnamese indigenous chickens found in this study in three haplogroups (A, B and E) out of the 13 haplogroups setting based on a study on whole mitochondrial genome (Miao *et al.*, 2012).

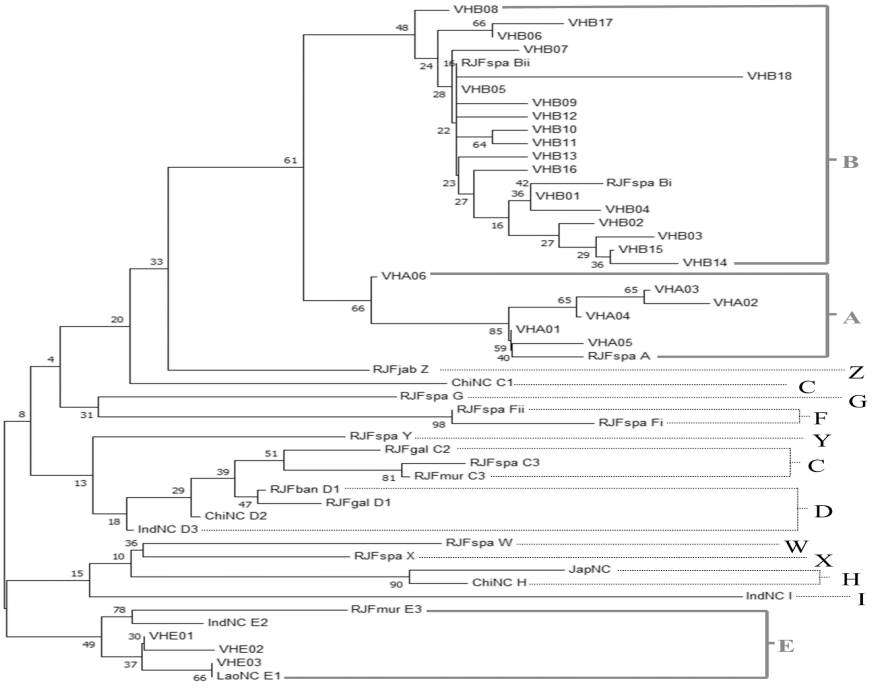
Haplogroup B was the major haplogroup which accounted for 18 haplotypes (66.67%) found in this study. Haplogroup A and Haplogroup E were lower with six (22.22%) and three (11.11%) haplotypes, respectively.

Haplogroup B was accounted for most of the individuals in the chickens studied with the exception of To. Haplogroup A consisted of only 10 individuals with four out of six haplotypes were singletons. Most of To individuals were sorted into Haplogroup E (90.48%) while the rest was grouped in Haplogroup B.

Table 6. mtDNA D-loop haplotypes distribution

Clade	Haplotypes	D	M	H	T	I	S	Total
B	VHB01	11	4	2	1	3	-	21
	VHB02	-	1	-	-	-	-	1
	VHB03	1	-	-	-	-	-	1
	VHB04	-	-	-	-	1	-	1
	VHB05	7	9	14	1	14	8	53
	VHB06	-	-	-	-	-	6	6
	VHB07	-	-	2	-	-	-	2
	VHB08	-	1	-	-	-	-	1
	VHB09	-	-	-	-	-	1	1
	VHB10	1	-	-	-	-	-	1
	VHB11	-	-	2	-	-	-	2
	VHB12	-	1	1	-	-	-	2
	VHB13	-	1	-	-	-	-	1
	VHB14	-	1	-	-	-	-	1
	VHB15	1	-	-	-	-	-	1
	VHB16	-	1	-	-	-	-	1
	VHB17	-	-	-	-	-	1	1
	VHB18	-	-	-	-	-	1	1
A	VHA01	1	-	-	-	3	-	4
	VHA02	1	-	-	-	-	-	1
	VHA03	1	-	-	-	-	-	1
	VHA04	-	-	1	-	-	-	1
	VHA05	-	2	-	-	-	-	2
	VHA06	-	-	-	-	1	-	1
E	VHE01	-	-	-	10	-	1	11
	VHE02	-	-	-	9	-	-	9
	VHE03	-	1	-	-	-	-	1
Total		24	22	22	21	22	18	129

Haplotype VHB05 contained the most individuals, at approximately 41.09%. Two major haplotypes from Sau Ngon chicken population were VHB05 (44.44%) and VHB06 (33.33%), the rest (VHE01, VHB05, VHB08, VHB12) were represented by only one individual. Most of Mong Tien Phong chicken individuals were grouped in haplotype VHB05 (40.91%) then followed by haplotypes VHB01 (18.18%) and VHA01 (9.1%). All other haplotypes of Mong Tien Phong chicken population were represented by one single sequence (VHB02, VHB06, VHB07, VHB09, VHB10, VHB11, VHE03). Haplotype VHB05 was also found in the majority of Mia chicken and Ho chicken (both accounted for 63.64%). The most frequent haplotypes found in Dong Tao chicken representative population were VHB01 (45.83%) and VHB05 (29.17%). The two major haplotypes of To chicken individuals were VHE01 (47.62%) and VHE02 (42.86%) while the rest (VHB01 and VHB03) represented by one single individual.



D, Dong Tao; M, Mong Tien Phong; H, Ho; T, To; I, Mia; S, Sau Ngon

Figure 2. An unrooted neighbor-joining tree displays the evolutionary relationship of relating the mtDNA D-loop haplotypes observed in the six Vietnamese indigenous chickens with reference sequences of 13 haplogroups (Miao *et al*., 2012; Teinlek *et al*., 2017)

4. DISCUSSION AND CONCLUSIONS

Indigenous chicken breeds of various geographical locations have been reported to have maternal lineages sharing among them (Liu *et al*., 2006; Oka *et al*., 2007; Teinlek *et al*. 2017). This study presented identical sequences counted as the same haplotypes from different breeds, which is in line with other reports. Therefore, domestic chickens are suggested to be closely related genetically regardless of breeds/ breeds and phenotypes. This might be the effect of human migration that causes long-distance gene flow and genetic material exchanges among chicken of different phylogeographical areas.

The phylogenetic result and the distribution of individuals in each haplotype revealed that the six representative Vietnamese indigenous chicken populations were each distributed into two to three haplogroups, which testified the existence of a contribution of multiple maternal lineages in all of them. All three haplogroups found in this study are also represented by red jungle fowls (Teinlek *et al*., 2017; Miao *et al*., 2012), indicating that red jungle fowls are the ancestor of domestic chickens including these six Vietnamese indigenous chicken breeds.

Haplogroups B and E appeared to be the two maternal lineages dominated the six Vietnamese indigenous chicken populations in this study. Haplogroup A, while accounted for more haplotypes than haplogroup E, contributed the least to the six chicken breeds. Approximately 75.97% of the Vietnamese indigenous chickens were found in haplogroup B, which distributes mainly in South Central/Southeast China and Southeast Asia and presumably originated from Yunnan and surrounding regions in China (Liu *et al*., 2006; Miao *et al*., 2012). This finding would be in agreement with historical records of human immigration from southern China to Vietnam. Yüeh people are inhabitants in the Southeastern coast of China and are the ancestors of the Cantonese, i.e., Guangzhou and Guangxi Southern Chinese people. By the 3rd century B.C., Yüeh people emigrated from Southern China to the Red River Delta of Vietnam and mixed with the indigenous Van Lang Vietnamese population (Taylor, 1983). Descriptions of immigration always state that people of a family moved together with their animals which could result in the introduction of chickens from Southern China into the North and South of Vietnam. The high percentage Haplogroup E is mainly distributed in Eurasian and South Asian domestic chickens and were accounted for 16.28% of the Vietnamese indigenous chickens and almost 90.5% of the To representative population. The matrilineal contributors of this haplogroup could have arisen from the Indian subcontinent and very likely spread to Southeast Asia.

Pairwise fixation indices between the six populations suggested that Dong Tao chicken, Mong Tien Phong chicken, Ho chicken and Mia chicken were closer related to each other than to those from To chicken and Sau Ngon chicken. Thus, suggested that the four former varieties were likely to be originated from a common ancestor belonged in haplogroup B and Sau Ngon chicken ancestor, while also belonged to haplogroup B, were different from the ancestor of the former group. The ancestor of To chicken breed might be a member of haplogroup E and had some genetic material exchanges with other haplogroups.

In conclusion, this study suggested that six Vietnamese indigenous chicken varieties, including Dong Tao, Ho, Mong Tien Phong, To, Mia and Sau Ngon chicken shared common multiple maternal lineages and possibly descended from the red jungle fowl. The ancestors of Vietnamese chicken might originate from Southern China (especially Yunnan and surrounding areas) and the Indian subcontinent and were introduced to Vietnam through human migration.

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GENETIC POLYMORPHISMS OF *DGAT1* AND *CAPN1* GENES, CANDIDATE GENES RELATED TO BEEF QUALITY, IN SOME CROSSBRED CATTLE POPULATIONS IN DAK LAK

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ABSTRACT

Diacylglycerol O-acyltransferase (*DGAT1*) and Calpain 1 (*CAPN1*) are candidate genes for beef quality traits. This study aimed to investigate genotype and allele frequencies of these genes in three crossbred cattle populations: BBB, Drought Master and Red Angus with local (LC) cattle in Dak Lak. Two hundred forty animals were genotyped for *DGAT1* (*Cfr*I) and *CAPN1* (*Btg*I) genes by PCR-RFLP method. The results showed that genotypic frequencies of *DGAT1* gen were ranged from 93.75 to 100% for AA genotype and from 0 to 6.25% for AK genotype. No animal with KK genotype of *DGAT1* gen was identified. The allelic frequencies of A and K alleles were rangned form 96.88 to 100% and 0 to 3.12%, respectively. There was no difference in allele frequencies between the populations. For the *CAPN1* gene, genotypic frequencies were ranged from 3.75 to 12.5% for CC genotype and from 0 to 43.75% for CG genotype and from 52.50 to 87.50% for GG genotype. The allelic frequencies of C and G were ranged from 12.50 to 25.62% and from 74.38 to 87.50%, respectively. There was significant difference in allele frequencies between (Drought Master x LC) and (BBB x LC), (Red Angus x LC) populations.

Keywords: *Genetic polymorphisms, DGAT1 and CAPN1 genes, crossbred cattle, Dak Lak*

1. INTRODUCTION[1]

The micromolar calcium-activated neutral protease (*CAPN1*) gene encodes a cysteine protease, calpain, that degrades myofibrillar proteins under postmortem conditions and appears to be the primary enzyme in the postmortem tenderization process. Regulation of calpain activity has been correlated with variation in meat tenderness (Geesink and Koohmaraie, 1999). Bovine *CAPN1* has been mapped to to chromosome 29. The majority of the SNPs were found in introns or were synonymous substitutions, except one substitution in exon 9 (C/G) and another in exon 14 (G/A) (SNP 316 y SNP 530, respectively). The SNP 316 (alleles C/G) determines the replacement of Ala by Gly in the amino acid 316 of the protein (domain II) and the other (alleles G/A) causes the change of Ile by Val in the position 530 (domain III). These SNPs have been associated with differences in beef tenderness in a wide range of Bos taurus breeds (Page *et al*., 2004; Kaupe *et al*., 2004). The diacylglyc- erol O-acyltra nsferase 1 (*DGAT1*) gene encodes the microsomal enzyme (*DGAT1*) in the triglyceride synthesis (Li *et al*., 2013). A lysine/alanine amino acid substitution in exon 8 region 232 (K232A) of *DGAT1* gene has been demonstrated to be associated with milk components (Cerit *et al*., 2014) and intramuscular fat content (Tait *et al*., 2014) in different cattle breeds. Both *CAPN1* and *DGAT1* genes have been shown to be important in regulating muscle and fa t metabolism of cattle (Schenkel *et al*., 2006; Curi *et al*., 2009; Li *et al*., 2013). In DakLak, directional selection in beef cattle to improve productivity, beef quality has been used to generate the crossbred between local cattle (LC) with imported breeds such as Drought Master (DrM), Red Angus (RA) and BBB. The objective of the current study was to examine the single nucleotide polymorphirm of the K232A substitution in the *DGAT1* gene and exon 9 (C/G) in *CAPN1* gene of these crossed catte populations.

2. MATERIAL AND METHODS

2.1. Samples collection and DNA extraction

A total of 240 ear tissue samples were collected from three crossbred cattle populations

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(80 samples per population), including (DrMxLC), (RAxLC) and (BBBxLC) in DakLak. Genomic DNA was extracted following the GeneJET Genomic DNA Purification Kit protocol (Thermo Fisher Scientific). DNA quality and quantity were checked by agarose gel (0.8%) and UV spectrophotometer (Nano drop machine).

2.2. Amplification of *DGAT1* and *CAPN1* genes

The *DGAT1* and *CAPN1* genes were amplified by PCR reaction with specific primers as repoted by De *et al*. (2004) and Soria *et al*. (2010) (Table 1).

Table 1. Primers sequence and SNPs for each gene

Locus	SNP	Forward (5′ - 3′)	Reverse (5′ - 3′)
DGAT1 (exon 8)	K232A (Lys/Ala)	TGGGCTCCGTGCTGGCCCTGATGGTCTA	TTGAGCTCGTAGCACAGGGTGGGGGCGA
CAPN1 (exon 9-316)	SNP316 (Ala/Gly)	CCAGGGCCAGATGGTGAA	CGTCGGGTGTCAGGTTGC

The components for each PCR reaction were: 2.5µl PCR 10X buffer, 2.5µl dNTP (2mM each), 2.5µl Mg2+ (25mM); 1µl primer (10pM each); enzyme ADN Taq polymerase (5 Ul/µl) 0,3µl, ADN 1,0µl, and ddH_2O for the total volume of 25µl. The amplification conditions were: 95^{0}C for 5min, 35 cycles at 94^{0}C for 45s, Tm for 50s, 72^{0}C for 1min, and a final extension at 72^{0}C for 5min. The Tm was specific for each candidate gene (66^{0}C for *DGAT1*, 62^{0}C for *CAPN1*).

2.3. Polymorphisms analysis by PCR-RFLP

PCR products of *DGAT1* and *CAPN1* genes were digested by restriction enzymes, *Cfr*I and *Btg*I, respectively, followed by the manufacturer's instructions. Polymorphisms was detected by agarose gel electrophoresis with a gel concentration of 2.5%.

2.4. Statistical analysis

Genotype and allele frequency were calculated by direct counting. Fisher's exact tests were performed to evaluate the significance of differences in allele and genotype frequency among studied populations. The Chi-square (χ^2) goodness of fit test was utilized to identify Hardy-Weinberg equilibrium by SPSS V.20.

3. RESULTS AND DISCUSSION

3.1. Polymorphisms of *DGAT1* gene

A 405bp fragment of *DGAT1* gene was amplified and consistent with previous report by De *et al.* (2004). PCR product after digesting by restriction enzyme *Cfr*I gave two alleles. A allele presented one band of 405bp and B allele presented two bands of 230 and 175bp (Figure 1).

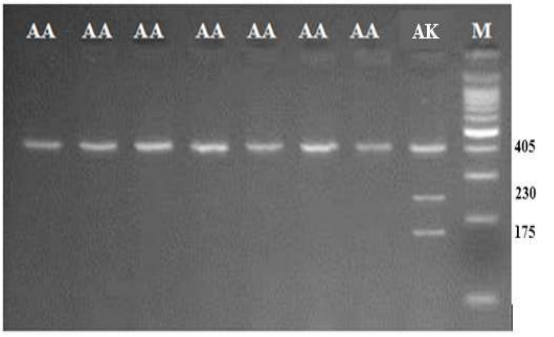

Figure 1. Genotyping of *DGAT1* gene by PCR-RFLP. M: **molecular weight standard 100bp ladder**

The results of genotyping in 204 animals identified two genotypes: AA and AK and no animal with KK genotype was identified. In (BBBxLC) and (DrMxLC) populations, the frequencies of AA genotype were 93.75 and 97.5%, respectively, the AK genotype frequencies were 6.25 and 2.50%, respectively. Only AA genotype was identified for (RAxLC) population. The frequency of A allele highly presented in three populations with to be 96.88, 100 and 98.75% for (BBBxLC), (RAxLC) and (DrMxLC), respectively. The frequency of K allele was low at 3.12, 0 and 1.25%, respectively. On average, A allele was 98.5%, and K allele was 1.5%. Fisher's exact test showed that allele frequency distribution between three populations was not significant difference. The allele distribution of (BBBxLC) and (DrMxLC) populations was in Hardy-Weinberg proportions (Table 2).

Table 2. Genotype and allele frequencies of *DGAT1* gene

Populations	Size	Genotype frequencies (%)			Allele frequencies (%)		$\chi 2(1, 0.05) = 3.841$
		AA	AK	KK	A	K	
BBBxLC	80	93.75	6.25	0	96.88[a]	3.12[a]	0.083
RAxLC	80	100	0	0	100[a]	0[a]	-
DrMxLC	80	97.50	2.50	0	98.75[a]	1.25[a]	0.013
Average	80	97.10	2.90	0	98.50	1.50	

Allele frequencies in the same column with different superscripts are significant difference at P<0.05

The substitution in exon 8 region 232 (K232A) of *DGAT1* gene has been demonstrated to be associated with milk components (Cerit *et al.*, 2014) and intramuscular fat content (Tait *et al.*, 2014) and the K allele has been proposed as the favorable allele. The polymorphism has also been reported to be associated with marbling in German HF and Charolais (Cha) cattle (Thaller *et al.*, 2003) and subcutaneous fat thickness in a Wagyu x Limousin (WxLm) cross (Wu *et al.*, 2005). In this study, the frequency of K allele was found to be very lower than that of A allele in all three populations. This results were similar to our previous study in Vietnamese yellow cattle populations and imported Brahman cattle population (Lan *et al.*, 2012) which showed the high level of A allele frequency and the low level of K (B) allele frequency. The A allele frequency was 100% for yellow cattle populations in Lang Son, Thanh Hoa, Ba Ria and 82% for Ha Giang cattle population. The K allele frequencies to be 18, 14, 15, 4 and 1% for Ha Giang, Phu Yen, Brahman (Br), U Dau Riu and Nghe An populations, respectively. However, the occurence of K allele frequency increased in HF cattle populations from deffirent countries such as 40% for HF population in New Zealand (Spelman *et al.*, 2002); 45% for HF in Germany (Thaller *et al.*, 2003); 47% for HF in Scotland was (Banos *et al.*, 2008), and 38% for HF in Egypt (Kaupe *et al.*, 2004). Especially, the B allele frequency was 63% for HF in France (Gautier *et al.*, 2007) and 86% for HF in Swiss (Näslund *et al.*, 2008). Selecting for greater fat concentration in milk seemed to lead to indirect selection for the K variant. This might be a reason for the high frequency of this variant in dairy cattle.

3.2. Polymorphisms of *CAPN1* gene

The 709bp fragment of *CAPN1* gene was amplified and consistant with reported by Soria *et al.* (2010). The PCR products contain one common and one polymorphic cut site for restriction BtgI enzyme. After enzyme digestion gave a common band of 87bp, and three polymorphic bands of 251 bp, 371 and 622bp. The G allele presented two bands of 622 and 87bp, allele C presented three bands of 371, 251 and 87bp. Three genotypes CC (371, 251 and 87bp), CG (622, 371, 251 and 87), and GG (622 and 87bp) were preseted in figure 2.

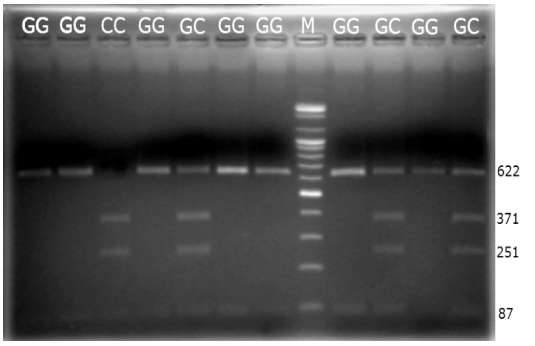

Figure 2. Genotyping of *CAPN1* gene by PCR-RFLP; M: molecular weight standard 100bp ladder

PCR-RFLP genotyping in 204 animals showed that the (BBBxLC) and (RAxLC) populations appeared three genotypes (CC, CG and GG) and the (DrMxLC) population appeared two genotypes (CC and GG). The G allele frequencies accounted in three populations to be 74.38% for (BBBxLC), 75.63% for (RAxLC) and 87.50 for (DrMxLC) population; while the C allele frequencies were 25.62, 24.32 and 12.50%, respectively. Fisher's exact test showed that the allele frequency distribution was significant difference between (DrMxLC) and (BBBxLC), (RAxLC) populations. The allele frequency distribution of *CAPN1* gen was in Hardy-Weinberg equilibrium for (BBBxLC) and (RAxLC) populations but was not for the (DrMxLC) population (Table 3).

Table 3. Genotype and allele frequencies of *CAPN1* gene

Populations	n	Genotype frequencies (%)			Allele frequencies (%)		$\chi 2$ (1, 0.05) = 3,841
		CC	CG	GG	C	G	
BBBxLC	80	3.75	43.75	52.50	25.62[a]	74.38[a]	1.747
RAxLC	80	3.75	41.25	55.00	24.32[a]	75.63[a]	1.130
DrMxLC	80	12.50	0.00	87.50	12.50[b]	87.50[b]	80.000
Average	80	6.70	28.30	65.00	20.80	79.2	27.626

Allele frequencies in the same column with different superscripts are significant difference at P<0.05

The C allele has been repoted that related to the meat tenderness trait (Corva *et al*., 2007; Page *et al*., 2004; Soria *et al*., 2010; White *et al*., 2005). However, in this study the frequency of C allele was identified to be lower than G allele in three populations. The results obtained in this study were similar to Page *et al*. (2004); Corva *et al*. (2007) and Soria *et al*. (2010) who reported that the frequencies of C lellele were lower than that of G allele in many beef cattle breeds (Table 4). Present results also support the study that was carried out by Li *et al*. (2013), reporting that the CC genotype was absent in Hereford, Limousin and Simmental populations. Similarly, Curi *et al*. (2009) and Allais *et al*. (2011) reported that the C allele and accordingly the CC genotype were rather low or absent in different cattle populations.

Table 4. Polymorphisms of *CAPN1* genes in different cattle populations

Breeds	Allele frequencies (%)		References
	G	C	
Angus	41	59	
Charolais	95	5	
Gelbvieh	100	0	
Hereford	94	6	Page *et al*.
Limousin	92	8	(2004)
RA	81	19	
Simmental	89	11	
Angus sires	61	39	
RA(RAxHd)	54	46	
(RAxHd)	59	41	Corva *et al*.
(LmxRA)	71	29	(2007)
Hd(HdxRA)	73	27	
RA sires	91	9	Soria *et al*.
(RaxBr) sire	81	19	(2010)
Br sires	100	0	

4. CONCLUSION

The frequencies of K allele for *DGAT1* gene and C allele for *CAPN1* gene, the favorable alleles related to meat tenderness and intramuscular fat content in beef cattle, were low in three crossbred cattle populations investigated. In oder to improve the frequencies of these favorable alleles, the sire with homogygous of KK for *DGAT1* and CC for *CAPN1* genes should be used for breeding programs.

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EVALUATING THE CARCASS YIELDS AND MEAT QUALITY OF NOI CROSSBRED CHICKENS

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ABSTRACT

This study was carried out with the aim of evaluating the carcass yields and meat quality of noi crossbred chickens in the G0 and G1 generations. A total of 60 Noi crossbred chickens at 13 weeks of age was divided into 2 equally sized generated groups (15 females and 15 males per each generation) selected for slaughter and meat yield and quality assessments. The results showed that live weight, weight and yields of internal organs such as gizzard, heart and liver, cecum and intestinal lengths were higher in G1 than in G0 (P<0.05), in contrast, the hot carcass weight and percentage of weight loss in G1 were lower than those in G0 (P<0.05). Male had higher live weight, carcass weight, heart weight and intestinal length than female, but lower breast meat yield than female (P<0.05). Besides, drip loss, pH of breast meat and thigh meat, and color values (L*, a*) were significantly higher in G1 than in G0. The pH value of breast and thigh meat at 30min, 24h and 48h, a* value of breast meat at 30min, 24h and 48h, and a* value of thigh meat at 48h were higher in male than female (P<0.05). In contrast, L* value of breast and thigh meat at 48h, and b* value of thigh meat at 48h were lower in male than female (P<0.05). The interaction between generation and gender was significantly different on drip loss of thigh meat at 48h; pH of breast and thigh meat at 30min, 24h and 48h; a* of breast meat at 24h and 48h; L* of breast meat at 48h and b* value of thigh meat at 30min (P<0.05). The lowest values of these above quality traits were on G0 Female, while G1 Male was lowest on b* value of thigh meat at 30min (P<0.05). There were significant differences in body measurements between G0 and G1 (P< 0.05)

Keywords: *Meat quality, selection, Noi crossed breed, sex, carcass yield.*

1. INTRODUCTION

In 2019, the total poultry meat of the whole country was 1302,5 thousand tons, ranking second after pork production, of which local chicken meat has contributed a part in the production (channuoivietnam.com, 2019). The Noi chicken is a native chicken breed that has been kept for a long time by the small holders, well adapted to the weather conditions in the South of Vietnam (Nguyen Van Thuong, 2004) and is currently being raised widely in both households with free-range and on semi-intensive farms (Le Thi Hoa, 2012). Noi chicken meat is considered a specialty product that is increasingly consumed by the domestic market and has great export potential, moreover, the demand for using this chicken breed for ornamental, cockfighting for entertainment also increases the value of this breed (Nguyen Van Quyen and Vo Van Son, 2008). However, crossbreeding with other chicken breeds for different purposes risks the degradation of this local breed. Therefore, optimizing the yield and meat quality of this chicken breed is being focused to contribute to raising income for farmers.

Selection and breeding is one of the methods used to improve the carcass yield and meat quality of livestock breeds. There have been many studies on yield and meat quality on different local chicken breeds such as Tau Vang chicken (Do Vo Anh Khoa *et al*., 2012), Ninh Hoa Ri and Luong Phuong chickens (Tran Quang Hanh and Pham The Hue, 2017) and Noi chicken (Nguyen Thi Thuong, 2015). However, the evaluation of chicken carcass yield and meat quality through selective breeding generations has not been reported, so this study was carried out to evaluate the performance and meat quality of the Noi crossbred lines at 13 weeks of age through 2 generations of selection.

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2. MATERIALS AND METHODS

2.1. Animals and management

The Noi crossbred chickens were raised from 1 day old to 13wks old at the experimental farm in Thuan Tien B hamlet, Thuan An commune, Binh Minh town, Vinh Long province, from February 2019 to February 2021. The experiment was performed with 60 crossbred chickens at 13 weeks of age in the G0 and G1 generations. The chickens have been fully vaccinated and dewormed according to the farm's procedures.

The feed provided for experimental chickens was a bran mixture with main ingredients including corn, broken rice, fish meal, soy protein, wheat bran, rice bran, amino acids, vitamin and mineral supplements, etc. Feed samples were taken and analyzed for nutritional composition according to AOAC (1986) and energy was measured by Bomb Calorimeter (IKAC600, Germany) at the Laboratory of Animal Nutrition and Feed, Department of Animal sciences, Faculty of Agriculture, Can Tho University. The nutritional value of feed for experimental chickens consisted of 2 rearing stages: (1) the stater period at 1-4 weeks of age was 3,000 kcal/kg ME and 21% CP, 0.82% calcium, 0.72% phosphorus; (2) the grower period at 5-13 weeks of age, 3,150 kcal/kg ME and 18% CP, 0.45% calcium, 0.65% phosphorus. Chicks were fed *ad-libitum* and water was available for free access.

2.2. Experimental design and data collection

Sixty crossbred female and male with each generation of 30 birds (15 roosters and 15 female/generation) were selected at 13 weeks of age, in good health, no infectious diseases and physical features without defects. Chickens selected for slaughter had the average weight of chickens for the whole experiment in each generation.

The slaughter process in chickens was carried out according to the method of Bui Huu Doan *et al.* (2011). Chickens selected for slaughter were weighed and given water only without feeding 24 hours before slaughter, the weight of chickens before and after bleeding; feathers, head, legs and internal organs were removed and the carcass was weighed. After slaughter and removing the skin and bones, chicken breast meat and thigh meat were stored in the refrigerator at 4^{o}C for 24 and 48 hours to evaluate the criteria for color and meat quality.

The parameters of meat yield include live weight before 24hrs without feeding, live weight, weight loss rate in 24hrs before slaughter, carcass weight, carcass percentage, heart weight, gizzard weight, liver weight and weight loss, the percentage of internal organs, breast weight, breast meat and the percentage of breast, breast meat, thigh and thigh meat and the ratio of thigh and thigh meat were recorded by Nhon Hoa 1 or 2kg balance, electronic balance (KD-TBED, Taiwan) with an error of 0.01g. Intestinal length and cecum length were measured by a tape measure.

The quality parameters of breast and thigh meat including drip loss, pH and meat color were recorded at 30min, 24hrs and 48 hours after slaughter. The drip loss of breast and thigh meat was calculated based on weight at different time points of breast and thigh meat. pH was measured with a handheld pH meter (Hanna Mettler, America) and meat color was measured with Konica Minolta Chroma Meter CR-400 (HI991001, Japan) with CIELAB parameters (L*, a* and b*) where a* takes a positive value for reddish and a negative value for green, b* gets a positive value for a yellowish color and a negative value for a bluish color. 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).

The parameters of the chicken's body appearance such as head width, neck length, breast length, breast circumference, breast depth, body length, thigh length and leg height were measured by measuring tape measure and body weight following FAO guideline (2012).

2.3. Statistical analysis

Experimental data were preliminarily processed by Excel 2016 software and statistically processed by Minitab 16 software with a general linear model (GLM), to determine the level of significant difference of the treatments by the Tukey test with 95% confidence.

3. RESULTS

3.1. Morphological traits of Noi crossbred chicks

The results of the body measurements of the crossbred chickens at 13wks of age between G0 and G1 were presented in Table 1 showing that the live weight, head width, neck length, chest length, chest circumference and chest depth were significantly different between two generations (P<0.05). In which, the parameters of live weight, head width, chest length, chest circumference and chest depth of chickens in G0 were lower than in G1, except that neck length in G0 was longer than G1. Similarly, the body morphological appearance parameters such as live weight (LW), head width (HW), chest circumference (CC), chest depth (CD) and thigh length (TL) between males and females were also statistically significant (P<0.05). Besides, HW, CC and TL are lower in females than males, whereas females have higher CD than males. The results of the interaction analysis between two factors, generation and sex showed that all other body measurements of chickens were not statistically significant with P>0.05.

Table 1. Some body morphological parameters of Noi crossbred chickens

Parameter	Generation		SEM/P	Sex		SEM/P	Generation x Sex				SEM/P
	G0	G1		Female	Male		G0Female	G0Male	G1Female	G1Male	
LW, g	1,004	1,084	24.95/0.03	985.2	1,102	24.95/0.00	937.3	1,069	1,033	1,135	35.29/0.67
HW, cm	26.08	27.87	0.51/0.01	26.23	27.72	0.51/0.05	25.93	26.23	26.53	29.21	0.72/0.10
NL, cm	15.62	13.81	0.29/0.00	14.94	14.49	0.29/0.27	16.03	15.21	13.85	13.77	0.41/0.37
CL, cm	10.19	12.04	0.23/0.00	10.91	11.31	0.23/0.24	10.00	10.37	11.83	12.25	0.33/0.94
CC, cm	24.01	24.72	0.24/0.05	23.89	24.85	0.24/0.00	23.34	24.68	24.43	25.01	0.34/0.28
CD, cm	6.87	8.09	0.21/0.00	7.92	7.04	0.21/0.00	7.58	6.16	8.27	7.91	0.30/0.09
CA,o	68.63	66.07	1.70/0.29	67.47	67.23	1.70/0.92	69.53	67.73	65.40	66.73	2.40/0.51
BL, cm	16.06	16.24	0.24/0.61	16.10	16.20	0.24/0.77	15.83	16.30	16.37	16.10	0.34/0.28
TL, cm	18.99	19.54	0.33/0.24	18.78	19.75	0.33/0.04	18.85	19.12	18.70	20.39	0.47/0.14
ShL, cm	9.89	7.58	1.61/0.31	9.54	7.93	1.61/0.48	11.72	8.07	7.367	7.80	2.27/0.37

Live weight (LW), head width (HW), neck length (NL), chest length (CL), chest circumference (CC), chest depth (CD), chest angle (CA), body length (BL), thigh length (TL), shank length (ShL)

3.2. Carcass yields of Noi crossbred chickens

Table 2. Carcass yields of Noi crossbred chickens

Parameters	Generation		SEM/P	Sex		SEM/P	Generation x Sex				SEM/P
	G0	G1		Femal	Mal		G0Female	G0Male	G1Female	G1Male	
LW before 24h slr, g/bird	1,074	1,110	24.56/0.30	1,028	1,156	24.56/0.00	1,000	1,148	1,057	1,163	34.74/0.55
LW, g/head/bird	1,004	1,084	24.95/0.03	985.2	1,102	24.95/0.00	937.3	1,069	1,033	1,135	35.29/0.67
Weight loss, %	6.54	2.38	0.55/0.00	4.27	4.66	0.55/0.62	6.21	6.88	2.33	2.43	0.78/0.71
Hot carcass wt, g/bird	716.4	679.3	21.22/0.22	661.0	734.7	21.22/0.01	661.7	771.0	660.3	698.3	30.01/0.23
Hot carcass yield, %	71.32	62.54	1.06/0.00	67.26	66.60	1.06/0.66	70.50	72.12	64.01	61.07	1.50/0.13
Breast weight, g/bird	119.6	123.3	6.96/0.70	129.6	113.2	6.96/0.10	119.5	119.7	139.8	106.8	9.85/0.09
Breast yield, %	16.83	18.02	0.82/0.31	19.50	15.35	0.82/0.00	18.09	15.57	20.91	15.13	1.161/0.16
Thigh weight, g/bird	149.1	155.5	7.40/0.54	146.3	158.3	7.40/0.25	137.1	161.1	155.4	155.6	10.47/0.26
Thigh yield, %	20.80	22.91	0.86/0.08	22.10	21.61	0.86/0.68	20.72	20.87	23.49	22.34	1.22/0.59
Gizzard weight, g/bird	16.86	20.26	0.86/0.00	17.78	19.34	0.86/0.20	15.20	18.53	20.36	20.16	1.20/0.15
Gizzard yield, %	2.28	3.03	0.12/0.00	2.70	2.61	0.12/0.59	2.31	2.24	3.10	2.97	0.18/0.85
Heart weight, g/bird	4.69	5.68	0.28/0.01	4.66	5.72	0.28/0.01	4.04	5.35	5.29	6.08	0.40/0.52
Heart yield, %	0.65	0.85	0.04/0.00	0.70	0.80	0.04/0.12	0.61	0.69	0.79	0.92	0.06/0.71
Liver weight, g/bird	20.67	27.28	0.99/0.00	22.88	25.08	0.99/0.12	19.13	22.22	26.63	27.94	1.41/0.52
Liver yield, %	2.90	4.18	0.19/0.00	3.48	3.60	0.19/0.64	2.90	2.90	4.06	4.31	0.27/0.64
Internal organ wt, g/bird	110.6	151.7	4.38/0.00	124.1	138.2	4.38/0.02	138.2	118.5	145.5	158.0	6.20/0.78
Intestinal length, cm	113.3	124.8	3.09/0.01	113.9	124.1	3.09/0.02	103.4^{b}	123.2^{a}	124.4^{a}	125.1^{a}	4.38/0.03
Cecum length, cm	13.43	28.13	1.11/0.00	20.27	21.29	1.11/0.52	12.85	14.00	27.70	28.57	1.57/0.92

Means with different superscript in a row are significantly different (P<0.05)

3.3. Meat quality traits of Noi crossbred chickens

The carcass yields of Noi crossbred chickens in Table 2 showed that there were significant differences between 2 generations on live weight (LW), weight loss, hot carcass weight, gizzard weight, heart weight, liver weight and their yields, internal organs weight, intestinal and cecum lengths (P<0.05). Live weight, weight and yields of internal organs such as gizzard, heart and liver, cecum and intestinal lengths were higher in G1 than in G0 (P<0.05), in contrast, the hot carcass weight and weight loss in G1 were lower than those in G0 (P<0.05). In addition, male had higher value of live weight, hot carcass weight, heart weight, internal organs weight and intestinal length than female, but lower value of breast yield as compared to female (P<0.05). There was an interaction between generation and sex on intestinal length (P<0.05) in G0 females had the lowest intestinal length compared with G1 female and male at G0 and G1. The other parameters of carcass yield did not show any interaction between these two factors (P>0.05).

The results of meat quality traits of Noi crossbred chickens at 13 weeks of age were shown in Table 3 showed that drip loss of breast meat at 24h and 48h; pH value of breast meat at 30min, 24h and pH value of thigh meat at 30min, 24h and 48h were significantly higher in G1 than in G0. Similarly, color values of thigh and breast meat at recorded times were higher in G1 compared to G0 (P<0.05), in which L* value of breast and thigh meat at 30min, 24h and 48h; b* value of thigh meat at 30min, 24h and 48h and a* value of breast meat at 30min in G1 were higher than G0, while only b* value of breast meat at 24h and 48h in G0 was found higher than G1.

Table 3. Drip loss, pH value, meat color of breast and thigh meat of Noi crossbred chickens

Parameters		Generation		SEM/P	Sex		SEM/P	Generation * Sex				SEM/P
		G0	G1		Female	Male		G0Female	G0Male	G1Female	G1Male	
Driploss	$Breast_{24h,}$ g	2.30	4.19	0.48/0.00	3.04	3.45	0.48/0.54	2.20	2.41	3.88	4.50	0.67/0.76
	$Breast_{48h,}$ g	3.93	6.00	0.66/0.03	5.25	4.69	0.66/0.55	4.13	3.73	6.36	5.64	0.93/0.86
	$Thigh_{24h,}$ g	1.27	1.47	0.20/0.48	1.13	1.62	0.20/0.08	0.94	1.61	1.32	1.63	0.28/0.53
	$Thigh_{48h,}$ g	2.09	2.23	0.33/0.77	1.74	2.58	0.33/0.08	1.14^{b}	3.05^{a}	2.34^{ab}	2.12^{ab}	0.47/0.03
pH	$Breast_{30min}$	5.59	6.09	0.04/0.00	5.69	5.99	0.04/0.00	5.35^{c}	5.84^{b}	6.04^{ab}	6.14^{a}	0.06/0.00
	$Breast_{24h}$	5.83	6.00	0.04/0.01	5.76	6.07	0.04/0.00	5.60^{b}	6.05^{a}	5.92^{a}	6.08^{a}	0.06/0.02
	$Breast_{48h}$	5.85	5.96	0.03/0.07	5.75	6.06	0.03/0.00	5.63^{c}	6.07^{a}	5.86^{b}	6.05^{ab}	0.05/0.02
	$Thigh_{30p}$	5.61	6.31	0.04/0.00	5.78	6.14	0.04/0.00	5.31^{c}	5.91^{b}	6.25^{a}	6.37^{a}	0.06/0.00
	$Thigh_{24h}$	5.85	6.28	0.04/0.00	5.87	6.26	0.04/0.00	5.59^{c}	6.11^{b}	6.16^{b}	6.41^{a}	0.05/0.02
	$Thigh_{48h}$	5.90	6.28	0.03/0.00	5.94	6.24	0.03/0.00	5.68^{b}	6.11^{a}	6.20^{a}	6.37^{a}	0.05/0.02
Meat color	Breast L^{*}_{30min}	44.97	54.15	0.84/0.00	48.79	50.32	0.84/0.20	44.26	45.67	53.32	54.98	1.19/0.91
	a^{*}_{30min}	2.52	3.67	0.24/0.00	2.73	3.45	0.24/0.04	1.93	3.11	3.54	3.79	0.35/0.19
	b^{*}_{30min}	7.04	7.14	0.58/0.90	7.14	7.03	0.58/0.89	6.68	7.39	7.60	6.67	0.82/0.32
	L^{*}_{24h}	50.77	55.71	0.74/0.00	53.08	53.40	0.74/0.76	50.68	50.85	55.48	55.94	1.05/0.89
	a^{*}_{24h}	3.61	2.84	0.32/0.09	2.65	3.81	0.32/0.01	2.41^{b}	4.81^{a}	2.88^{b}	2.80^{b}	0.45/0.00
	b^{*}_{24h}	8.99	6.59	0.67/0.01	8.20	7.38	0.67/0.39	9.02	8.97	7.38	5.80	0.95/0.43
	L^{*}_{48h}	48.62	54.82	0.65/0.00	52.99	50.44	0.65/0.00	51.51^{b}	45.72^{c}	54.47^{ab}	55.16^{a}	0.92/0.00
	a^{*}_{48h}	4.13	3.14	1.03/0.50	1.91	5.36	1.03/0.02	0.96^{b}	7.29^{a}	2.85^{ab}	3.42^{ab}	1.46/0.05
	b^{*}_{48h}	8.20	6.77	0.47/0.03	8.05	6.93	0.47/0.10	8.41	7.99	7.68	5.86	0.66/0.30
	Thigh L^{*}_{30m}	45.42	54.87	1.12/0.00	50.53	49.76	1.12/0.63	45.28	45.56	55.78	53.96	1.59/0.51
	a^{*}_{30m}	5.21	9.27	0.57/0.00	6.48	8.00	0.57/0.06	4.71	5.71	8.24	10.30	0.80/0.51
	b^{*}_{30m}	6.96	6.50	0.61/0.60	6.60	6.86	0.61/0.76	5.62^{b}	8.29^{a}	7.57^{a}	5.43^{b}	0.87/0.00
	L^{*}_{24h}	48.57	53.43	0.87/0.00	50.80	51.20	0.87/0.75	48.16	48.99	53.45	53.41	1.23/0.72
	a^{*}_{24h}	7.61	11.33	0.52/0.00	9.31	9.63	0.52/0.66	7.27	7.94	11.35	11.31	0.72/0.63
	b^{*}_{24h}	8.62	8.49	0.78/0.90	8.81	8.29	0.78/0.63	9.20	7.99	8.39	8.58	1.11/0.52
	L^{*}_{48h}	47.47	53.59	0.75/0.00	52.51	48.56	0.75/0.00	50.0	44.94	55.02	52.17	1.06/0.30
	a^{*}_{48h}	5.75	11.05	0.46/0.00	7.32	9.48	0.46/0.00	4.87	6.63	9.77	12.33	0.65/0.54
	bđ$^{*}_{48h}$	7.14	8.90	0.66/0.06	9.65	6.39	0.66/0.00	9.31	4.97	9.99	7.80	0.94/0.25

The results on the effect of gender showed that pH value of breast and thigh meat at 30min, 24 and 48h; a* value of breast meat at 30min, 24 and 48h, and a* value of thigh meat at 48h were higher than female (P<0.05). In contrast, L* value of breast and thigh meat at 48h, and b* value of thigh meat at 48h were lower in male than female (P<0.05). The interaction between generation and gender was significantly different on drip loss of thigh meat at 48h; pH of breast and thigh meat at 30min, 24 and 48h; a* of breast meat at 24 and 48h; L* of breast meat at 48h and b* value of thigh meat at 30min (P<0.05). The lowest values of these above quality traits were on G0Female, while G1Male was lowest on b* value of thigh meat at 30min (P<0.05).

4. DISCUSSION

Body weight is an important parameter of carcass yield of live poultry combined with body morphological appearance (Bui Huu Doan *et al.*, 2011). The increase in live weight, weight of internal organs, and body measurements of G1 Noi crossbred chickens compared to G0 showed that initially successfully selected chicken breeds with higher productivity and economic efficiency. In addition, the difference in body weight and body morphological appearance between male and female may be due to the skeletal structure and muscle fiber diameter of males are larger than that of females (Bui Huu Doan *et al*., 2011). Besides, the study found a large difference in gut length of males that are longer than females or the intestinal length in G1 higher than G0 generation is explanned by using commercial feed as a basal diet in the experiment, so the nutrient digestion and absorption process was mainly in intestine, thereforce, the feed absorption and metabolism in male chicks were more efficient than female chicks as well as adaptation over time of breeding. Indeed, Noi crossbred chickens raised selectively in this experiment had a heavier body weight than Noi chickens of Nguyen Thi Thuong (2015) also recorded at 13 weeks of age. This difference is mainly due to the variety of local chicken breeds and also proves that this Noi crossbred brings higher economic value to farmers. In addition, the results of this experiment are similar to those of Lawrie (1991), Guni and Katule (2013) and Pripwai *et al.* (2014) also found male chickens were higher than female chickens on most carcass traits. These differences are caused by sex hormone factors, male chicks only focus on growth, while females, beside using nutrition for growth they also distribute to develop reproductive functions, which lead to their growth lower than cocks.

The quality of chicken is also assessed by the quality of each part of the carcass (Bui Huu Doan *et al*., 2011) and is influenced by many different factors such as genetics, feed (Fletcher, 1999; Xiong *et al*., 2019). The results of meat quality of Noi crossbred chickens in this experiment show the initial effectiveness of the selection process by the improvement of pH value and color index of the G1 generation as compared to the parent generation-G0. The pH values of breast meat (5.59-6.09) and thighs (5.61-6.31) of Noi crossbred chickens in this experiment were similar to those reported by Liu and Niu (2008) and Jaturasitha *et al.* (2008), however, the interesting result of pH value of breast and thigh meat of Noi crossbred chickens in this experiment was the increase in post-slaughter compared with other reports which decreased pH of meat in post-slaughter on White Lueyang (WL), Arbor Acres (AA) and WL*AA F1 crossed chicken (Liu and Niu, 2008), Black-boned chickens, Thai local chickens, Bresse and Rhode Island Red chickens (Jaturasitha *et al.*, 2008). Changes in pH during slaughter are associated with changes in meat quality (Le Bihan-Duval *et al*., 2008). After slaughter, muscle glycogen is converted to lactic acid and a decrease in muscle deoxygenation leads to a sharp drop in pH, and in poultry that are not stressed at slaughter, the pH reaches its final value. after about 30 min of slaughter is between 5.6 and 6.0 (Ali *et al*., 1999). An increase in post-slaughter pH was found in beef when stored at 4°C for 7-14 days, Stanistic *et al*. (2012) suggested that it is the result of alkylation taking place by the presence of spoilage microorganisms or by the release of basic products of proteolysis during endogenous alteration. after slaughter. Research results of Craig *et al.* (1999); Santos *et al.* (2004) found an increase in the pH value over time, which could be the result of proteolysis during chicken aging. According to Ali *et al*. (1999) suggested that the slower rate of pH decline was the result of anaerobic glycolysis,

because the abrupt relaxation in muscle due to shear would reduce the demand for high concentrations of ATP in the tissue. However, the results showed that the pH of breast meat and thigh meat of Noi crossbred chickens in this experiment were in a normal state, there was no protein breakdown or meat decay, so the cause of the increase in pH value of breast meat and thigh meat in the experiment is still unknown. In addition, the exudate in breast meat and thigh meat of experimental chickens was also lower than that of Black-boned chickens 8.26 and 4.22%, Thai local chickens 10.39 and 3.2%, Bresse chicken 8.43 and 3.66%, and Rhode Island Red chicken 11.14 and 4.09% (Jaturasitha et al., 2008). However, the breast meat L* index of Noi crossbred chickens was similar to that reported by Liu and Niu (2008) and lower than that of Black-boned chickens, Thai local chickens, Bresse and Rhode Island Red chickens (Jaturasitha *et al*., 2008). The difference between the study results may be due to differences in breed, rearing conditions, slaughter process.

5. CONCLUSION

The carcass yield of selection Noi crossbred chickens in G1 generation was significantly improved in the live weight and meat quality than in G0 generation.

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USING FEMALE HYBRID PIGS BETWEEN GF337 AND GF24 AS A SOW FOR REPRODUCTION

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ABSTRACT

The objective of this experiment is to investigate the reproductive performance of GF24 sows and hybrid sows GF337xGF24 from crossbred between GF337 sire and GF24 dam under industrial production system. Reproductive performance was recorded from 53 sows (37 GF24 and 16 GF337xGF24) at Anh Duong farm, Cao Bang province from January 2019 to March 2021 with 4 parities. At birth and at weaning, number of piglets was counted while litter weight was recorded. The total number of piglets at birth, at weaning and litter weight at weaning of GF24 sows was higher than that of GF337xGF24 (P<0.05). Inversely, individual bodyweight at birth and survival rate to weaning of GF24 were lower than GF337xGF24 (P<0.05). Additionally, litter weight at birth was not significantly different between two groups of sows (P>0.05). In all parity, number of piglets at birth, weaning and litter weight at weaning of GF24 tended to be higher than GF337xGF24 except second parity. While, SRW of GF24 was lower than GF337xGF24 but this difference was not significant (P>0.05) except the third parity (P<0.05). Although reproductive traits of GF337xGF24 sows was lower than GF24 sows, however all traits of both sows were higher than technical requirements of exotic breeding pigs according to TCVN9111:2011. These results suggest that GF337xGF24 pigs can be used for reproduction as alternative approach in short term in case of deficit. Further study could be done to investigate production performance of offspring from GF337xGF24 sows.

Keywords: *Fattening pigs, swine, reproductive performance, ASF.*

1. INTRODUCTION

African Swine Fever (ASF) was first recognized in East Africa in the early 1900s as a disease causing high mortality in domestic pigs (Montgomery, 1921). Since 2007, this disease has been reported in multiple countries across Africa, Asia, and Europe, in both domestic and wild pigs (OIE, 2021)

In Vietnam, the first ASF outbreak was confirmed in 2019 (Le *et al.*, 2019). In the end of December 2019, ASF has recorded in 8,527 communes in 667 districts of all provinces with a total of 6 million pigs culled (GSO, 2021). As sequence, the deficits of a gilts were detected in the pigs breeding market, especially for the farms of small and middle scale. Using fattening female pigs for the reproductive purpose was an alternative approach in this crisis (Nguyên Huân and Hưng Giang, 2020). GF24 and GF337 are well known as dam- and sire lines of Green Feed Company (GF). These pigs were used to produce fattening pigs as recommended by GF (Le Dinh Phung *et al.*, 2019). However, these crossbreeding pigs had been using as sows due to ASF outbreak. The reproductive performance of sows based on these crossbreeding pigs is not confirmed in any research. Reproduction of GF24 sows was mentioned in the studies of Van Ngoc Phong *et al.* (2018) and Hoang Thi Mai *et al.* (2019) while production and of their offspring was reported by Le Dinh Phung *et al.* (2019) and Le Dinh Phung *et al.* (2020).

The objective of this experiment is to investigate the reproductive performance between GF24 sows and sows from hybrid pigs between GF337 sire and GF24 dam at Anh Duong pig farm in Cao Bang province.

2. MATERIAL AND METHODS

The experiment was carried out at Anh Duong farm, Cao Bang province from January 2019 to March 2021. A total of 53 sows (37 GF24 and 16 GF337xGF24) that mated from January to July

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2019 were selected. GF337xGF24 was the offspring from GF337 sire and GF24 dam. The animals were reared in the industrial production system.

The reproductive performance was recorded from first to fourth parity with the following traits: duration of cycle (DC, day), total number of pig born (NB, piglet), number born alive (NBA, piglet), individual bodyweight at birth (BW, kg), litter weight at birth (LBW, kg), survival rate at birth (SRB, %), number to weaning (NW, piglet), individual bodyweight at weaning (WW, kg), litter weight at weaning (LWW, kg) survival rate to weaning (SRW, %) and weaning age (WA, day). At birth and at weaning, number of piglets was counted while litter weight was recorded. Individual bodyweight at birth and weaning was an average of litter weight over number of piglets. The piglets were weaned at an average age of 24 days.

The feed rations were starter (21% protein, 3,300 kcal ME), gestation (14.0% protein, 3,000 kcal ME) and lactation (16.5% protein, 3,200 kcal ME). The vaccination program was applied for prevention of enzootic pneumonia, porcine reproductive and respiratory syndrome, classical swine fever, Aujeszky, porcine parvovirus, and foot and mouth disease.

The data presented in the tables are sample size (n), arithmetic mean (Mean), standard deviation (SD). A linear model including the fixed effects of Sow and Parity was adjusted to the data. $Y_{ijk} = \mu + Sow_i + Parity_j + e_{ijk}$; *where, Y_{ijk} is reproductive traits of; μ is overall mean; Sow_i is fixed effect of sow group i (GF337xGF24 and GF24); $Parity_j$ is fixed effect of parity j (first, second, third and fourth); e_{ijk} is residual errors*. The means of the various sows group and parity were compared using Duncan test. Later, the reproductive traits were compared between two groups of sows by parity using the same model mentioned above without Parity effect. The data were analyzed using Minitab software 16.

3. RESULTS

Reproductive performance of GF24 and GF337xGF24 sows is shown in Table 1 showing that the total number born, at weaning and litter weight at weaning of GF24 sows was higher than that of GF337xGF24 (P<0.05). Inversely, BWB and SRW of GF24 were lower than GF337xGF24 (P<0.05). Additionally, litter weight at birth was not significantly different between two groups of sows (P>0.05). NB, NBA and NW of GF337xGF24 were 2.14, 2.03 and 1.06 piglets, respectively lower than GF24. NBA, NW, LBW and LWW of both sows are presented in the Figure 1 and 2. NBA and NW were lowest at the first parity and increased over parities (Figure 1). NBA was highest at the fourth parity (13.1) while NW at the third parity (12.6). The similar trend was observed for LBW and LWW (Figure 2).

Performance of each group of sows was also presented from first to fourth parity (Table 2). In all parity, number of piglets at birth, weaning and litter weight at weaning of GF24 tended to be higher than GF337xGF24 except second parity. While, SRW of GF24 was lower than GF337xGF24 but this difference was not significant (P>0.05) except the third parity (P<0.05). Duration of cycle was not different between two groups of sows through parities (P>0.05). For GF24, NB, NBA and NW increased from the first to fourth parity. However this trend was not observed for GF337xGF24 sows. These values were dropped in the third parity (Table 2).

Table 1. Reproductive performance of sows

Variable	GF24		GF337xGF24		RMSE
	n	Mean	n	Mean	
DC	102	150.46	45	148.18	16.63
NB	139	13.96^a	61	11.82^b	2.57
NBA	139	13.13^a	61	11.10^b	2.30
BW	119	1.29^b	46	1.40^a	0.14
LBW	119	16.87	46	15.96	3.09
SRB	139	94.47	61	94.7	6.47
NW	137	11.43^a	57	10.37^b	1.62
BWW	118	6.65	57	6.68	0.64
LWW	118	76.56^a	57	69.16^b	11.29
SRW	137	88.97^b	57	94.98^a	11.72
AW	137	24.04	57	23.77	2.85

Means with differing letters in each row within an effect differ (P<0.05), duration of cycle (DC, day), total number of pig born (NB, piglet), number born alive (NBA, piglet), individual bodyweight at birth (BWB, kg), litter weight at birth (LWB, kg), survival rate at birth (SRB, %), number to weaning (NW, piglet), individual bodyweight at weaning (BWW, kg), litter weight weaning (LWW, kg) survival rate to weaning (SRW, %) and weaning age (WA, day).

Table 2. Reproductive performance of GF24 and GF337xGF24 sows according to parity

Variable	1st parity					2nd parity					3rd parity					4th parity				
	GF24		GF337x24		RMSE	GF24		GF337x24		RMSE	GF24		GF337x24		RMSE	GF24		GF337x24		RMSE
	n	Mean	n	Mean		n	Mean	n	Mean		n	Mean	n	Mean		n	Mean	n	Mean	
DC	-	-	-	-	-	36	155.1	14	146.4	19.12	35	148.9	16	152.3	20.00	31	146.8	15	145.5	6.14
NB	37	13.16^{a}	16	10.25^{b}	2.30	36	13.36	14	12.21	2.71	35	14.57^{a}	16	11.69^{b}	2.60	31	14.90	15	13.27	2.62
NBA	37	12.62^{a}	16	9.94^{b}	2.18	36	12.89	14	11.71	2.66	35	13.51^{a}	16	10.88^{b}	2.14	31	13.58^{a}	15	12.00^{b}	2.14
BWB	34	1.23	1	1.26	0.15	23	1.25^{b}	14	1.44^{a}	0.18	32	1.36	16	1.35	0.12	30	1.30^{b}	15	1.43^{a}	0.10
LWB	34	15.06	1	11.3	3.06	23	16.08	14	16.61	2.79	32	18.60^{a}	16	14.61^{b}	3.14	30	17.67	15	17.11	2.76
SRB	37	95.94	16	97.52	5.25	36	93.39	14	96.17	5.66	35	93.26	16	94.29	7.43	31	91.83	15	90.77	7.49
NW	37	10.89^{a}	16	8.75^{b}	1.60	34	11.12	14	11.21	1.85	35	12.00^{a}	16	10.81^{b}	1.54	31	11.77	11	11.00	1.26
BWW	24	6.59	16	7.04	0.79	30	6.62	14	6.82	0.70	33	6.62	16	6.42	0.42	31	6.74	11	6.36	0.55
LWW	24	71.79^{a}	16	61.38^{b}	10.96	30	73.97	14	76.21	12.80	33	79.79^{a}	16	70.31^{b}	10.77	31	79.32^{a}	11	69.82^{b}	10.13
SRW	37	88.06	16	89.09	14.46	34	89.43	14	96.31	11.96	35	90.13^{b}	16	99.48^{a}	8.88	31	88.23	11	95.3	10.56
AW	37	23.68^{b}	16	26.06^{a}	2.43	36	23.67^{a}	14	22.14^{b}	2.19	34	24.03	16	24.5	2.97	30	24.97^{a}	11	21.45^{b}	3.17

Means with differing letters in each row within an effect differ (P<0.05), duration of cycle (DC, day), total number of pig born (NB, piglet), number born alive (NBA, piglet), individual bodyweight at birth (BWB, kg), litter weight at birth (LWB, kg), survival rate at birth (SRB, %), number to weaning (NW, piglet), individual bodyweight at weaning (BWW, kg), litter weight weaning (LWW, kg) survival rate to weaning (SRW, %) and weaning age (WA, day).

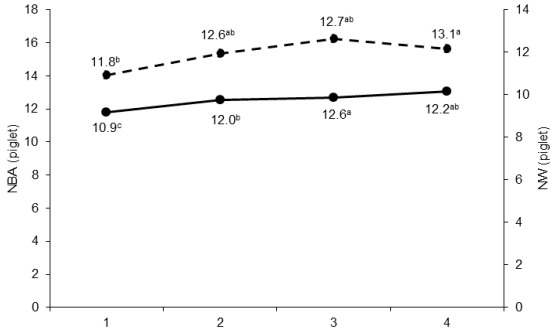

Figure 1. NBA (— —) and NW (—) of GF24 and GF337x24 sows by parities

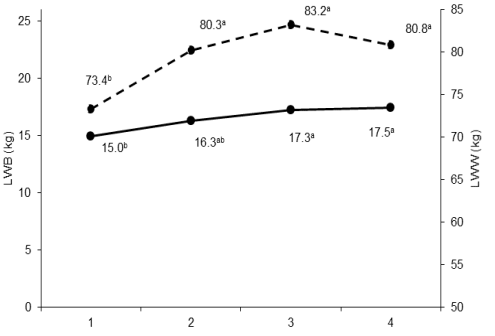

Figure 2. LWB (—) and LWW (— —) of GF24 and GF337x24 sow by parities

4. DISCUSSION

Performance of two groups of sows in our study was high. NBA, NW, LBW and LWW of both sows were higher than those of Landrace and Yorkshire according to TCVN9111:2011 (MARD, 2011). NBA and NW of GF24 in present study were consistent with the study of Van Ngoc Phong *et al.* (2018) and Hoang Thi Mai *et al.* (2019) on the same sows, while BW was higher. NBA and NW of GF24 was higher than Landrace but lower than Yorkshire sows in the study of Nguyen Thi Hong Nhung *et al.* (2020).

Recent studies of Van Ngoc Phong *et al.* (2018) and Hoang Thi Mai *et al.* (2019) on GF24 sows shown that NB, NBA and NW of GF24 sows were higher than that of GF337xGF24 sows in our study while BWB were similar. All reproductive traits of Landrace and Yorkshire sows in study of Nguyen Thi Hong Nhung *et al.* (2020) on Landrace and Yorkshire sows were also higher than GF337xGF24 sows except DC. Reproductive performance of GF337xGF24 sows was consistent with study of Trinh Hong Son and Nguyen Thi Huong (2019) on hybrid sows between Landrace and Yorkshire

Reproductive performance of GF24 sows by parity was consistent with the results published

by Aherne and Kirkwood (2001), Hoang Thi Mai *et al.* (2019), Nguyen Thi Hong Nhung *et al.* (2020), Serenius and Stalder (2006), Tretinjak *et al.* (2009). When using GF337xGF24 fattening pigs as sows, NB, NBA and NW were inconsistent with the results of above authors. These result shown that the reproductive traits of GF337xGF24 sows were not stable in comparison with GF24 sows.

This study was focused to investigate the reproductive performance of GF337xGF24 sows and the results shown that all traits were higher than technical requirements of exotic breeding pigs according to TCVN9111:2011. However, the production performance of offspring from these sows as fattening pigs is unknown. Further study could be done to investigate production performance of offspring from GF337xGF24. The information related to production performance of GF337xGF24 offspring is important for economic point of view.

5. CONCLUSION

Number of piglets at birth, at weaning and litter weight at weaning of GF24 sows was higher than that of GF337xGF24 ($P<0.05$). Inversely, survival rate to weaning of GF24 were lower than GF337xGF24 ($P<0.05$). Additionally, litter weight at birth was not significantly different between two groups of sows ($P>0.05$). Although reproductive traits of GF337xGF24 sows was lower than GF24 sows, however all traits of both sows were higher than technical requirements of exotic breeding pigs according to TCVN9111:2011. These results suggest that GF337xGF24 pigs can be used for reproduction as alternative approach in short term in case of deficit. Further study cloud be done to investigate production performance of offspring from GF337xGF24 sows.

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ALLELE AND GENOTYPIC FREQUENCIES OF GENES ASSOCIATED TO BODY CONFORMATION IN KAZAKHSTAN KUSHUM HORSES

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ABSTRACT

Kazakhstan Kushum horses were formed from local breeds with imported breeds like Thoroughbred, Trotter, Russian Don ... Horses are tall stature, providing meat, herding cattle, and serving the army. The aim of study was to investigate the genotype distribution and the allele frequency of the genes that can carry the point mutation, associated to body conformation by PCR-RFLP method in the *HMGA2* gene g.81481064 *C>T* (high mobility group AT-hook), related to withers height, the *LASP1* gene g.23259732 G>A (*LIM* and *SH3* protein 1), related to higher stature, and the *ZFAT* gene g.75550059 *C>T* (zinc finger and AT hook domain containing), associated to withers height. As a result, the distribution of *TT, TC* and *CC* genotypes in the *HMGA2* gene were 86.4, 13.6 and 0% respectively, the frequency of recessive *C* allele caused a decrease in withers height of 0.07. Genotypes of *GG, GA* and *AA* in *LASP1* gene were 13.6, 72.8 and 13.6%, the frequency of recessive *A* allele causing stature increase was 0.5. The *ZFAT* gene has *CC, TC* and *TT* genotypes, respectively, 72.7, 27.3 and 0%, frequency of recessive *T* allele, causing withers height increase is 0.14. Thus, the recessive *C* allele in the *HMGA2* gene has a very low frequency, indicating that this population may be under strong selection pressure for particular body conformation, expressed through the high frequency in recessive allele A=0.5 belongs to *LAST* gene. In addition, this population continued to show signs of a recessive allele belonging to the *ZFAT* gene, increasing withers height. Therefore, the Kushum horse has a big stature, and tends to be suitable for long-distance travel.

Keywords: *HMGA2, LASP1,* and *ZFAT gene, Kushum horses, stature.*

1. INTRODUCTION

The Kushum is one of the Kazakhstan original horse breeds established in the Ural region of West Kazakhstan between 1931 and 1976 through crossing between mares of local horses and stallions of Thorough-bred, Trotter and Russian Don breeds (Dmitriez and Ernst, 1989). The original goal of the breeding of Kushum horses was to improve the body size, endurance performance, and gait of the local horses to match the military demand for war horses before World War II. In the later periods, the horses have been used mainly for milk and meat production (Dmitriez and Ernst, 1989). This breed has a big body, hardiness and well adapted to the semi-desert grassland environment. They have important roles in the local community such as herding cattle, goats, sheep and serve the army. However, molecular genetic characteristics related to diversity in stature, physical activity... have not been studied.

Horses serve humans by their physical activity. Under strong selection pressure during their short evolutionary time has resulted in genotypic homogeneity within individuals, however, there is still considerable variation between horse breeds (Petersen *et al*., 2013). They have distinctive characteristics related to physical activity such as body composition and movement pattern. Recently, there have been many molecular genetic diversity studies in horses, including studies related to genome-wide analysis (Petersen *et al*., 2013). As a result, gene polymorphisms are closely related to physical fitness, body structure and locomotion characteristics in different horse breeds

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(Albertsdóttir *et al*., 2011; Andersson *et al*., 2012; Makvandi-Nejad *et al*., 2012; Signer-Hasler *et al*., 2012). The allele frequencies of genes differ markedly among horse breeds, such as those for riding, racing and pulling (Makvandi-Nejad *et al*., 2012; Petersen *et al*., 2013; Promerová *et al*., 2014).

The genetic basis of variation in horse body size was investigated by Signer-Hasler *et al*. (2012) and Makvandi-Nejad *et al*. (2012), through four loci explain 83% of size variation in the horses namely *HMGA2, ZFAT* and *LASP1* and *LCORL* gene.

The indicators of length, width and depth of chest increased significantly (13.2%), the *CC* genotype of the *HMGA2* gene g.81481064 *C>T* increased in size in the anterior and posterior phenotypes by 29.4 and 21.9%, respectively, when compared with the *TT* genotype, while the *TT* genotype increased in length circumference body 6.9% (Sevane *et al*., 2016). Point mutations at *HMGA2* have an important role in determining the height in horses and many other animals (Weedon *et al*., 2008; Boyko *et al*., 2010). The SNP g.81481064 *C>T* polymorphism in *HMGA2* is strongly associated with wither height in different horse breeds, the *C* recessive allele of this SNP causes reduced wither height (Makvandi-Nejad *et al*., 2012).

The *LASP1* gene and the *LIM* and *SH3* proteins are involved in vertebrate development, and may affect animal stature. Among them, *LASP1* gene expression can affect cartilage tissue formation and osteogenic differentiation. (Joos *et al*., 2008; Hermann-Kleiter *et al*., 2009; Lin *et al*., 2004). The SNP g.23259732*G>A* polymorphism in *LASP1* is strongly associated with wither height in different horse breeds (Junior *et al*., 2018). A mutation (*G>A*) in the *LASP1* gene represent differences in the height of the animals, the *A* allele shows an association with the tall horses, while the *G* allele, even in heterozygotes, causes reduced height in Brazilian horses. Jun *et al*. (2014) studied Marwari horses and identified *LASP1* as a candidate gene for stature.

In addition, the *ZFAT* gene encodes a DNA-binding protein and functions as a transcriptional regulator involved in cell survival and natural death (Tsunoda *et al*., 2010). In the region from 74,795,013 to 76,254,733bp, including the *ZFAT* gene was identified as a candidate region for the study of wither height in horses. The *ZFAT* gene is associated with wither height in several horse breeds (Makvandi-Nejad *et al*., 2012; Signer-Hasler *et al*., 2012). In which, the SNP g.75550059 *C>T,* point mutation was strongly associated with wither height in different horse breeds. The *T* recessive allele of this SNP causes an increase in wither height (Makvandi-Nejad *et al*., 2012). Furthermore, Signer-Hasler *et al*. (2012) suggested that SNP point mutations in Fraches-Montagnes horses are strongly associated with wither height and other body compositions.

In this study, SNP polymorphisms of three genes *HMGA2* g.81481064 *C>T*, *LASP1* g.23259732 *G>A*, and *ZFAT* g.75550059 *C>T* related to stature were analyzed in 22 Kushum horses.

2. MATERIALS AND METHODS

The 22 blood samples were randomly collected from the population of Kushum horses in Zhanibek and Kaztalov regions, Kazakhstan. Blood was collected from the jugular vein and stored in a vacuum tube containing anticoagulant EDTA. DNA extraction from white blood cells was performed by the phenol-chloroform method. Functional genes such as *HMGA2, LASP1* and *ZFAT* were amplified via PCR using primer pairs (Table 1) and genotype identification by PCR-RFLP (Table 3). PCR reactions were performed in a 10µl mixture, consisting of 10ng DNA, 0.2µM primers, 0.25 µmol/l dNTPs, 2×PCR GoTaq DNA buffer, 1U Go Taq DNA polymerase (Toyobo, Osaka), Japan). The thermal cycling is shown in Table 2. After amplification, PCR products and restriction enzyme cut products were electrophoresed in 1.5% agarose gel, TAE buffer (15-45 min/75-135V), stained with Gelred and observed with UV transilluminator. Allele and genotype frequencies were calculated according to the Hardy Weinberg equilibrium (HWE) principle, based on the difference between predicted and detected values (p=P+H/2, q=Q+H/2), where p and q are allele frequencies.

Table 1. Primer sequences and restriction enzymes for genotyping

Gene	Primer (5′ - 3′)	Fragment lengths (bp)	Sources
HMGA2	F: TGATTTCAGTGTTGCTTCTCT R:TTTATGTTGTTATCTGCCTGTG	246	Sevane *et al*. (2016)
LASP1	F: ACACCCCAACACATACAACCC R: CAGGGGCATGTGCAGCTA	177	This study
ZFAT	R: GCAGAGACCCTTTGAGACC R: GCACCATTTATGTTCCTTCA	389	Sevane *et al*.(2016)

Table 2. PCR conditions

Gene	Step 1	Step 2	Step 3	Final
HMGA2	95°C, 10 minutes 1 cycle	95°C - 45 seconds; 58°C - 60 seconds; 72°C - 30 seconds; 35 cycles	72°C - 10 minutes 1 cycle	8°C - ∞ 1 cycle
LASP1	95°C, 10 minutes 1 cycle	95°C - 45 seconds; 55°C - 30 seconds; 72°C - 30 seconds; 35 cycles	72°C - 10 minutes 1 cycle	8°C - ∞ 1 cycle
ZFAT	94°C, 30 seconds 1 cycle	95°C - 30 seconds; 53°C - 60 seconds; 72°C - 30 second; 35 cycles	72°C - 10 minutes 1 cycle	8°C - ∞ 1 cycle

Table 3. Restriction enzyme with cleavage site, incubation temperature and time of incubation

Gene	Cleavage site	Restriction enzyme	Incubation temperature (°C)	Incubation time	Genotyping
HMGA2g.81481064 C>T	5…AC/GT..3	*HpyCH4III*	37	1 hour	T = 247bp; C = 137, 110 bp
LASP1 g.23259732 G>A	5…C/CGC…3	*Aci1*	37	1 hour	G = 48, 129bp ; A = 177 bp
ZFAT g.75550059 C>T	5…TG/CA…3	*HpyCH4V*	37	1 hour	T = 267, 122bp; C = 389 bp

3. RESULTS AND DISCUSSION

3.1. Variation in *HMGA2 gene*

Genotypic diversity affecting horse stature and body size from the *HMGA2* g.81481064 *C>T* gene was analyzed on 22 Kushum horses. The results showed that the recessive allele *C*, which causes a decrease in wither height, has a very low frequency (0.07). Therefore, this population may have experienced high phenotypic selection pressure during stallion screening.

The *HMGA2* gene plays a crucial role in determining height in horses (Weedon *et al*., 2008; Boyko *et al*., 2010). The SNP g.81481064 *C>T* in *HMGA2* is strongly associated with wither height in different horse breeds, the *C* recessive allele of this SNP causes reduced wither height (Makvandi-Nejad *et al*., 2012). Then, Frischknecht *et al*., (2015) described the SNP c.83G>A (p.G28E) in *HMGA2* to be strongly associated with wither height in Shetland horses and the *C* allele of this SNP to be related to decrease in wither height.

From Table 4, we see that there are 19/22 Kushum horses with genotype *TT*, 3 have genotype *TC*, no individual has homozygous recessive *CC* of *HMGA2* gene. The distribution of *TT, TC* and *CC* genotypes in the *HMGA2* gene is 86.4%, 13.6% and 0%, respectively, the frequency of recessive allele *C* causes a decrease in wither height by 0.07. Thus, this population of Kushum horses has a fairly homogenous dominant genotype, possibly as a result of the breeding process that screened for recessive traits that cause reduced wither height in horses.

Table 4. Genotype distributions and allele frequencies of genes

Gene	Genotype distributions			Allele frequencies		HWE*
HMGA2 g.81481064 C>T	*TT*	*TC*	*CC*	*T*	*C*	0.12
	19	3	0	0.93	0.07	
LASP1 g.23259732 G>A	*GG*	*GA*	*AA*	*G*	*A*	NA
	3	16	3	0.5	0.5	
ZFAT g.75550059 C>T	*CC*	*TC*	*TT*	*C*	*T*	0.55
	16	6	0	0.86	0.14	

** HWE: Chi-square values in Hardy Weinberg Equilibrium*

3.2. Variation in *LASP1* gene

Results of *LASP*1 gene analysis in Table 4 shows the presence of three genotypes: *GG, GA* and *AA*. In which, the highest frequency was genotype *GA* (16 horses, 72.8 %) and the lowest was *GG* and *AA* (3 horses, 13.6%). The genotypes of *GG, GA* and *AA* in the *LASP1* gene are 13.6, 72.8 and 13.6%, respectively, the frequency of recessive allele *A* causing increased horse stature is 0.5. Thus, this horse population carries fifty percent recessive *A* allele that has the potential to increase body size.

The *LASP1* gene may functioned as an adaptive molecule involved in cell signaling or bone cell structural organization. The *LASP1* gene and its *LIM* and *SH3* proteins are involved in vertebrate bone development, and may affect animal stature. Among them, the expression of *LASP1* can affect cartilage tissue formation and osteoblast differentiation. (Joos *et al*., 2008; Hermann-Kleiter *et al*., 2009; Lin *et al*., 2004).

The SNP g.23259732 *G>A* polymorphism in *LASP1* was strongly associated with wither height in different horse breeds. The recessive *A* allele of this SNP increases the height of horses (Makvandi-Nejad *et al*., 2012). The results were then confirmed by Junior *et al*. (2018), who suggested that in the *LASP1* gene, mutant alleles (*G >A*) resulted in height differences of animals. The *A* allele showed an association with tall horses, the *G* allele was associated with reduced wither height in Brazilian horses, and the author proposes this SNP as a molecular marker for height assessment in horses. Jun *et al*. (2014) also evaluated the stature of the Marwari horse and identified *LASP1* as a candidate gene for assessing horse stature. Therefore, the recessive allele *A* = 0.5 of the *LASP1* gene g.23259732 *G>A* reflects the potential to increase the size and physique of this Kushum horse breed.

3.3. Variation in *ZFAT* gene

ZFAT gene polymorphism analysis in the Table 4 showed the presence of three genotypes: CC, TC and TT at 72.7, 27.3 and 0%, respectively, and the frequency of the recessive allele T causing increased wither height is 0.14.

The ZFAT gene is associated with body height in some horse breeds. In which, the SNP g.75550059 C>T polymorphism was strongly associated with wither height in different horse breeds. The T recessive allele of this SNP is associated with increased wither height (Makvandi-Nejad et al., 2012). Furthermore, Signer-Hasler et al. (2012) suggested that a SNP polymorphism g.74798143 A>G belongs to the ZFAT gene in Fraches-Montagnes horses, which is closely related to wither height and other body compositions, recessive allele related to wither height increase. Thus, this population of Kushum horses has signs of carrying a recessive allele in the ZFAT gene that helps increase wither height.

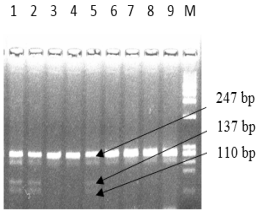

Figure 1. Genotype *HMGA2*: 1,2,5(*TC*); 3,4,6,7,8,9(*TT*)

M: Marker (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72bp). Wells 1-9 are, respectively, the product of the HpyCH4III enzyme that cuts the HMGA2 fragment 247bp amplified from the total DNA of the 1st-9th horse.

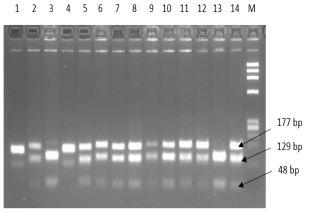

Figure 2. Genotype *LASP1*: 1,2,4,5, 6,7,8,9,10,11,12,13(*GA*); 3,13(*GG*)

M: Marker (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72bp). Wells 1-14 are, respectively, the product of the Aci1 enzyme that cuts the LASP1 fragment 177bp amplified from the total DNA of the 1st-14th horse.

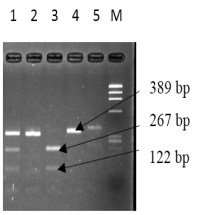

Figure 3. Genotype *ZFAT:* *2,4,5*(*CC*); 1(*TC*); 3(*TT*)

M: Marker (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72bp). Wells 1-5 are, respectively, the product of the enzyme HpyCH4V that cuts the ZFAT fragment 389bp amplified from the total DNA of the 1st-5th horse. Well 3, 5 are the control with genotypes are TT and CC

4. CONCLUSION

The Kushum horse population in this study carried the recessive allele *C*, which causes reduced wither height, belonging to the HMGA2 gene, with very low frequency, suggesting that this horse population may have experienced great tall stature selection pressure during the process of forming new breed. Moreover, clearly shown by the high frequency of recessive allele A, helping increased wither height, belonging to the LAST1 gene. In addition, the population continued to show signs of carrying a recessive allele of the ZFAT gene, which increased the horse's wither height. Thus, the Kushum horse has a tall stature, tends to be suitable for long-distance travel, and is suitable for field surveying.

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POLYMORPHISM IN *LCORL, MSTN,* AND *DMRT3* GENES ASSOCIATED TO BODY CONFORMATION AND LOCOMOTION TRAITS IN KUSHUM HORSES

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ABSTRACT

Kushum were bred in Western Kazakhstan by crossbreeding of local mare with imported breeds such as Trotter, Thoroughbred and Russian Don. This breed has a tall and flexible body, playing an important role in meat and milk production and serving the army. In this study, we investigated the genotypic distribution and allele frequency of genes that could carry point mutations related to body conformation and locomotion traits by PCR-RFLP method, such as *LCORL* g.105547002 *C>T* (associated to withers height), *MSTN* g.66493737 *C>T* (related to high endurance stamina), and *DMRT3* g.22999655 *C>A* (altered the pattern of locomotion and strong positive impact on trotting performance). As a result, genotypic distributions of *TT, TC,* and *CC* in *LCORL* were 50, 45 and 5%, respectively, and the frequency of recessive *C* allele related to increase withers height by 0.27. And genotypic distributions of *TT, TC* and *CC* in *MSTN* were 82, 18 and 0%, accordingly, and the frequency of *T* allele associated with high endurance stamina was 0.91. Thus, these horses may have experienced phenotypic selection pressure in the process of forming new breed. Moreover, genotypes of *CC, CA,* and *AA* in *DMRT3* were distributed respectively by 77, 18 and 5%, and the frequency of recessive *A* allele, associated to locomotion by 0.14. Therefore, Kushum horse might be the result of the introgression of *A* allele from Trotter stallions. As a result, the discovered alleles of these geneses might be a trace of a breeding history and many Kushum horses do not carry recessive mutations that change the trotting pattern, do not develop muscle, but increase withers height, tend to be suitable for long distance travel.

Keywords*: DMRT3, LCORL, MSTN gene, Kushum horse, conformation and locomotion traits.*

1. INTRODUCTION

The Kushum horse breed established in the Ural region of West Kazakhstan between 1931 and 1976 through crossing between the local mare and stallions of Russian Don, Trotter, and Thorough-bred breeds (Dmitriez and Ernst, 1989). The original aim of the breeding of Kushum horses was to improve the endurance performance, body size, and gait of the local horses to match the military demand for war horses before World War II. In the later periods, the horses have been used mainly for meat and milk production (Dmitriez and Ernst, 1989). This breed has a big body, hardiness and well adapted to the semi-desert grassland environment. They have important roles in the local community such as herding cattle, goats, sheep and serve the army. However, molecular genetic characteristics related to body conformation and locomotion traits... have not been studied.

Horses have mainly worked for people by their physical performance. Therefore, horse breeds have been intensively selected for particularly traits related to physical performance, such as body composition and locomotion traits. As a result, many different breeds with different physical traits, such as stamina, muscular power, pattern of locomotion and body composition, have been formed (Petersen *et al.*, 2013).

Recently, the genes associated with physical traits of horses have been identified by genome-wide association studies (GWAS) (Hill *et al.*, 2010; Andersson *et al.*, 2012; Signer-Hasler *et al.*, 2012; Makvandi-Nejad *et al.*, 2012), and have disclosed that allele frequencies of these genes significantly differ among breeds with different

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aims, such as breeds for draughting, racing, and riding (Makvandi-Nejad *et al.*, 2012; Petersen *et al.*, 2013; Promerová *et al.*, 2014). Particularly, Double-sex and mab-3-related transcription factor 3 (*DMRT3*), ligand dependent nuclear receptor corepressor-like/non-SMC condensing I complex, subunit G (*LCOR*L), and myostatin (*MSTN*), are of particular interest regarding the physical performance of horses. Since a single nucleotide polymorphism (SNP) of the *LCORL g.105547002 C>T* is associated with withers height of the horse (Signer-Hasler *et al.*, 2012; Makvandi-Nejad *et al.*, 2012), and SNP of *MSTN g.66493737 C>T* is associated with body composition and racing performance of racehorses (Tozaki *et al.*, 2010; Hill *et al.*, 2010), and a nonsense mutation of the *DMRT3 g.22999655 C>A* has major effects on the locomotion pattern of the horse (Andersson *et al.*, 2012).

Since Kushum horses in Kazakhstan are mainly used for meat and milk production, herding cattle, and serving the army, and their body sizes are fairly tall. Their genetic characteristics related to body composition and physical performance are of particular interest. We, therefore, discovered genotype distributions and allele frequencies of these three genes in Kazakhstan, as an initial step in genetic studies to understand genetic bases for body composition and physical performance of Kazakhstan Kushum horses.

2. MATERIALS AND METHODS

Total 22 blood samples were randomly collected from the population of Kushum horses in Kaztalov and Zhanibek regions, Kazakhstan. Blood was collected from the jugular vein and stored in a vacuum tube containing anticoagulant EDTA. DNA extraction from white blood cells was performed by the phenol-chloroform method. Functional genes such as *LCORL, MSTN* and *DMRT3* were amplified via PCR using primer pairs (Table 1) and genotype identification by PCR-RFLP (Table 3). PCR reactions were performed in a 10µl mixture, consisting of 10ng DNA, 0.2µM primers, 0.25 µmol/l dNTPs, 2×PCR GoTaq DNA buffer, 1U Go Taq DNA polymerase (Toyobo, Osaka), Japan). The thermal cycling is shown in Table 2. After amplification, PCR products and restriction enzyme cut products were electrophoresed in 2% agarose gel, TAE buffer (15-45 min/75-135V), stained with Gelred and observed with UV transilluminator. Allele and genotype frequencies were calculated according to the Hardy Weinberg equilibrium (HWE) principle, based on the difference between predicted and detected values (p=P+H/2, q=Q+H/2), where p and q are allele frequencies.

Table 1. Primer sequences and restriction enzymes for genotyping

Gene	Primer (5′ - 3′)	Fragment lengths (bp)	Sources
LCORL	F: GCCATCTATTTGCATGTTCTTG R: GGCAAGTTCATAGGCTGGTTC	347	Metzger *et al.* (2013
MSTN	F: ATTTGATAGCAGAGTCATAAAGGAAAAGTA R: CTGCGATCCTGCTTTACCCA	132	Polasik *et al.* (2015)
DMRT$_3$	R: CGACAAAGACACCGACCAGA R: CCGATCCCACGGACCATT	485	Han *et al.* (2015a)

Table 2. PCR conditions

Gene	Step 1	Step 2	Step 3	Final
LCORL	94°C, 30 seconds 1 cycle	94°C - 30 seconds, 60°C - 30 seconds. 72°C - 40 seconds, 35 cycles	72°C - 5 minutes 1 cycle	8°C - ∞ 1 cycle
MSTN	95°C,10 minutes 1 cycle	95°C - 45 seconds, 55°C - 45 seconds. 72°C - 45 seconds, 35 cycles	72°C - 5 minutes 1 cycle	8°C - ∞ 1 cycle
DMRT3	94°C 5min 1 cycle	94°C - 20 seconds, 65°C 20 seconds. 72°C - 20 seconds, 35 cycles	72°C - 5 minutes 1 cycle	8°C - ∞ 1 cycle

Table 3: Restriction enzyme with cleavage site, incubation temperature and time of incubation

Gene	Cleavage site	Restriction enzyme	Incubation temperature (°C)	Incubation time	Genotyping
LCORL g.105547002 C> T	*5...AG/CT…3*	*AluI*	37	1 hour	*C* = 292, 55; *T* = 347bp
MSTN g.66493737 C> T	*5…GT/AC…3*	*RsaI*	37	2 - 3 hour	*T* = 132; *C* = 102, 30bp
DMRT3 g.22999655C> A	*5..C/TNAG..3*	*DdeI*	37	1 hour	*C* = 485; *A* = 413, 72bp

3. RESULTS AND DISCUSSION

3.1. Variation in *LCORL gene*

LCORL gene has been identified as the gene associated with carcass weights of cattle (Setoguchi *et al.*, 2009; Eberlein *et al.*, 2009) and human height (Weedon *et al.*, 2008); Gudbjartsson *et al.*, 2008). In horses, Makvandi-Nejad *et al.* (2012); Signer-Hasler *et al.* (2012) revealed that an SNP of *LCORL* is strongly associated with withers height in various horse breeds. The *C* allele of this SNP is the minor allele associated with increased withers height. It is also associated with other body composition traits (Tozaki *et al.*, 2016).

As shown in Table 4, the genotyping results of *LCORL* gene in Kushum horses indicated that three genotypes and the distribution of *TT*, *TC* and *CC* genotypes were 50, 45 and 5%, respectively, the frequency of recessive allele *C* was 0.27. Comparing with native horses in Myanmar, Kazakhstan, and Japan, these populations are remarkably small body, bearing the recessive *C* allele of *LCORL* gene were 0.13, 0.10 and 0.03 respectively, Kushum *C* allele is larger than those by 0.27 (Okuda *et al.*, 2016; Paul Ripon Chandra, 2019; Nguyen Ba Trung, 2020). Metzger *et al.* (2013) also reported that the *TT* genotype was highly associated with all pony breeds up to the limit value of 1.48 m for the withers height. Horses ranging from 1.30m to 1.60m at the withers showed *TT* and *CT* genotypes, while the taller and heavier horses showed predominantly the *CC* genotype, associated with greater stature. Levels of transcripts in the *LCORL* gene were 40% lower in the *CT* heterozygote relation to the *CC* homozygote, whereas this genotype presented 56% less transcription than the *TT* genotype. Thus, these Kushum horses may have experienced phenotypic selection pressure during stallion screening for higher stature.

Table 4. Genotype distributions and allele frequencies of genes

Genes	Genotype distributions			Allele frequencies		HWE*
LCORL g.105547002 C>T	*TT*	*TC*	*CC*	*T*	*C*	0.47
	11 (0.5)	10 (0.45)	1 (0.05)	0.73	0.27	
MSTN g.66493737 C>T	*TT*	*TC*	*CC*	*T*	*C*	0.22
	18 (0.82)	4 (0.18)	0	0.91	0.09	
DMRT3 g.22999655C>A	*CC*	*CA*	*AA*	*C*	*A*	1.14
	17 (0.77)	4 (0.18)	1 (0.05)	0.86	0.14	

* *HWE: Chi-square values in Hardy Weinberg Equilibrium*

3.2. Variation in *MSTN* gene

MSTN is a negative regulator of skeletal muscles growth, and many mutations in *MSTN* gene have been shown to be associated with muscle hypertrophy phenotypes in cattle, dogs, mice as well as humans (Grobet *et al.*, 1997; Schuelke *et al.*, 2004; Mosher *et al.*, 2007). In horses, an SNP of *MSTN* (g.66493737 *C>T*) was initially revealed to be strongly associated with racing performance and body composition of racehorses (Hill *et al.*, 2010; Tozaki *et al.*, 2010; Tozaki *et al.*, 2011), and later reported to be associated with proportions of muscular fiber types (Petersen *et al.*, 2013). *T* allele of *MSTN* associated with high endurance stamina. The *C* allele is the minor allele associated with higher proportions of Type 2B muscular fiber and lower proportions of Type 1 muscular fiber.

As shown in Table 4, the genotyping results of *MSTN* in Kushum horses indicated that two

genotypes and the distribution of *TT*, *TC* and *CC* genotypes were 82, 18 and 0%, respectively, the frequency of allele *T* was 0.91.

In dogs, the whippet racing dogs that are *TC* for a *MSTN* polymorphism have significantly greater racing ability than both *CC* wild-type dogs and *TT* for the mutation that have an increased musculature that is detrimental to performance (Grobet *et al.*, 1997; Schuelke *et al.*, 2004; Mosher *et al.*, 2007). It also has suggested that an intronic variant in *MSTN* is predictive of the best race distance for the Thoroughbred (Hill *et al.*, 2010).

The horses with the *TT* genotype in *MSTN* gene were revealed to show high endurance stamina in long-distance races, while those with the *CC* genotype were released to show high speeds in short-distance races (Hill *et al.*, 2010). Since the initial aim of breeding of Kushum horses was mainly for military use, endurance for long-distance locomotion might have been crucial for this breed. While the *T* allele is the major allele in many horse breeds, the sharply high frequency of the *T* allele (0.91) of *MSTN* is concordant with such a demand for Kushum horses.

3.3. Variation in *DMRT3* gene

The ability of horses to perform alternate gaits, ambling, as well as common gaits, walk, trot and canter/gallop, is an important locomotion trait, since it affects smoothness of the locomotion. The gait trait has a strong genetic basis and horses of only limited breeds can perform the ambling gaits. Recently, a nonsense mutation of C to A in DMRT3 (p.Ser 301 STOP) was studied to have major effects on the gaitedness of the horse (Andersson et al., 2012) and the horses possessing the minor A allele are more likely to perform ambling gaits.

DMRT3 gene polymorphism analysis in Table 4 showed the presence of three genotypes of CC, AC, and AA at 77, 18 and 5%, respectively, and the frequency of the recessive allele A was 0.14. The presence of the minor alleles for *DMRT3* gene has been reported in Iranian horses at frequencies of 0.3 to 0.10 and some of Japanese horses at frequencies of 0 to 0.72 (Promerová *et al.*, 2014) and 0 to 0.82 in Chinese horses (Han *et al.*, 2015). According to Amano *et al.* (2018), animals exhibiting pace and pace/trot had *AA* for *DMRT3* in high frequencies (100 and 81.8%, respectively) confirming strong association between *A* allele of this SNP and pace in horses.

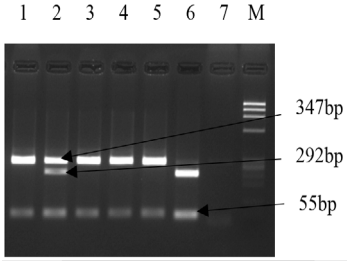

Figure 1. Genotype *LCORL*: 1,3,4,5 (*TT*); 2 (*TC*); 6 (*CC*)

M: Marker (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72bp). Wells 1-6 are, respectively, the products of the AluI enzyme that cuts the LCORL fragment 347bp amplified from the total DNA of the 1st-6th horse.

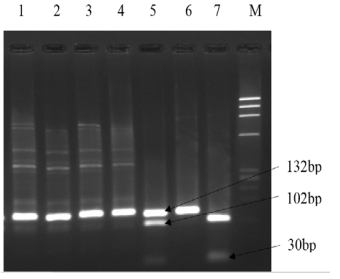

Figure 2. Genotype *MSTN*: 1,2,4,6 (*TT*); 5 (*TC*); 7 (*CC: control*)

M: Marker (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72bp). Wells 1-7 are, respectively, the products of the RsaI enzyme that cuts the MSTN fragment 132bp amplified from the total DNA of the 1st-7th horse.

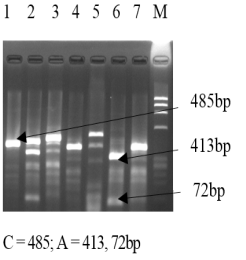

Figure 3. Genotype *DMRT3*: 1,4,7 (*CC*); 2,3,5 (*CA*); 6(*AA*)

M: Marker (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72bp). Wells 1-7 are, respectively, the products of the enzyme DdeI that cuts the DMRT3 fragment 485bp amplified from the total DNA of the 1st-7th horse.

In the present study, we detected *A* allele in Kushum horses, with frequency is lower than *C* allele. Since the frequencies of *A* alleles in most horse breeds are generally very low, except in those with the ability to perform an ambling gait (Promerová *et al.*, 2014), the discovered frequency of *A* allele (0.14) in Kushum horse is relatively high. While we have

no documentation on the ability of Kushum horses to operate an ambling gait, one of the initial aim of the cross with the Trotter during breeding was to upgrade the gait of Kushum horses (Dmitriez and Ernst, 1989). Therefore, the comparatively high frequency of the *A* allele might be the result of the introgression of this allele from Trotter stallions, which are known to perform an ambling gait and show a high frequency of the *A* allele (Jäderkvist *et al.*, 2014; Prmerová *et al.*, 2014). In addition to, gaited horses may have been chosen at the early stages of breeding to upgrade their locomotion traits as military horses, although such a selection may have no longer been performed in later stages of breeding, since these horses were mainly used for milk and meat production subsequently. Thus, the discovered frequency of the *A* allele of *DMRT3* might be a trace of such a breeding history of Kazastan Kushum horses.

4. CONCLUSION

The Kushum horses in this study carried a sharply high frequency of the *T* allele of *MSTN* and relatively high *C* allele of *LCORL* gene, which help increased the endurance stamina in long-distance races, and wither height and other body composition traits accordingly, suggested that these horses may have experienced phenotypic selection pressure during stallion screening in the process of forming new breed. Moreover, the found high frequency of the *A* allele of *DMRT3* might be the result of the introgression of this allele from Trotter stallions. Thus, the discovered allele frequency of these geneses might be a trace of such a breeding history and many Kushum horses do not carry recessive mutations that change the trotting pattern, do not develop muscle, but increase withers height, tend to be suitable for long distance travel.

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INFLUENCE OF CRYOPRESERVATION AND DEVELOPMENTAL STAGES OF EMBRYOS ON SAANEN GOAT EMBRYOS DURING COLD STORAGE IN VIETNAM

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ABSTRACT

The study was aimed to evaluate the influence of different cryopreservation methods and developmental stages of goat embryos on Saanen goat embryos during cold storage in Vietnam. Goat embryos were subjected to two methods: (1) Cryotop and (2) Microdrop. The number of embryos viable after Cryotop was higher than those after Microdrop (100% compared to 86.02%, P<*0.05*). However, there was no difference in developmental stages, embryo re-expansion, and hatching blastocysts after thawing between the two methods (1) and (2) (75.24% and 80.16%, 12.68% and 16.98%, P<*0.05*, respectively). This study did not observe any difference in survival rates of embryos and hatching blastocysts after thawing while freezing at morulae and blastocyst stages (75.34 and 86.72%, and 12.98 and 20.91%, P<*0.05*, respectively). The results showed the morulae and blastocysts of the Saanen goat were successfully fast-freezing procedure by Cryoptop and Microdrop.

Keywords: *Saanen goat embryos, fast-freezing, Cryoptop, Microdrop*.

1. INTRODUCTION

In animal husbandry, embryo cryopreservation and embryo transfer might facilitate the protection of endangered animal species, contribute to conservation, and improve reproduction ability at low cost through embryo bank establishment. Cryopreservation aims to maintain and store embryos at certain stages and enable their development after thawing. While stored at low temperatures, intracellular enzyme activities, cellular respiration, metabolism, embryo division discontinue. Therefore, embryos might be preserved without any genetic damage (Massip, 2001).

Embryo cryopreservation plays a crucial role in causing multiple ovulations and embryo transfer. Besides, embryo cryopreservation makes embryo preservation procedures and genetic material transportation more accessible and more convenient. Goat embryos are usually cryopreserved by conventional slow-freezing procedure with different cryoprotectants. Ethylene Glycol (EG) is a cryoprotectant used in the slow-freezing process; the survival rate of goat embryos after cryopreservation and thawing using EG is higher than using Glycerol or Dimethyl sulfoxide (DMSO). However, the survival rate of goat embryos after cryopreservation-thawing by slowing freezing method usually lower than the vitrification method (Guignot *et al*., 2006). The survival ability of goat embryos after cryopreservation-thawing was improved by the vitrification method containing EG and DMSO. According to a finding reported by Köse *et al.* (2016), the survival rate of goat embryos *in vivo* after cryopreservation-thawing by the vitrification method was 59.4%. At the same time, the survival rate of embryos of Grades I quality *in vivo* was 78.6%.

Vitrification is a physical process that converts liquid radioactive and chemical waste into a solid, stable glass without ice crystal formation (Vajta *et al*., 2009). The vitrification process forms an amorphous glass due to rapid cooling by direct immersion of the embryos, which contains high concentrations of cryoprotectants for ice formation prevention. Vitrification is an optimum approach to the ultra-rapid cryopreservation method because it might avoid cryodamage and ice formation. The procedure time from dehydration of the

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specimen in a concentrated solution to the end of the process occurs quickly.

The vitrification approach has several practical advantages compared to conventional slow-freezing procedures, such as less complex, easy-to-use, low cost, does not require specialized or expensive equipment, which provides the more significant potential for cryopreservation by intracellular ice crystal formation prevention. Conventional slow cryopreservation usually uses individual cryoprotectants consisting of Glycerol, EG, or DMSO. Meanwhile, the vitrification procedure employs their combination such as Glycerol + EG, Glycerol + DMSO, etc. (Prentice and Anzar, 2011). The combination of different cryoprotectants improves the survival ability of embryos after the cryopreservation-thawing process.

There have been some reports on bovine and porcine embryos except for goat embryo cryopreservation so far in Vietnam. A successful goat embryo cryopreservation procedure enhances reproduction efficiency, genetic material from valued breeds. Derived from those practical needs, we researched the next developmental potential of cryopreserved goat embryos *in vitro* by the vitrification approach.

2. MATERIAL AND METHODS

Saanen goat embryos were collected *in vivo*. Embryo culture media were prepared using a solution of M199-HEPES medium supplemented with 20% fetal bovine serum (FBS) and antibiotics. Cryopreservation solutions consisted of two different solutions vitrification solution VS1 including M199-HEPES medium supplemented with 10% Ethylene Glycol, 10% Dymethylsulfoxide and 10% FBS, and VS2 including M199-HEPES medium supplemented with 16.5% Ethylene Glycol, 16.5% Dymethylsulfoxide, 0.5M Sucrose and 10% FBS.

2.1. Goat embryo vitrification by Cryotop

The vitrification procedure by Cryoptop was followed steps: goat embryos were initially kept in embryo culture media for 5 min. Then the embryos were transferred to VS1 solution for 45 sec. After 45 sec in VS1 solution, the embryos were transferred to VS2 solution, then placed by Cryotop sheet and plunged directly in liquid nitrogen. The time from the embryos in VS2 solution to liquid nitrogen was 25 sec. The embryos were stored in liquid nitrogen until thawing.

2.2. Goat embryo vitrification by Microdrop

The vitrification procedure by Microdrop was followed steps: goat embryos were initially kept in embryo culture media for 5 min. Then the embryos were transferred to VS1 solution for 45 sec. After 45 sec in VS1 solution, the embryos were transferred to VS2 solution, then drew up into a pipette, dropped on a metal surface pre-cooled with liquid nitrogen to form immediately 1-2 µl vitrification droplets containing embryos. The time from transferring embryos to vitrification solution to forming vitrification droplets on the metal surface was 25 sec. The vitrification droplets containing embryos were stored in cryovials in liquid nitrogen.

Thawing media was prepared in three different solutions: solution T1 containing M199-HEPES medium supplemented with 0.5M Sucrose and 20% FBS; solution T2 containing M199-HEPES medium supplemented with 0.25M Sucrose and 20% FBS; solution T3 containing M199-HEPES medium supplemented with 0.15M Sucrose and 20% FBS.

2.3. Thawing of frozen embryos by Cryoptop

The thawing procedure after vitrification by Cryoptop was the following: thawed embryos were taken out of liquid nitrogen and immediately deposited in T1 solution at 38.5^0C for 30 sec. Then the thawed embryos were transferred to T2 solution and T3 solution for 2 min and 3 min, respectively, to remove all the cryoprotectants.

2.4. Thawing of frozen embryos by Microdrop

The thawing procedure after vitrification by Microdrop was the following: cryovials containing vitrification droplets were taken out of liquid nitrogen then immediately dropped on a metal surface pre-cooled with liquid nitrogen formed previously. The vitrification droplets containing cryopreserved embryos were transferred to T1 solution at 38.5^0C for 30 sec.

Then the thawed embryos were transferred to T2 solution and T3 solution for 2 min and 3 min, respectively, to remove all the cryoprotectants.

2.5. Embryo culture after cryopreservation-thawing

Embryo culture media after cryopreservation-thawing were prepared using a solution of SOF medium supplemented with FBS. After thawing, the embryos were washed three times with SOF solution supplemented with FBS. Then, the embryos were cultured for 24-48 h in SOF medium supplemented with FBS in an atmosphere of 38.5^{0}C, 5% CO_2, and 5% O_2 in humidified air to evaluate the survival ability and the development of embryos after cryopreservation-thawing.

2.6. Statistical analysis

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

3. RESULTS AND DISCUSSION

3.1. Influence of cryopreservation methods on goat embryo preservation

In this experiment, we studied two cryopreservation methods: (1) vitrification by Cryoptop and (2) vitrification by Microdrop. The evaluation was based on the number of embryos collected, the number of regenerated and re-expanded embryos, the number of hatching blastocysts after cryopreservation-thawing (Figure 1).

Table 1. Rates of survival and regenerated embryos *in vitro* after vitrification by Cryotop and Microdrop

Group	Total embryos	Retrieved % (Mean±SE)	Regeneration and re-expanded embryos % (Mean±SE)	Hatching embryos % (Mean±SE)
Microdrop	28	24 (86.02±4.72)^a	18 (75.24±4.58)^a	3 (12.68±3.57)^a
Cryotop	30	30 (100)^b	24 (80.16±3.96)^a	5 (16.98±4.01)^a

Numbers with different superscripts in the same column differed significantly (P<0.05)

The results showed that there was a significant difference between the two methods in the number of survival embryos collected after cryopreservation-thawing. Table 1 illustrated that 24 of 28 (86.02%) embryos in Microdrop were collected after thawing, while that in Cryoptop was 100% (*P<0.05*). This finding was higher than the results reported by Van *et al.* (2018) but lower than a finding of Huang *et al.* (2006). According to results found by Van *et al.* (2018), there were 69.7% and 98.7% of survival embryos after vitrification by Microdrop and Cryoptop, respectively. Meanwhile, Huang *et al.* reported that 100% of embryos viable after vitrification-thawing by Microdrop. The pipette usage during Micropdrop formation in the vitrification process might explain the result differences. Embryo loss in vitrification by Microdrop was due to that embryo stick into the pipette, which was a possible disadvantage of vitrification by Microdrop compared to Cryoptop (Van *et al.*, 2018).

Although the rate of regenerated and re-expanded embryos and the rate of hatching blastocysts in the Cryoptop group were higher than in the Microdrop group (80.16% compared to 75.24%; 16.98% compared to 12.68%, respectively), the differences were not statistically significant (P>0.05).

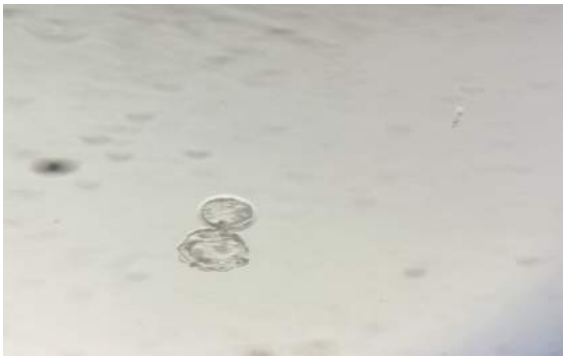

Figure 1. Re-expanded embryos and hatching blastocysts after the freezing-thawing process

In the vitrification method by Microdrop, the volume of vitrification droplets containing cryopreserved embryos was approximately 2-3µl. On the report of Huang *et al.* (2006), a smaller vitrification solution volume (2µl) might lessen the zona pellucida damage while exposuring directly to liquid nitrogen; therefore, it could enhance the survival rate of embryos after cryopreservation-thawing. Dinnyes *et al*. (2004) also reported that the vitrification droplets volume over 2 µl might elevate crack on zona pellucida or damage on cell membrane.

Cryopreservation rate is one of several vital factors affecting the embryo or oocyte vitrification success (Vajta and Kuwayama, 2006). The decrease in vitrification droplet containing cryopreserved samples volume might enhance the freezing-thawing rate during the embryo or oocyte vitrification process (Huang *et al.,* 2006). The Cryotop vitrification method offered a new perspective to minimize the vitrification droplet containing cryopreserved samples volume, there fore, embryos placed into Cryotop with a slight amount (about 0.1µl) (Kuwayama *et al*., 2005).

Small volume containing cryopreserved samples would increase the freezing-thawing rate, prevent cells from cold damage and reduce cryoprotectant concentration. Hence, the cells might rapidly cross restricted temperature (-5^{0}C to -15^{0}C), water moves out of cells and gets frozen.

Our study used a mixture of cryoprotectants, including Ethylene Glycol (EG) and Dimethylsulfoxide (DMSO), with a concentration of 16.5% each in vitrification solution. Based on the study of Huang *et al.* (2006), the vitrification solution containing 16.5% EG + 16.5% DMSO during embryo cryopreservation improved the survival rate of goat embryos after the freezing-thawing process. The cryoprotectant concentration in the vitrification solution also was one of the significant factors effecting the vitrification effectiveness. Less toxic cryoprotectants or several different protectants together were considered to reduce the specific cryoprotectant toxicity of each (Huang *et al.,* 2006).

Ethylene Glycol has a molecular weight lighter than Glycerol. It has high membrane permeability, soluble in water and alcohol, has the same effect as Glycerol, and has been used as an alternative to Glycerol in cryopreservation. When moving into cells, the EG molecules lie alternately between water molecules, which makes water frozen in a small form, reduces the expansion of water crystals, and prevent cell membrane damage. Thus, EG keeps soluble substance concentration stable, remains the osmotic pressure, and limits protein destruction during cryopreservation. The another advantage of EG is that EG removal seems unnecessary because EG, by itself could easily permeate out of the cells.

Despite no differences in the survival and regenerated embryos rate after the freezing-thawing process between Microdrop and Cryotop, the disadvantage of Microdrop vitrification led to the loss of a certain embryos number; we suggest using the Cryoptop vitrification procedure for goat embryo cryopreservation.

3.2. Influence of developmental stages on goat embryo preservation

To evaluate the effect of developmental stages on goat embryo preservation, we cryopreserved goat embryos at two stages: (1) morulae and (2) blastocysts. The efficiency of cryopreservation was evaluated based on criteria: the number of survival and regenerated embryos, the number of hatching blastocysts after the freezing-thawing process.

The differences in the number of survival embryos, the number of hatching blastocysts were not significant ($p>0.05$). However, the blastocysts group had a higher number of survival embryos and hatching blastocysts than those of the morulae group (86.72% compared to 75.34%, 20.91% compared to 12.98%, respectively). Similar results were found in the report of Huang *et al.* (2006), which showed that cryopreservation in the blastocyst stage had a higher number of survivals than in the morulae stage.

Table 2. Influence of developmental stages on goat embryo preservation

Developmental embryos	Total vitrified	Surviving % (Mean±SE)	Renerated and re-expanded % (Mean±SE)	Hatched % (Mean±SE)
Morula/compact	16	12 (75.34±3.92)[a]	8 (50.21±3.78)	2 (12.98±4.02)[a]
Blastocyt	29	25 (86.72±4.16)[a]		6 (20.91±3.89)[a]

The cold damage tolerance ability of blastocysts is better than morula might be explained that after embryo cavity formation, embryo membranes fight against osmosis pressure and toxicity of cryoprotectants (Huang *et al.*, 2006). The variation of cell types, and the increase in Na/K ATPase activity occurring during trophectoderm formation effect positively on cryoprotectant activities (Naitana *et al*., 1996).

Another important factor contributing to embryo survival ability after freeing-thawing process is the embryos size. Morula is larger than the blastocysts; this might be why permeability and cryoprotectant removal during the freezing-thawing process of morulae were lower than that of blastocysts (Tachikawa *et al*., 1993).

4. CONCLUSION

Successful goat embryo cryopreservation *in vivo* by fast-freezing Cryotop and Microdrop with the survival embryo rates after the freezing-thawing process was 80.16% and 75.24%, respectively.

Goat embryos might be preserved at morula stage or blastocyst stage.

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EFFECTS OF VITAMIN AND TRIBUTYRIN SUPPLEMENTATION IN DIET ON GROWTH AND FEATHER PECKING OF BEN TRE NOI CHICKEN

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ABSTRACT

An experiment was conducted to evaluate the effects of vitamin or tributyrin supplementations in the diet or drinking water on growth performance and feather pecking of Ben Tre Noi chicken. A total of 240 male Noi chicken at 5 weeks' old was allocated in a completely randomized design with 3 treatments, 4 replications with 20 chickens/pen as an experimental unit. The 3 treatments (NT) were (1) Cont: Basal diet without supplementation both vitamin or tributyrin products in feed or drinking water; (2) VIT: Basal diet + 2g vitamin/l drinking water; (3) TBU: Basal diet + 2g tribtyrin/kg feed. Results showed that, final body weight of chickens was highest from chickens in the VIT (1,602.3 g/head), followed by TBU (1,569.1 g/head) and lowest in Cont (1,511.2 g/head). So, there was a trend lower average daily gain (ADG) of chickens in cont (19.09 g/head/day) compared to that of chickens in TBU (20.0 g/head/day) and VIT (20.58 g/head/day). Similarly, there was a trend higher feed intake (FI) of chickens fed VIT (63.29 g/head/day) to compared with TBU (60.16 g/head/day) and Cont (59.86 g/head/day). As a result, feed conversion ratio (FCR) of chickens feed Cont (3.09kg feed/kg gain) was almost similar with chickens feed TBU (3.01kg feed/kg gain) and VIT (3.02kg feed/kg gain). The feather pecking rate of chickens supplemented with 7% VIT and 10% TBU reduced significantly to compare with 20% Cont. There was no significant difference in carcass evaluation characteristics of chickens in all treatments. In conclusion, supplementation vitamin product in drinking water or tributyrin in diet of Ben Tre Noi chickens tended to improve final weight, and reduced the feather pecking rate of chickens in supplemented treatments to compare with chickens in control group.

Keywords: *Growth performance, feather pecking, Ben Tre Noi chicken, tributyrin, vitamin.*

1. INTRODUCTION

Poultry production has an important role in Vietnam agriculture, accounting for 512.6 million heads of total poultry production in the country, in which chicken production occupy 409.5 million heads in early of year 2021, and increase around 7% compared with that in year 2020 (GSO, 2021). Besides the development of raising industrial chicken breeds in large farms, there is a strong development of local chicken breed such as Noi and Ac chickens raising in large and also in small farms. The production systems of small poultry producers show a significant variety from very low input systems, because of low performance and high morbidity and mortality. Therefore, the farmers are usually supplemented antibiotics in the chicken diet to prevent and treat disease of chickens, but the antibiotic residue in meat may be affect to human health. So, from the year 2018, the using of antibiotics has been banned to supplement in animal feed as a growth stimulant, and not be used to prevent animal diseases from 2020 (Department of Livestock Production, 2017). This situation has been pressing the farmers to find other supplements in order to improve the chicken health. So, vitamin or organic acid are priority substitutes for the antibiotic, which does not leave residues and safety for meat production. Therefore, this research had been concentrated on using tributyrin or vitamin supplementation in feed or drinking water in the opening housing system, and in small scale farm. The objectives of this study were to

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evaluate the growth, feed efficiency and pecking rate of Ben Tre Noi chickens in the absence of preventive antibiotic use in the process.

2. MATERIALS AND METHODS

2.1. Animals and experimental design

The experiment was conducted during a period of 9 weeks in an experimental farm, in Thoi Lai district, Can Tho city. Ben Tre Noi chickens (male) were raised in an open-sided house, in 12 pens (each 2x1.3m) separated by netting. Feed and water were provided continuously from feeders and automatic drinkers. Prior to starting the experiment, the chickens were vaccinated against common diseases (Gumboro, H_5N_1 and fowl pox). The experimental time lasted 10 weeks, from 5 to 14 week old of chickens.

2.2. Experimental treatments and feed

Feed formulation and composition are showed in Table 1. Feed ingredients include maize meal, rice bran, broken rice, fish meal, soya meal, bone and shellfish meal, AAs and mineral premix. CP and ME were satisfied the chicken nutrient requirement.

Table 1. Chemical composition of basal diets

Ingredients and composion		5-9wks	9-14wks
Ingredients, %	Maize meal	40.4	43.2
	Broken rice	15.0	15.0
	Rice bran	14.2	15.5
	Fish meal	7.00	5.0
	Soya meal	19.00	17.0
	Lysine	0.03	0
	Methionine	0.10	0
	Bone meal	2.0	2.0
	Shellfishmeal	1.5	1.5
	Premix	0.77	0.8
Chemical composition and Metabolisable energy, %	ME, kcal/kg feed	3,000	3,050
	EE	4.10	4.01
	CP	18.5	17.1
	CF	4.23	4.45
	NFE	67.5	69.3
	Ca	1.55	1.52
	P	0.52	0.51

Tributyrin product, which have 50% of acid butyric was a white powder, and provided by Menon Animal Nutrition Technology Co., Ltd, and was supplemented in the feed. Vitamin product (Vitazin) was a water form and supplemented into drinking water. The experiment was arranged as a completely randomized design with 3 treatments and 4 replicates, each replicate consisted of a pen with 20 male chickens.

Table 2. Composition of Vitazin and tributyrin

Ingredients	VIT	TBU,%
Vitamin A (min), IU	10.000.000	-
Vitamin E (min), mg	2.000	-
Vitamin C (min), mg	-	-
Vitamin D3 (min), IU	2.500.000	-
Calcium pantothenol (min), mg	-	-
Vitamin B5 (min)	10.000	-
Methionine (min), mg	40.000	-
Vitamin H (min)	250	-
Zinc (min), mg	13.500-16.500	-
Butyric acid	-	50

2.3. Management and measurements

The chickens were weighed as one group of 20 birds in each pen. This was done at the beginning of the experiment and every week, always in the early morning before feeding. The data collections were average daily gain (g/head/day), average daily feed intake (g/head/day), feed conversion ratio (kg feed/kg gain) by the week. Carcass characteristics, disease incidence and pecking rate also were evaluation.At the end of the experiment (14 weeks of age), four chickens/pen were selected to be slaughtered. The chickens were chosen for a 12-hour fasting (for water only) before surgery. Carcass parameters in chickens were slaughter weight, carcass weight, thigh and breast meat weigh.

2.4. Experimental design and treatments

Treatments were:

1/Cont: Basal diet (BD) without any supplementation (Control)

2/ VIT: BD + 2 ml/l drinking water

3/ TBU: BD + 2g tributyrin/kg feed

2.5. Analysis methods

The chemical composition of feed was determined according to AOAC (1990). Crude protein was determined by the Kjeldahl method.

Total ash was the residue after ashing the samples at 550 °C, and the ether extract (EE) was determined by Soxhlet extraction.

2.6. Data analysis

Collected data was analyzed by ANOVA using the General Liner Model (GLM) of Minitab Statistical Software Version 16. Tukey pair-wise comparisons were used to determine differences between treatment means at P<0.05. The statistical model used is as follows: $Y_{ij} = \mu + \alpha_i + e_{ij.}$ Where: Y_{ij} is growth performances or feed efficiency; 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 treatment.

3. RESULTS AND DISCUSSIONS

3.1. Growth performance and feed efficiency

Live weight of chickens in treatments during the period of 9 weeks of experiment presented in Table 3 and 4 showing that there was a small improvement of final weight of chickens fed VIT and TBU diets to compare with chickens in control group, which led to little higher ADG of chickens fed VIT and TBU diets than that in Cont diet, even though the different was too small. These results are consistent to Rahman *et al.* (2012), who showed that it is necessary to provide enough nutrients and vitamins in the diet for improvement the growth performance. And also research from Hoseein *et al*. (2013) found that supplementing vitamins to chicken diets showed higher weight gain of chickens than control, because their presence in the intestinal tract helps increase the absorption of the intestinal parenchyma, participates in the coenzymes to promote the breakdown and metabolism of substances in the body. Also research of Moravej *et al.* (2012) found that vitamins participating in the structure of many enzymes in the system catalyze biological reactions to maintain all normal life activities such as growth, reproduction, and antibody production.

Beside, research of Sheikh *et al*. (2011) showed that the organic acid changes the structure of the small intestine as it increases the velocity of the villi in all segments of the small intestine especially in the ileum, thus improving absorption and feed efficiency. And according to research from Nguyen Thi Thuy *et al.* (2018), acid butyric could reduce intestinal pH, increased enzyme activity, so it should improve the digestion and absorption of protein and change pH of the intestinal environment, allowing absorb nutrients and prevent disease too.

Table 3. Liveweight of experimental chickens

Weeks of age	Treatments			SEM	P
	Cont	TBU	VIT		
5	308.33	309.2	305.47	4.81	0.15
6	425.55^{ab}	414.83^{b}	434.15^{a}	3.79	0.03
7	532.5^{b}	530.21^{b}	560.12^{a}	10.30	0.04
8	650.11	670.32	668.33	15.98	0.32
9	781.66	810.33	814.25	14.18	0.41
10	939.17	935.83	956.67	14.53	0.65
11	1072.5^{ab}	1059.1^{b}	1108.3^{a}	10.45	0.04
12	1185.8	1180.1	1260.1	27.09	0.64
13	1350.2	1379.1	1420.3	33.63	0.65
14	1511.2	1569.1	1602.3	38.53	0.69

Table 4. Average daily gain (g/head/day)

Weeks of age	Treatments			SEM	P
	Cont	TBU	VIT		
5-6	16.75^{b}	15.09^{b}	18.38^{a}	0.63	0.04
6-7	15.28^{b}	16.45^{ab}	17.98^{a}	0.76	0.01
7-8	16.79^{b}	20.00^{a}	15.48^{b}	1.13	0.03
8-9	18.81	20.00	20.85	1.85	0.26
9-10	22.50^{a}	17.98^{b}	20.35^{ab}	1.21	0.02
10-11	19.05^{b}	17.62^{b}	21.67^{a}	2.05	0.03
11-12	16.19^{b}	17.26^{b}	21.67^{a}	2.14	0.04
12-13	23.45^{b}	28.43^{a}	22.86^{b}	2.08	0.03
13-14	23.02	27.14	26.00	1.93	0.67
ADG_{5-14}	19.09	20.00	20.58	1.61	0.17

Feed intake of chickens feed VIT treatments also higher than that in TBU and Cont chickens. According to Jang *et al.* (2014), all vitamin deficiencies lead to metabolic disorders, because these vitamins may also help chickens stimulate appetite, then grow quickly and play an important role in oxidation of substances, and also it may increase feed intake of chickens. In addition, vitamins are important components of coenzyme-A function, which important in fat synthesis, and results in higher weight gain of supplemented treatments with vitamin.

Table 5. Average daily feed intake (FI)

Weeks of age	Treatments			SEM	P
	Cont	TBU	VIT		
5-6	30.15	31.17	30.9	0.57	0.47
6-7	40.47	41.10	43.28	0.77	0.09
7-8	44.12	47.09	47.44	2.01	0.11
8-9	54.4	57.85	55.83	1.72	0.58
9-10	58.04	59.88	59.76	2.55	0.65
10-11	67.14	69.88	75.86	3.44	0.14
11-12	67.13^{b}	68.76^{b}	77.98^{a}	2.37	0.03
12-13	84.64	78.95	82.14	5.73	0.69
13-14	92.69	84.81	96.43	5.27	0.12
$ADFI_{5-14}$	59.86	60.16	63.29	5.15	0.46

Feed conversion ratio over the 9-week period was almost not very different for the 3 treatments, but the trend to be lower in supplemented treatments than control. It may be because vitamin or acid butyric are not only stimulate digestion, increase weight gain but also prevent the growth of pathogenic, destroy harmful bacteria in the intestinal tract, as results it improve the feed conversion ratio.

Table 6. Feed conversion ratio of chickens

Weeks of age	Treatments			SEM	P
	Cont	TBU	VIT		
5-6	1.80^{ab}	2.07^{a}	1.68^{b}	0.12	0.02
6-7	2.65	2.50	2.41	0.13	0.12
7-8	2.63^{b}	2.35^{b}	3.06^{a}	0.13	0.04
8-9	2.89	2.89	2.68	0.32	0.31
9-10	2.58^{c}	3.33^{a}	2.94^{b}	0.11	0.03
10-11	3.52	3.97	3.50	0.32	0.42
11-12	4.14^{a}	3.98^{ab}	3.60^{b}	0.22	0.03
12-13	3.61^{a}	2.67^{b}	3.59^{a}	0.26	0.04
13-14	4.03^{a}	3.12^{b}	3.71^{ab}	0.27	0.02
FCR_{5-14}	3.09	3.01	3.02	0.28	0.20

3.2. Carcass evaluation

The carcass yield of Ben Tre Noi chickens in this research ranged from 67-68%, there was no difference in thigh and breast proportion of chickens in different treatments with or without addition of supplementation products. Overall observation of the carcass parameters found that, the addition of tributyrin or vitamin into the diets of Ben Tre Noi chickens was almost no effect on carcass proportions, thigh meat and breast of male chickens.

Table 7. Carcass characteristic evaluation of male chickens in the treatments

Variables	Treatments			SEM	P
	Cont	TBU	VIT		
Slaughter weight (g)	1566	1558	1550	23.3	0.08
Carcass weight (g)	1060	1051	1049	20.9	0.36
Carcass yield (%)	68.1	67.5	67.7	0.71	0.31
Thigh weight (g)	338.8	341.0	342.0	8.12	0.65
Thigh proportion/carcass (%)	32.0	32.4	32.5	0.68	0.46
Thigh meat weight (g)	226.2	234.2	231.5	6.54	0.74
Thigh meat proportion/carcass (%)	21.5	22.3	22.1	0.42	0.65
Breast weight (g)	236.2	228.3	230.0	9.11	0.56
Breast proportion/carcass (%)	22.3	21.8	22.0	0.70	0.91
Breast meat weight (g)	188.6	182.3	178.5	5.46	0.26
Breast meat proportion/carcass (%)	17.8	17.4	17.1	0.40	0.46
Liver weight (g)	32.2	32.1	33.1	1.52	0.63

3.3. Feather pecking rate

The addition of vitamin or tributyrin has prevented the development of harmful microorganisms in the intestines, leading to increased digestibility of chickens, reduced morbidity and mortality (Fatufe and Matanmi, 2011). The morbidity number of the experimental chickens is quiet lower than control group. However, the mortality was almost not significant different in all treatments. In this experiment, almost chickens died mainly due to some diseases such as coccidiosis and *E. coli*. The morbidity number of chicken in this experiment was high, it may be because chickens were raised in openhouse, be affected by changing of

weather and no antibiotic supplied in the diets. So, when vitamin or tributyrin supplementation in the diet inhibits the pathogenicity of harmful bacteria, enhance immunity and reduce poultry mortality. Because, the addition of acid butyric to the diet reduced pH to below 3.5, then to limit the activity of harmful bacteria and enhance the activity of beneficial bacteria. Therefore, it is possible to change the number of intestinal bacteria, as well as the growth of beneficial bacteria in the gut of the chicken by feeding (Adil and Magray, 2012), so this is the reason why supplemented chickens reduced number of morbidity and mortality.

Table 8. Feather pecking, morbidity and mortality

Variables	Treatments		
	Cont	TBU	VIT
Initial chicken number	80	80	80
Final chicken number	75	76	76
Number of morbidity	15	10	8
Number of mortality	5	4	4
Number of feather pecking	16	8	6
Feather pecking rate, %	20.0	10.0	7.0

4. CONCLUSIONS

Supplementation of vitamin or tributyrin in drinking water or in feed trend to improve final live weigh and reduce morbidity and feather pecking rate of Ben Tre Noi chickens raising in open house.

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EFFECTS OF PROBIOTIC SUPPLEMENTATION IN LOW CP DIET ON GROWTH AND *E.COLI* IN FECES OF GRIMAUD DUCK

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ABSTRACT

An experiment (Exp) was conducted to determine the effects of two probiotic products (ProbiP and Lactozyme) supplementation in low CP diet on growth performance, carcass quality and *E.coli* in feces of Grimaud super meat duck. The experiment was conducted in a completely randomize design with 3 treatments and 3 replications with 20 ducks/pen (10 male + 10 female). The treatments were:1/ Cont: Control diet (optimum CP diet) without any product supplementations; 2/ PRO: 1% lower CP diet (Low CP) + 2g ProbiP/kg feed; 3/ LAC: Low CP diet + 2g Lactozyme /kg feed. Results showed that the average daily gain (ADG) of ducks fed Cont (66.58 g/head/day) was lowest, and the highest was found in LAC (69.15 g/head/day) and PRO (67.10 g/head/day). Average daily feed intake (ADFI) was almost similar in supplemented diets (153 g/head/day) to compare with control diet (150.9 g/head/day). Therefore, feed conversion ratio (FCR) of ducks fed LAC (2.10 kg feed/kg gain) was better than that in Cont (2.13 kg feed)/kg gain) and PRO (2.17 kg feed/kg gain). The density of *E.coli* bacteria in feces reduced in PRO (1.11 and 1.45 x10^6 CFU /g feces) and LAC (1.16 and 1.55 x10^6CFU /g feces) compared to Cont (2.23 and 2.14 x10^6CFU /g feces) at 21 and 42 days, respectively. There was no difference in the slaughter parameters such as carcass proportion, thigh and breast meat percentage among ducks in 3 treatments. In conclusion, supplementation of Lactozymee and ProbiP in 1% lower CP diet of Grimaud duck diets tended to improve weight gain and reduce *E.coli* density in the feces compared with ducks feed optimum CP diet without any probiotic supplementation.

Key words: *Probiotic, ProbiP, Lactozyme, E.coli, Grimaud duck.*

1. INTRODUCTION

Duck raising is a traditional activity of farmers in the Mekong Delta, local duck breeds with low production have been popularly raised many years ago. Recently, many super meat duck breed are being imported by companies for rearing and breeding production, these breeds are raising on industrial and household scales. The Grimaud duck breed was imported into our country in 2009, so far this breed is raising popularly in the Mekong Delta because of its rapid growth and high meat percentage (Hoang Hai Chau and Tran Thanh Son, 2016). However, because of farming environmental conditions at the household scale are not good, so the farmers must use antibiotics in food or drinking water to prevent and treat diseases for ducks. Antibiotics have been used as promoters for growth and well-being for decades, but they have been banned in many countries (Mehdi *et al.*, 2018; Roth *et al.*, 2019). Hence, natural strategies such as functional feed additives such as probiotics, prebiotics or synbiotics are efficacious promising alternatives to antibiotics (Bozkurt *et al.*, 2014; Dhama *et al.*, 2015). One of the substances that can replace antibiotics is probiotic. Recently, there have been many studies using some kinds of probiotics added to broiler diets by indirect or direct effects that can be used instead of antibiotics mixed in the feed. However, there have not been many studies on supplementing these products on ducks, especially Grimaud super meat ducks. Therefore, this study concentrated on using ProbiP and Lactozymee supplementation in the lower CP diet to compare with optimum CP diet in small scale farming, in order to evaluate the growth ability, feed efficiency, number of *E.coli* in the feces of Grimaud ducks under the condition of not using preventive antibiotics in the rearing process.

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2. MATERIALS AND METHODS

2.1. Animals and experimental feed

The experiment was conducted during a period of 7 weeks in a householder in Thoi Lai district, Can Tho city. Grimaud ducks were raised in an open-sided house, in 9 pens (2m x 2m) separated by netting. Feed and water were provided continuously from feeders and long drinkers.The supplemented products were ProbiP and Lactozymee which was supplied from Vemedim company. The experiment was carried out on 180 Grimaud ducks at 1 day old (90 male and 90 female) vaccinated against diseases such as hepatitis (2 days old), cholera (14 and 28 days old), H_5N_1 (20 days old) and hemophilia at 24 days of age. Feed chemical compositions are showed in Table 1. Feed ingredients include maize meal, rice bran, broken rice, fish meal, soya meal, bone and shellfish meal, amino acids and mineral premix. CP and ME in control diet were satisfied the ducks nutrient requirement each period 0-3 and 4-7 weeks age.

Table 1. Chemical composition of experimental diet

Chemical composition	Cont 0-3wks	Cont 4-7wks	Low CP 0-3wks	Low CP 4-7wks
ME (kcal/kg TA)	2,750	2,800	2,800	2,800
DM,%	86.0	86.0	87.0	86.0
CP	21.0	17.0	20.0	16.0
CF	6.03	6.20	6.10	6.20
EE	3.31	3.78	3.42	3.78
Methionin	0.71	0.51	0.71	0.52
Lysine	1.10	0.81	1.10	0.81
Ca	1.22	1.10	1.23	1.10
P	0.78	0.72	0.78	0.72

Table 2. Composition of ProbiP and Lactozymee

Ingredients	Lactozymee	ProbiP
Pediococcus acidilactic	-	10^{11} CFU
Phytase	124700 FYT	-
Protease	6000 UI	-
Amylase	2000 UI	-
Cellulase	18000 UI	-
Xylanase	14000 UI	-
Lactobacillus acidophilus	10^8 CFU	-
Bacillus Subtilis	10^8 CFU	-
Saccharomyces cerevisiae	10^8 CFU	-
Lactose	*Enough for 1kg*	*Enough for 1kg*

2.2. Eexperimental design

The experiment was arranged in a completely randomized design with 3 treatments, each treatment was repeated 3 times, each repetition was 1 cage with 20 ducks (10 males and 10 females). The treatments were:

1/ Control diet (Cont): Optimum CP diet without supplements

2/ PRO: Low CP diet (1% lower than optimum CP diet) + 2g ProbiP/kg feed

3/ LAC: Low CP diet (1% lower than optimum CP diet) + 2g Lactozymee/kg feed

2.3. Measurements and fecal sampling

The ducks were weighed as one group of 20 birds in each pen. This was done at the beginning of the experiment and every week, always in the early morning before feeding. The data collections were average daily gain (g/head/day), average daily feed intake (g/head/day), feed conversion ratio (kg feed/kg gain) by the week. *E.coli* in feces at 21 and 42 days old and carcass characteristics evaluation also were collection. At the end of the experiment (7 weeks of age), four ducks/pen (2 male and 2 female) were selected to be slaughtered. The ducks were chosen for a 12hr fasting (for water only) before surgery. Carcass parameters in ducks were slaughter weight, carcass weight, thigh and breast meat weigh, and abdominal fat.

2.4. Analysis methods

The chemical composition of feed was determined according to AOAC (1990). Crude protein was determined by the Kjeldahl method. Ether extract (EE) was determined by Soxhlet extraction. At the weeks 21st and 42nd , the feces in the caecum were collected immediately from 4-5 ducks (about 50g feces/bag) of each replication (pen) after exsanguination, placed into sterile centrifuge tubes, put on ice and transported 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; v: The volume (ml) of dilution to grow in each plate.*

2.6. Statistical analysis

Collected data was analyzed by ANOVA using the General Liner Model (GLM) of Minitab Statistical Software Version 16. Tukey pair-wise comparisons were used to determine differences between treatment means at P<0.05. The statistical model used is as follows: $Y_{ij} = \mu + \alpha_i + e_{ij.}$ Where: Y_{ij} is growth performances or feed efficiency; 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 treatment.

3. RESULTS AND DISCUSSIONS

3.1. Growth performance and feed efficiency

The weight of ducks at the beginning of the experiment was around 52-53 g/head, at the end of the experiment (7wks old), there was a little higher in LAC (3,400.2 g/head) and PRO (3,340.2 g/head) than in control animals (3,330.3 g/head). This initially have showed that ducks fed 1% lower CP diet supplemented with Lactozymee or probiP also have good effect on growth ability to compare with ducks fed optimum CP diet without probiotic supplementation. The above results are also consistent with the study of Ahmed *et al.* (2021), research shows that when adding probiotic to the 14% CP diet improved the performance of ducks caused by reduced CP diet to performance due to the 18% CP diet without probiotic supplementation. This results showed the positive impacts of probiotics especially in the lower CP requirement diet, it is expected that dietary inclusion of probiotics reduces the negative effects of low dietary protein. So, it may be explained the final weight of ducks supplemented Lactozymee was highest because the composition of Lactozymee was probiotic mixture. In fact, research of Best *et al*. (2017) showed that using the correct mixture probiotic is more beneficial than using each type separately as a result of combining the roles of each species in the mixture, and probiotics have become popular among commercial poultry producers to improve overall poultry health and performance.

Table 3. Body weight of Grimaud duck (g/head)

Weeks of age	Treatments			SEM	P
	Cont	PRO	LAC		
1 day	53.2	52.1	52.3	0.61	0.12
1	230.4	233.6	238.9	7.25	0.15
2	650.2	660.3	654.5	13.9	0.34
3	1,102.2	1,175.1	1,186.5	28.2	0.12
4	1,540.4^{b}	1,645.3^{a}	1,689.2^{a}	28.5	0.04
5	2,024.1^{a}	2,118.2^{b}	2,207.2^{a}	25.1	0.03
6	2,689.2^{b}	2,702.1^{b}	2,798.2^{a}	22.7	0.04
7	3,330.3	3,340.2	3,400.2	26.7	0.08

Duck weight gain in the treatments increased gradually with age, and in the first 2 weeks there was a tendency to be higher than in the supplemented treatments, and similarly in the following weeks. In the present study, the addition of probiotics to the diets trend to little improving the ADG of birds. Especially, feeding probiotics to birds fed with the 1% lower CP diet resulted in return of their performance to the level of that in birds fed with the optimum diet not supplemented with probiotics. This resulted in a higher weight gain in the treatments adding probiotics than in the control treatment, although the difference was not statistically significant. It can be explained that when probiotics are added to the diet into the digestive tract, they produce organic acids which cause reduction of intestinal pH, change intestinal villi morphology and change the pH environment, suppress pathogenic bacteria, allow nutrient absorption (Sari *et al*, 2019). In addition, the appropriate use of lower protein diets has become common to solve the problem of protein cost, solve the problem of environmental problems related to excreted nitrogen and allow alternative feedstuffs (Ravangard *et al*., 2017). However, excessive protein deficiency in the diet may impair poultry performance (Jiang *et al*., 2018), but in such a case the 1% CP lower is quiet small, so the positive impacts of probiotics is more effective than this reduction, and growth promoters may offset the effects of dietary

protein deficiency. Therefore, the inclusion of probiotics in duck diets boosts the performance, utilization of feed protein, and immunity (Salim *et al.*, 2013).

Table 4. ADG of experimental ducks (g/head/day)

Weeks of age	Treatments			SEM	P
	Cont	PRO	LAC		
0-1	25.31	25.93	26.66	1.35	0.67
1-2	59.97	60.96	65.20	3.32	0.45
2-3	64.54^{b}	73.54^{a}	75.93^{a}	3.44	0.04
3-4	62.57	67.13	71.86	4.56	0.14
4-5	69.16	67.60	74.03	3.64	0.56
5-6	95.01	83.41	84.43	4.56	0.45
6-7	91.54	91.13	85.97	3.56	0.07
1-7	66.58	67.10	69.15	3.17	0.23

The results in Table 5 showed that the daily feed intake of ducks between treatments over weeks of age was not statistically significant different. In order to meet the body's requirement for maintenance and growth, the amount of feed consumed increases over time. At week 1, the feed intake of ducks ranged from 35.2 to 36.3 g/day, the feed intake increased rapidly according to the duck's growth rate and was highest at week 7 from 239-250.3 g/head. Feed intake of ducks in all 3 treatments were almost similar, this result is consistent with the study of Sari *et al.* (2019), who showed adding probiotics to the diet also showed no difference in the amount of feed consumed by ducks in all treatments.

Table 5. Feed intake of ducks in the experiment

Weeks of age	Treatments			SEM	P
	Cont	PRO	LAC		
0-1	35.2	36.1	36.3	0.55	0.15
1-2	89.3	88.3	90.1	0.71	0.29
2-3	134.4^{b}	150.4^{a}	151.2^{a}	4.21	0.03
3-4	150.4	153.5	154.2	5.19	0.74
4-5	167.7	170.2	176.2	8.12	0.45
5-6	240.1	226.3	230.2	7.32	0.42
6-7	240.1	250.3	239.4	7.21	0.78
1-7	150.9	153.8	153.5	6.62	0.67

The improved FCR may be one of the reasons for the promoted growth in groups fed on probiotic diets. This research is in agreement with research of Ravangard *et al.*(2017), who found the improvement in the growth and nutrient utilization by the addition of probiotics under the optimal or suboptimal nutritional levels. The observed decrease in FCR may be associated with the improved intestinal status owing to modulating the intestine structure and secretion impacts (Yadav and Jha, 2019), and the internal villus function is to absorb nutrients, whereas the blind end allows for an increased retention time for dietary content within the digestive tract (Reynolds *et al.*, 2020). The results of intestinal enzyme activity showed improvement with supplementation of probiotics, which explains the improvement in FCR and growth. So, probiotics resulting in raising duck immunity can be linked to the improved intestine at the structural or microbial levels that induce nutrient digestion, absorption (Haghighi *et al.*, 2005).

Table 6. FCR of ducks in the experiment (kg/kg)

Weeks of age	Treatments			SEM	P
	Cont	PRO	LAC		
0-1	1.39	1.39	1.36	0.04	0.57
1-2	1.49	1.45	1.38	0.11	0.89
2-3	2.08	2.05	1.99	0.06	0.32
3-4	2.40^{a}	2.32^{a}	2.11^{b}	0.05	0.04
4-5	2.42^{ab}	2.52^{a}	2.38^{b}	0.05	0.04
5-6	2.53^{b}	2.71^{a}	2.73^{a}	0.06	0.03
6-7	2.62	2.74	2.78	0.07	0.06
1-7	2.13	2.17	2.10	0.08	0.18

3.2. *E.coli* in feces of experimental ducks

Table 7. *E.coli* content in feces of ducks (10^{6}CFU/g)

Ingredients	Treatments			SEM	P
	Cont	PRO	LAC		
At 21 days old	2.23^{a}	1.11^{b}	1.16^{b}	0.22	0.04
At 42 days old	2.14^{a}	1.45^{b}	1.55^{b}	0.15	0.02

The results on the amount of *E.coli* in duck feces in the treatments are presented in Table 7. When probiotics was added to the diet, the amount of *E.coli* in the feces was statistically significantly lower compared with no addition. I may be because probiotic produce organic acid which cause reduction intestinal pH, but disease bacteria are often active at high pH, the pH is suitable for the activity of *E.coli* (4.3) while beneficial bacteria such as Lactobacillus are active at low pH (<3.5). Thus, it will limit

the activity of harmful bacteria and enhance the activity of beneficial bacteria (Adil *et al.* 2012).

3.3. Carcass evaluation

The carcass yield of ducks ranged 72-74%, there was no difference in thigh and breast proportion of ducks in different treatments with or without addition of supplementation products. Overall observation of the carcass parameters found that, the addition of ProbiP or Lactozymee into the diets of Grimaud ducks was almost no effect on carcass proportions, thigh meat and breast of male and female ducks compared to control ducks. Howerver, there is a trend of decreasing fat percentage of ducks fed the diet supplemented with probiotics. This finding may be linked to the improvement in feed utilization as a result of the increased feed intake and activity of digestive enzymes. Moreover, the accumulation of nutrients in tissues is dependent on feed intake, intestinal absorption, and metabolism. This research is in agreement with research from Pourakbari *et al.* (2016) and Ravangard *et al.* (2017), the carcass weight showed a positive linear trend with the probiotic level, and probiotic supplements reduce the percentage of abdominal fat.

Table 8. Carcass characteristic evaluation of Grimaud ducks in the treatments

Ingredients	Male			SEM	P	Female			SEM	P
	Cont	PRO	LAC			Cont	PRO	LAC		
Slaughter weight, g	3.300	3.370	3.440	47.3	0.06	3.120	3.230	3.250	44.1	0.07
Carcass weight (g)	2.400	2.437	2.505	38.1	0.07	2.307	2.405	2.415	38.2	0.06
Carcass yield (%)	72.73	72.31	72.82	0.54	0.35	73.9	74.46	74.31	0.75	0.08
Thigh meat weight (g)	285.5	295.2	299.9	8.45	0.06	275.5	280.8	281.6	11.2	0.34
Thigh meat proportion/carcass (%)	11.90	12.11	11.97	0.25	0.08	11.94	11.68	11.66	0.41	0.67
Breast meat weight (g)	510.5	511.2	530.1	15.6	0.12	470.5	482.3	495.5	14.2	0.33
Breast meat proportion/carcass (%)	21.27	20.98	21.16	0.32	0.23	20.39	20.05	20.52	0.41	0.21
Abdominal fat, g	19.2	17.7	17.9	0.39	0.06	19.3^{a}	18.1^{b}	17.8^{b}	0.32	0.03
Abdominal fat,%	0.80	0.72	0.70	0.07	0.07	0.84	0.75	0.74	0.08	0.11

4. CONCLUSIONS

Supplementation of Lactozymee and ProbiP in 1% lower CP diet of Grimaud duck diets tended to improve weight gain, reduce *E.coli* density in the feces and abdominal fat compared with ducks feed optimum CP diet without any probiotic supplementation.

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EFFECT OF PROBIOTIC ACTISAF ON GROWTH PERFORMANCE, SOME LARGE INTESTINAL BACTERIUM COUNTS AND SMALL INTESTINAL MORPHOLOGY IN CHICKENS

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ABSTRACT

A total of 240 one day-old (LP×Mia) broiler chicks were raised over 63 days. Chicks were wing-banded, weighed individually and randomly divided into two groups each having two replicates. Chicks of group 1 (control group) were fed the starter and finisher diets that did not supplemented with probiotic. The chicks of experimental group were fed the control starter and finisher diets supplemented with 1 g of probiotic per kg feed. Weekly body weight, feed consumption and feed conversion were measured. All chicks were kept under similar environmental, managerial and hygienic conditions. The results demonstrated that probiotic supplementation significantly increased the body weight and daily weight gain of broiler chicks from ages (3-9wks). Improved feed conversion was noticed in birds fed a diet supplemented with probiotic. There was no significant difference in mortality rate between two groups. Colon *E. coli, Salmonella, Clostridium perfringens* and total aerobic bacterial counts were decreased by the probiotic administration. The height and the width of duodenum between control group and experimental group showed no significant difference. Jejunum villi height was increased while jejunum villi wideness was decreased in the chicken supplemented probiotic in the diet. We concluded that use of selected commercial probiotic resulted in improved performance parameters and good for the intestinal health of chickens.

Keywords: *Chickens, growth performance, large intestine, probiotic Actisaf, small intestine.*

1. INTRODUCTION

The presence of antibiotic residues in poultry meat and eggs may have deleterious effects on human consumers. The residues of antibiotics can cause resistance of human flora and pathogenic microbes to those groups of antibiotics. Moreover, cross-resistance to antibiotics used in the therapy of humans and other animals could also result (Edens, 2003; Pelicano *et al*., 2004).

A lot of researchers have partially replaced antibiotics with probiotics as therapeutic and growth promoting agents. It was reported that probiotics have a good impact on the poultry performance (Koenen *et al*., 2004; Mountzouris *et al*., 2007), improve microbial balance, synthesize vitamins (Fuller, 1989), decrease pH and release bacteriocins (Rolfe, 2000), improve feed consumption in layers and broilers (Nahashon *et al*., 1994). Most of the previous researches on probiotic utilization in poultry focused on the use of multispecies probiotics and various strains of *Lactobacillus*.

The probiotic microbes have the capacity to inhibit the development of pathogenic microorganisms in the gut of poultry (Getachew, 2016). Supplementation of probiotic products allows manipulation of the GI Microbiota. For example, Listeria monocytogenes is one of the pathogenic microbes that affect the poultry GI tract. Administration of multi-strain probiotic containing different *Lactobacillus* species and *Bacillus amyloliquefaciens* prevents the establishment and spread of this bacterium in the GI tract of broiler chickens (Neveling *et al*., 2017). In another study, the administration of commercial probiotic preparation formulated from different species of *Lactobacillus* and *S. cerevisiae* reduced the stress of *E. coli* K88 infected Hubbard broiler chicks and reduces

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E.coli proliferation in GI tract (Mohamed and Younis, 2018).

According to Forkus *et al*. (2017), the production of the antimicrobial peptide known as Microcin J25 by engineered *E.coli* inhibits colonization of *Salmonella* enterica in the turkey GI tract. *Clostridium perfringens* is a pathogenic microbe that causes necrotic enteritis in poultry and negatively affects poultry health and productivity (Yan *et al*., 2017; Kibrnesh *et al*., 2019).

2. MATERIALS AND METHODS

2.1. Materials

240 one day-old crossbreds of Luong Phuong (LP) and Mia F_1(LP×Mia) broiler chicks.

Actisaf probiotic contain *Saccharomyces cerevisiae* sc in a concentration of 10^{10} CFU/g.

2.2. Methods

2.2.1. Experimental design

A total of 240, one day-old F_1(LP×Mia) broiler chicks, obtained from hatchery, were grown over 63-day period. The chicks were wing-banded, weighed individually and the randomly seperated into two treatment groups following completely randomized design. There were 60 chicks per replicate and two replicates per treatment group. Feed and water were provided *ad libitum*.

A probiotic commercially identified as Actisaf was used as a feed additive in this study. The bacterial flora in the Actisaf probiotic has mentioned to be *Saccharomyces cerevisiae* sc in a concentration of 10^{10} CFU/g. Chicks of control group were fed the starter and finisher diets that did not supplemented with probiotic. The chicks of experimental group were fed the control starter and finisher diets plus 1g of a commercial probiotic Actisaf per kg of ration. Diets were formulated to provide the recommended requirements for broiler.

2.2.2. Growth performance

Measurements of broiler performance including body weight, daily weight gain, daily feed consumption and mortality rate were determined. All chicks in each group were weighed individually at hatch from 1 to 9 weeks of age. Daily weight gains were then calculated for the periods: hatch from 1 to 9wks of age. The feed offered to each room was recorded daily with an automatic weighing machine. At the end of each week feed residues were weighed, feed consumption was therefore recorded on a weekly basis and then calculated as feed consumed per day over the periods: hatch from 1 to 9wks of age. The feed conversion ratios could then be calculated for the time periods: hatch from 1 to 9wk expressed as feed conversion ratio: feed consumed/weight gain. Mortality rate was weekly determined as a cumulative percentage, all dead chicks were removed daily (morning) and weighed. Their feed consumption was estimated and discounted from the total feed given to the group during that week.

2.2.3. Intestinal bacteria counts

Six chicks in each group were slaughtered at 63d of age. The substances contained in the colon were determined bacteria counts including *Escherichia coli, Salmonella, Clostridium perfringens, Saccharomyces cerevisiae* sc and total aerobic bacterial counts, according to the standards of ISO 13349/2001, ISO 7937/2004. ISO/Dis 11290/1994 and ISO 4833/2003.

2.2.4. Intestinal morphometric parameters

Intestinal morphometric variables were evaluated by light microscopy, where a bird of each replication was sacrificed at 63d of age after fasting for 12hrs. From each bird, a sample from the medial region of the duodenum was collected, opened and immediately fixed in Bouin solution for 24hrs. They were washed in 70% alcohol to remove the Bouin solution and were subsequently dehydrated in ascending series of alcohols, cleared in xylene and embedded in paraffin. Histological sections were made and stained according to the methodology of Giannenas *et al.* (2010). Analyses were made by the program Image J® 1. The variables evaluated were height and width of the villi, being held 12 readings per intestinal region.

2.3. Statistical analysis

Data were analised using two-way ANOVA at 5 or 1% of probability, using the Statistical Analysis System (SAS, 2004) software. The statistical assumption of residual normality was

evaluated using the Shapiro-Wilk while Levene's test was used for homogeneity of variances. Data were subjected to a one-way analysis of variance using GLM procedure.

3. RESULTS AND DISCUSSION

3.1. Weekly body weight means of chickens

Table 1 demonstrated that the probiotic tested in this study significantly improved the body weight of the chickens. The effect of probiotic started at two weeks of age. At 3 weeks of age, the probiotic supplementation showed significant increase in the body weight compared with the control group. This positive effect of probiotic on body weight persisted until 9wks of age. The differences in the body weight became greater towards the end of the trial period. The average body weight of the chicks supplemented with probiotis Actisaf was higher than 39.87 g/bird as compared with the control group.

Table 1. Weekly body weight of chickens (g/bird)

Age in week	Exp group (n=120)		Cont group (n=120)	
	Mean±SE	CV(%)	Mean±SE	CV(%)
0	34.98±0.74	11.61	34.84±0.67	10.60
1	75.28±0.72	5.21	73.67±0.83	6.18
2	138.94^{a}±0.65	2.55	132.77^{b}±1.06	4.39
3	216.08^{a}±1.17	2.96	206.12^{b}±1.73	4.60
4	317.61^{a}±1.02	1.77	290.24^{b}±1.57	2.97
5	428.08^{a}±1.30	1.66	385.94^{b}±1.58	2.25
6	571.31^{a}±1.56	1.50	518.11^{b}±1.37	1.45
7	730.09^{a}±1.40	1.05	658.16^{b}±1.28	1.06
8	958.94^{a}±2.98	1.70	875.89^{b}±2.55	1.60
9	1237.41^{a}±2.10	0.93	1134.28^{b}±3.01	1.45
Average	470.87^{a}±1.36	3.09	431.00 ^{b}±1.57	3.65

Means within rows with no common letters are significantly different (P<0.05).

Pham Kim Dang *et al*. (2016) studied the effect of probiotic heat resistance *bacillus* on growth performance of Ross 308 chickens, from new hatch to 45d showed the body weight of the chicks supplemented with probiotis was higher than that as compared with the control group. Some other reults of studies showed significant differences of the body weight of the chicks supplemented with probiotis compared with the control group from the age of week 3 of chicks (Alkhalf *et al*., 2010; Tran Duc Hoan *et al*., 2020).

3.2. Daily weight gain of chickens

The results of the abow table showed that, the effect of probiotic on the broiler daily weight gain is consistent with its effect on body weight in this study. The probiotic treatment groups showed a significant increase in the daily weight gain at 3, 4, 5, 6, 7, 8 and 9wks of age. The birds fed on the probiotic showed higher daily weight gains than the control group. This finding is in agreement with several reports demonstrating that probiotic supplemented to the birds improve the body weight and daily weight gain (Khaksefidi and Ghoorchi, 2006; Liu *et al*., 2007; Mountzouris *et al*., 2007). However, the results obtained in this study concerning body weight and daily weight gain are contrary to the findings of other studies. Mohan *et al*. (1996) reported that the beneficial effect of probiotic on chicken occurred only after the 4th week of growth.

Table 2. Daily weight gain of chickens (g/bird/day)

Age in week	Exp group (n=120)		Cont group (n=120)	
	Mean±SE	CV(%)	Mean±SE	CV(%)
0-1	5.76±0.10	13.06	5.55±0.1	14.43
1-2	9.09±0.08	6.42	8.44±0.13	12.11
2-3	11.01±0.08	5.89	10.47±0.12	8.71
3-4	14.50^{a}±0.09	4.97	12.02^{b}±0.12	7.72
4-5	15.78^{a}±0.09	4.17	13.70^{b}±0.26	14.09
5-6	20.46^{a}±0.12	4.64	18.88^{b}±0.23	9.06
6-7	22.68^{a}±0.19	6.39	20.01^{b}±0.21	8.08
7-8	32.69^{a}±0.31	7.24	31.10^{b}±0.30	7.36
8-9	39.78^{a}±0.39	7.50	36.91^{b}±0.37	7.50

Daily weight gain was also significantly influenced by supplemented probiotic. Pham Kim Dang *et al*. (2016); Doan Van Soan and Tran Duc Hoan (2017) reported, the results of daily weight gain of chickens supplemented with probiotic was higher than that as compared with the control group, and showed clearly significant differeces at 8-9; 9-10 weeks of age.

3.3. Daily feed consumption and feed conversion ratio of chickens

Concerning the average feed consumption, the birds supplemented with probiotic consumed significantly more feed than control group.

From 2 to 9wks of age, there were a significant differences in the daily feed consumption between the probiotic groups and the control group. These findings are in agreement with those of Willis *et al*. (2007) who observed significant differences in feed consumption and efficiency of broiler chickens receiving the probiotic. The results of the pressent study can be noticed that the probiotic treatment groups showed less feed conversion ratio than control group. These results showed that there were no significant differences in the means of feed conversion ratio between probiotic groups and control group at 1 and 2wks of age. However, there were significant differences between probiotic groups and control group from 2 to 9wks of age. These findings are in agreement with the findings of Maiorka *et al*. (2001) who found that the use of a synbiotic composed of *Saccharomyces cerevisiae* and *Bacillus subtilis* improved feed conversion compared with antibiotic and control treatments at 45 days of age. Also, Khaksefidi and Ghoorchi (2006) concluded that feed conversion of birds fed diet supplemented with 50 mg/kg of probiotic were significantly better from 22 to 42d than birds fed the control diets.

Table 3. Daily feed intake and feed conversion ratio

Age in week	Exp group (n=120)		Cont group (n=120)	
	FI (g/bird/week)	FCR (kg)	FI (g/bird/week)	FCR (kg)
0-1	48.28	1.20	48.28	1.24
1-2	89.75[a]	1.41	86.75[b]	1.47
2-3	182.81[a]	2.37	179.81[b]	2.45
3-4	253.82[a]	2.50	248.82[b]	2.96
4-5	292.38[a]	2.65	286.38[b]	2.99
5-6	339.37[a]	2.37	332.37[b]	2.51
6-7	360.52[a]	2.27	352.52[b]	2.52
7-8	379.28[a]	1.66	375.28[b]	1.72
8-9	421.86[a]	1.51	416.86[b]	1.61
Average	263.12[a]	1.99	258.56[b]	2.16

3.4. Mortality percentage of chickens

Concerning mortalities, cumulative mortality rates were lower in the birds fed the probiotic than the control group over the period 0-9 weeks of age. The supplementation of probiotic of chicks reduced the mortality 2.62% as compare to the chicks not supplemented with probiotic in this study. With similar trials with chicks given *Lactobacillus* preparations, the effects on mortality were inconsistent (Zulkifli *et al*., 2000). Yo¨ru¨k *et al*. (2004) found that supplementation of probiotic (containing *Lactobacillus, Bifidobacterium, Streptococcus,* and *Enterococcus* species) during the late laying period in layer hens reduced mortality. O'Dea *et al*. (2006) reported that there were no significant differences in broiler mortality between the probiotic treatments and the control group in any of the trials.

Table 4. Mortality percentage of chickens

Age of week	Exp group (n=120)		Cont group (n=120)	
	Number of mortality	(%)	Number of mortality	(%)
0-1	1	0.83	2	1.67
1-2	0	0	1	0.85
2-3	0	0	0	0
3-4	1	0.84	3	2.56
4-5	2	1.69	1	0.88
5-6	1	0.86	0	0
6-7	0	0	1	0.88
7-8	0	0	0	0
8-9	0	0	0	0
Total	5	4.22[b]	8	6.84[a]

3.5. Large intestinal bacteria counts

The results of table 5 indicated that, there were a significant differences of the parameters of some intestinal bacteria such as *E. coli, Salmonella* and *Clostridium perfringens* in the chickens of control group as compared with experimental group. The parameters of *E. coli*/g of feces in colon of the experimental chickens were lower than the control chickens 1,18 Log10 CFU/g. The parameters of *Salmonella*/g of feces in colon of the experimental chickens were lower than the control chickens 0,90 Log10 CFU/g, and The parameters of *C. perfringens*/g of feces in colon of the experimental chickens were lower than the control chickens 0,97 Log10 CFU/g. Whereas, the parameters of benificial bacteria *Saccharomyces cerevisiae* sc and total aerobic bacterial counts of the experimental chickens were higher than the control chickens 0,59 và 0,46 Log10 CFU/g. These results demonstrated that, the supplementation of probiotic increased the benificial bacteria and limited the harmful bacteria.

Table 5. Large intestinal bacteria counts of chickens (Log10 CFU/g)

Parameters	Exp group (n=120)	Cont group (n=120)	F
E. coli/g of feces	4,21±0,19	5,29±0,24	**
Salmonella/g of feces	1,62±0,03	2,61±0,04	**
C. perfringens/g of feces	4,11±0,20	5,08±0,25	**
Saccharomyces cerevisiae/g of feces	5,43±0,24	4,84±0,22	**
Total aerobic bacterial counts/g of feces	5,89±0,21	5,43±0,26	ns

Edens (2003) reported that the inclusion of desirable microorganisms (probiotics) in the diet allows the rapid development of beneficial bacteria in the digestive tract of the host, improving its performance. As a consequence, there is an improvement in the intestinal environment, increasing the efficiency of digestion and nutrient absorption processes (Kibrnesh *et al*., 2019). on the other side, probiotics also help boost the immune system (Yan *et al*., 2017; Uraisha *et al*., 2019).

3.6. Small intestinal morphology

The present study showed that, there were no significant differences between the height and the width of duodenum villi of chickens supplemented probiotic Actisaf and the control group. Whereas, there were no significant differences between the height and the width of jejunum villi of chickens supplemented probiotic Actisaf as compared with non supplementation of probiotics in the diet. The height of jejunum villis of chickens supplemented probiotic was higher than the control chickens as 33.52%. In contrast, the width of jejunum villis of chickens supplemented probiotic was smaller than the control chickens as 6.25%. Edens *et al*. (1997) showed that *in vivo* and *ex vivo* administration of *Lactobacillus reuteri* resulted in an increased villus height, indicating that probiotics are potentially able to enhance nutrient absorption and thereby improve growth performance and feed efficiency.

Table 6. Small intestinal morphology of chickens

Position	Size of villis (mm)	Exp. group (n=12)	Cont. group (n=12)	F
Duodenum	Height	1,31±0,09	1,26±0,10	ns
	Width	0,34±0,12	0,31±0,14	ns
Jejunum	Height	1,85±0,08	1,73±0,06	**
	Width	0,30±0,05	0,34±0,04	**

4. CONCLUSIONS

Supplementation of the probiotic Actisaf to broilers improves growth performance. The supplementation of probiotic increased the benificial bacteria and limited the harmful bacteria Probiotic Actisaf increased the height of jejunum villis of chicks and decreased the width of jejunum villis of chickens. It is worth to mention that no any antibiotic was supplemented to or injected in the chickens from the first day until the end of the experiment.

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APPLICATIONS OF PMSG AND HCG IN ASSISTED REPRODUCTIVE TECHNOLOGY IN ANIMALS

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ABSTRACT

This article presents an overview of the structure, functions, biological characteristics and applications of PMSG and hCG in assisted reproduction technology in animals. Equine Chorionic Gonadotropin (eCG) is a hormone produced in the reproductive membranes of pregnant mares, commonly known as pregnant mare serum (Pregnant Mare's Serum Gonadotropin-PMSG). Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced during pregnancy by developing embryos and later by syncytium (syncytiotrophoblast, part of the placenta). Its role is to prevent the destruction of the corpus luteum and thus maintain progesterone secretion. In animal husbandry, an increase in the reproductive performance of domestic animals is very important. The combination of PMSG and hCG with reasonable doses has caused ovulation, stimulating reproduction effectively on many subjects such as mice, goats, pigs and cows. In Vietnam, there were studies on the effect of sex hormones on the fertility of cows and pigs. Preliminary research results on the impact of PMSG and hCG on some wild animals showed an increase in reproductive efficiency in captivity. Therefore, the research on the effects of sex hormones on the reproductive ability of domesticated wild animals is grounded and very necessary. That is order to improve reproductive performance, to raise breeding efficiency, to both exploit and preserve the ex-situ conservation of rare wildlifes in captivity.

Keywords: *Assisted reproductive technology (ART), hCG, PMSG.*

1. INTRODUCTION

More than 90 years ago, equine chorionic gonadotropin (eCG) was discovered (Murphy, 2012). It is as a factor found in circulation of the pregnant mare during the first third of gestation. eCG is a variant of equine luteinizing hormone (LH). This hormone is secreted from endometrial cups within the pregnant mare uterus aging from 40 to 130 days into their maturationIt has the peculiar property of provoking both follicle-stimulating hormone (FSH) and LH activity in non-equid species. The biological basis for this dual activity is believed to be the result of promiscuity of the mammalian FSH receptors, imparting the capacity to respond to this equine LH-like hormone. The role of eCG in the mare is that it induces accessory corpora lutea to better support early gestation. eCG solely exhibits luteinizing hormone like activity, however in other animal classes it has FSH and LH like activity. Thus, eCG are applicated in domestic species, including induction of puberty, reversal of anestrus, superovulation, and improvement of fertility (Murphy, 2012).

According to Laurence (2010), hCG was discovered more than 100 years ago. In early pregnancy, human chorionic gonadotropin (hCG) is produced primarily by differentiated syncytiotrophoblasts, and represents a key embryonic signal, essential for thre maintenance of pregnancy. hCG is a member of the glycoprotein hormone family that includes LH, thyroid-stimulating hormone (TSH), and FSH. It is a 237 amino acid (AA) heterodimer. hCG is comprised of α-(93-AA) and β-(145-AA) subunits. Now a days, the function of hCG is still marked as being pro-gesterone promotion and other important placental, uterine and fetal functions in pregnancy. The research showed that there are at least 4 indepen-dent variants of hCG, each produced by different cells with separate biological functions. All the molecules share a common hCGb-subunit AA sequence. There is hCG, produced by differentiated syncytiotro-phoblast cells or more specifically

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villous syncytiotro-phoblast cells as pregnancy progresses (Laurence, 2010).

In assisted reproductive tecnology, conservation of endangered species, transgenesis, or cloning by nuclear transfer, oocyte supply is crucial for the preservation of genetic resources. So that, the collection as many unfertilized eggs as possible at any time of the day, is an essential tool needed for experimental animals, domestic species and endangered wildlife (Martemucci and D'Alessandro, 2011). The method of superovulation induction by administering both PMSG and hCG was investigated on mice (Corbin and McCabe, 2002; Popova *et al*., 2002; Cornejo-Corte *et al*., 2006; Luo *et al*., 2011), pig (Schilling and Cerne, 2008), dairy cattle (Rensis *et al*., 2010). Pregnant mare's serum (PMS) is used to mimic follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG) is used to micic lutenizing hormone. The times that the PMSG and hCG are admistered relative to each other affect both the developmentatl uniformity and the number of eggs that are recovered from superovulated female on animals (Leã and Esteves, 2014).

2. THE STRUCTURE AND FUNCTION OF PMSG

2.1. The structure of PMSG

Pregnant Mare's Serum Gonadotropin (PMSG) secreted from the mare's endometrium on days 40-130 of pregnancy. It is essentially a glycoprotein hormone, consisting of two subunits that are alpha and beta. The alpha subunits are common to all glycoprotein hormones (LH, FSH, TSH). The beta subunits are species-specific (Murphy, 2012).

In the early days of studies, serum from pregnant horses injected into experimental animals stimulated ovarian growth. The biologically active component of this hormone was named pregnant serum gonadotropin (PMSG) to reflect the occurrence in the 2-5 months of pregnancy. Some time later, this ovarian stimulant was determined to be present in the horse uterus, leading to the suggestion that it was of fetal origin. In the early years of 1970s, studies have confirmed that the origin of this hormone is from fetal chorionic cells invading the uterine epithelium to form endometrial cups. This finding led to renaming of the PMSG hormone to chorionic gonadotropin (eCG) (Murphy, 2012).

Protein analysis have shown that eCG is synthesized and secreted in the same way of pituitary gonadotropin (FSH, LH). It is metabolized after glycosylation. The AA sequence showed that the main structure of eCGß is identical to LHß. Both display a carboxyl terminal extension of 30 AAs, and this tail is strongly glycosylated. Subsequent studies demonstrated that they are two products of a single gene (Sherman *et al*., 1992). Glycosylation distinguishes them, since the LH chain is supplemented with sialylated oligosaccharides and sulfates, while the sialylated forms dominate the stronger glycylated eCGß molecule (Matsui *et al*., 1991). Especially the binding of sialic acid, this difference leads to one of the most important properties is the prolonged biological halflife of PMSG, six times longer than LH (Martinuk *et al*., 1991). The chorion begins to develop during the early embryonic phase in the 7th week of pregnancy and begins to penetrate the endometrium to form the endometrial tubes. At this time, eCG can be detected in the horse serum. eCG ususlly peaks on days 70-80 of pregnancy (Murphy, 2012).

2.2. The biological activity of eCG

In equids, eCG has only LH-like activity and is known as equine chorionic gonadotropin. In non-equine species, PMSG has both FSH and LH activity and stimulates follicular development, ovulation, and corpus luteum formation (Murphy, 2012). eCG can act as the LH hormone in horses, although less powerful than eLH. The remarkable role of eCG has been explored in many commercial and experimental settings. It has the ability to express FSH activity in other species. The biological basis for this phenomenon is not fully understood, but is largely explained by the double action of the structural determinants of eCG or of LH and FSH receptors in non-horse species (Saint-Dizier *et al*., 2004).

One of the characteristics of eCG is its half-length. When injected into mice, eCG displays a half-life in excess of 5hrs; while in sheep, this value is 21hrs. In cattle, the half-life is es-

timated to be 45.6hrs (Murphy, 2012). The basis for this elongation is the strong glycosylation, which comprises about 45% of its molecular weight (Matteri *et al.*, 1987). Both chains are glycosylated at the asparagine (N-glycosylated) and serine-threonine (O-glycosylated) bonds and the C-terminal extension of the chain is strongly glycosylated, where the carbohydrate chains contain sialic acid, resulting in a long half-life. Removing sialic acid from eCG reduces the half-life, and when removed 80% reduces the half-life to less than 60 minutes (Martinuk *et al.*, 1991). However, the eCG glycosylation model not only determines its existence in serum but also its biological activity in the target tissue (Murphy and Martinuk, 1991). Because the structure is capable of binding to receptors, especially in FSH receptors, it is the basis for the double action (roles such as LH and FSH) of eCG. This effect is enhanced by its extended half-life.

3. THE STRUCTURE AND FUNCTION OF HCG

3.1. The origin and structure of hCG

Human chorionic gonadotropin (hCG) is a glycoprotein consisting of 244 AAs with a molecular weight of 36.7kDa. Its dimensions are 75×35×30 A^0 (7.5×3.5×3nm), including a β subunit with 145 AAs and an α subunit with 92 AAs. There are 2 oligosaccharides bound to N on the α subunit of hCG and 2 oligosaccharides linked to N on the β subunit of hCG. There are also 4 O-linked oligosaccharides O on the C-terminal peptide of hCG subunit (Laurence, 2009).

3.2. The biological activity of hCG

hCG has many functions: promoting progesterone production by luteal corpus cells, angiogenesis, fusion of white blood cells and differentiation to produce lymphocytes; interfering with any maternal immune or macrophage activities on invasive placental cells; causing uterine growth in parallel with fetal development; preventing any uterine spasms during pregnancy; induces growth and differentiation of umbilical cord; endometrial signaling about implantation; activity on receptors in the mother's brain causing hyperplasia; and it also seems to promote fetal organ growth during pregnancy (Lea and Esteves, 2014).

hCG interacts with LH-CG receptors and promotes corpus luteum retention during early pregnancy, in order to release the progesterone hormone. Progesterone thickens the uterine and capillary blood vessels and helps maintain fetal growth. hCG can repel immune cells of the mammals, protecting the fetus in the first trimester (Laurence, 2009).

hCG acts like a strong luteinizing hormone (LH) which is effective in prolonging the lifetime of the corpus luteum (CL). hCG increases progesterone synthesis, induces ovulation throughout the oestrus cycle, and promotes the formation of lutea corpora when applied in the early luteal phase. It alters cystic wave dynamics, increases frequency of dominant follicular cycles. According to Rensis *et al.* (2010), hCG acts on ovarian cells independently of pituitary hormones and its effects last longer than endogenous production of LH. hCG could be advantageous used for livestock in place of gonadotropin-releasing hormone (GnRH).

The research showed that there are at least 4 variants of hCG, each made up of different cells with distinct biological functions. All molecules have the same hCGβ AA chain. These are molecules that promote progesterone production by ovarian luteal cells and have various biological functions (Laurence, 2010). The hCG function raises blood sugar to promote the growth of lymphocyte cells and the penetration of these cells, which occurs during the transplant of pregnancy, growth and invasion of cancer cells (Laurence, 2009).

4. USING PMSG AND HCG HORMONES IN ASSISTED REPRODUCTIVE TECHNOLOGY

PMSG is often used in combination with progestogens to induce ovulation in livestock before artificial insemination. PMSG tends to be widely used because it has a long half-life. In addition, it stimulates maturation of spermatas and spermatogenesis in males. The most common use of PMSG is to exploit its FSH-like activity in stimulating oestrus in immature animals. PMSG is used to stimulate the ovaries in animals for embryos during the embryo transfer process. Its long half-life provides the advantage of supercharging by a single injection, as it tends to stimulate the ovaries, resulting in mul-

tiple viable cysts and viable embryo production. PMSG displays both FSH and LH activity, which are necessary for the maturation and ovulation of follicles in mammals (Murphy, 2012).

Studies of using PMSG and hCG alone or in combination have been evaluated based on ovarian and uterine weight; follicle development, ovulation and the number of ovulation was carried out very early. Lunn and Bell (1968) have shown that a combination of both PMSG and hCG can cause ovulation. This event is associated with uterine stimulation but usually occurs in the absence of marked increase in ovarian weight or follicular hemorrhage. The effects of PMSG and hCG cause ovulation on a wide range of both hormonal dosages. With low doses of PMSG the number of eggs produced is independent of the dose of hCG.

PMSG and hCG are usualy used in superovulation as involves hormonal treatments to induce follicular development and oocyte release. Corbin and McCabe (2002) examined the responses of different groups of mice to suggest hormonal treatments for super ovulation. The effectiveness of hormonal treatments is assessed by the criteria: mating rate, ovulation rate, total ovulation per female and fertilization rate. Research has demonstrated that treatment with PMSG + hCG is an effective method of causing hyper ovulation reactions in most of the test criteria. The authors' results also showed that different rats have different levels of sensitivity and response to different protocols of the hormone super ovulation. This is the scientific basis for conducting research to determine the appropriate dosage of hormonal use on other subjects, including the endangered wildlife.

Similar to this research direction, to assess the effectiveness of using PMSG instead of FSH, Popova *et al.* (2002) implemented protocols for superovulation using a single injection of PMSG or FSH in immature Sprague-Dawley mice (SD). The monitoring criteria included: total number of eggs collected, fertilization rate, *in vitro* embryos development, sensitivity of zygotes in introducing foreign DNA into the pronucleus and their development in the body after implant into the fallopian tubes of receiving individuals. Female SD mice were stimulated with 15IU PMSG or FSH 10mg after administration of hCG at doses of 20 and 30IU to each individual. The results of this study demonstrated that the treatment of immature SD mice of PMSG was as effective as FSH treatment, and was therefore more suitable for gene transfer technology due to its lower cost, ease of treatment and more rational (Popova *et al*., 2002).

To determine the therapeutic dose to optimize super ovulation in mice, Cornejo-Corte *et al*. (2006) conducted research to produce a large number of good quality embryos suitable for the development of mouse embryonic stem cells (RES). The authors assessed the ovulation kinetics of three mouse strains: Wistar, Fisher and ACI/N. Experimental animals were treated with 50IU PMSG, and 50IU hCG after 50hrs. The evaluation parameters for super ovulation were: the percentage of mated female mice, the percentage of pregnant rats and the average number of embryos collected per head. The results of these experiments indicated that the best dose combination is 50IU for each hormone. Subsequent experiments, the Wistar mouse was designed to test which of the four hormonal treatments combined (30/30, 30/50, 50/30, 50/50IU PMSG/hCG) to generate numbers amount of workpiece reached the highest quality. The quality of embryos was assessed according to the following criteria: uniform development of embryos, embryo morphology, intrauterine and viable embryo-embryo life. The results of these experiments showed that 30/50IU PMSG/hCG is the therapeutic dose that produces the best quality of embryos (Cornejo *et al*., 2006).

Schilling and Cerne (2008) published results of oestrous treatment in gilts at 400IU PMSG and 200IU hCG. Five experiments were conducted on a Yugoslav industrial pig farm with more than 2,500 Landrace Swedish sows. There are 93-100% of pigs treated oestrus 3-7 days after injection. In animals that control oestrus not observed before 6 months of age; 33% showed symptoms of estrus for 6-6.5 months. The conception rate of experimental animals was very high: more than 80% of the fertilized sows formed in the first cycle caused (82.1% of all treated animals, 76. 7% delivered after six months of treatment (in the first cycle) and 80% after injection for 5.5

months (in the second cycle) compared with 23.3 and 40% of the control group. The combination of PMSG and hCG can be used in to stimulate reprodutive in sows by getting, high conception rate, low postpartum loss, short lactation period, high conception rate after weaning Early milk and higher profitability can result in premature oestrus, fertilization and simultaneous delivery in larger groups of animals.

An assessment describing the clinical use of hCG to improve the reproductive performance of dairy cattle was implemented (Rensis *et al.*, 2010). The authors described the developments in the therapeutic use of hCG and the research addressing the benefits of introducing hCG in oestrus pathogenesis and ovulation synchronization. The author's research was based on the results of previous findings related to ovarian response when using hCG for treatment, which can be explained by the understanding of advances in clinical application of hCG.

Luo *et al.* (2011) examined different weight ranges and hormone dosages to determine the hyper-ovulation protocols for 6 rats commonly used in genetic engineering: C57BL/6N HSD, B6 (Cg)-Tyrc-2J/J, B6D2F1/HSD, FVB/NHsd, BALB/cAnNCr, and CRL-CD1 (ICR). Mice from each strain were divided into groups based on weight, corresponding to 3, 4, 5 and 6 weeks of age. Mice were treated with 5IU PMSG and 5IU hCG. The authors concluded that the reaction to inducing ovulation could be optimized based on mouse line, weight and dosage and injection time.

A study was conducted to compare eight different superovulation protocols for golden hamsters using two concentrations of hCG given at two time intervals PMSG injection and two time intervals of oocyte harvesting. Fifty-six female golden hamsters were randomly and equally assigned into eight superovulation groups. Hamsters were superovulated initially with PMSG followed by hCG. In conclusion administration of 40IU PMSG followed by 45IU hCG injection at 55 and 57hrs post PMSG injection followed by oocyte recovery after 16-18hrs gave the highest response in oocyte recovery and maturation in golden hamsters (Kazhal *et al*., 2013).

Masoumeh *et al.* (2018) determined the effect of human chronic gonadotrophin (hCG) and pregnant mares serum gonadotrophin (PMSG) on ovary. This study used 20 rats that were randomly divided to experimental and control groups. The results demonstrated that hCG and PMSG hormones will signifcantly increased the number of stimulated oocyte in the ovary but did not have any significant role on the ovary weight or volume ($P<0.05$). These results confirmed the inductive role of PMSG and hCG hormones on folliculogenesis.

In Vietnam, Tran Tien Dung (2004) used a combination of PMSG and hCG reproductive hormones to treat postpartum oestrus in foreign sows in Yorkshire and Landrace. The study results showed that the proportion of sows in oestrus accounted for 80%, the time of oestrus, conception rate, number of litter per litter of sows cured were satisfactory. With the treatment regimen of PMSG 3,000 IU/sow, if the oestrus is injected with hCG 1,000 IU/sow, the results will be good with the time of estrus after 5.8 days, conception rate is 91.67%, the number of births was born with 9.8 heads. Do Van Thu *et al.* (2013) used a combination of PMSG and Prosolvin (PGF2α) to mass oestrus in combination with artificial insemination to improve the productivity and quality of the herds on both the yellow and Sind crossbred breeds. With the procedure of injecting cows with two doses of PGF2α (2ml) 11 days apart in combination with injecting PMSG (500IU) in the second nose, determining the conception rate by the method of rectal pregnancy examination. The results showed that the oestrus rate was 84.9%, the conception rate was 82.88% and the delivery rate was 93.52%. To cause ovarian hyperplasia, Hoang Nghia Son *et al.* (2013) used PMSG at a dose of 2,500IU to match FSH use (200mg). The number of corpus luteum and number of embryos collected in PMSG treated group was less than that of FSH group, however, higher than in previous studies. The team also pointed out that the combination of ovarian ultrasound to determine the follicle stage to select the optimal time and treatment method for hormone stimulation to obtain the best amount of eggs and embryos in the method of creating eggs and embryos *in vivo*. Nguyen Ngoc Tan and Bui Ngoc Hung (2017) evaluated the effectiveness of diffe-

rent hormone treatments on inseminated cows. The result of 7-day CIDR (progesterone-containing) loop therapy, combining the hormones GnGH and PGF2α were effectively produced oestrus and ovulation in dairy cows.

Although the treatments in each study are described in detail fortraditional livestock like pigs or cattle, no studies have been done on wildlife. Preliminary to research on the use of sex hormones to improve reproductive performance of wildlife in captivity, Nguyen Thanh Binh (2015) assessed the impact of hCG and PMSG on reproduction results of common palm civets (*Paradoxurus hermaproditus*). The results showed that the use of sex hormones significantly improved the reproductive ability of civets in the indicators of pregnancy and litter size. An autoimmune study performed on large molds (*Rhizomys pruinosus*) also showed an increase in reproductive efficiency in captivity (Nguyen Thanh Binh, 2016). This makes sense in increasing the number of breeds in captivity, which is a reliable scientific basis for further studies to assess the effect of hormones on wild animals. Nguyen Thị Thu Hien *et al.* (2020, 2021) carried a study to evaluate the effectiveness of different doses of PMSG, hCG on the time of occurrence, duration of oestrus and reproductive performance of the delayed oestrus civets. The results showed that the duration of oestrus averaged from 1.1 to 2.6 days after injecting sex hormones. The formula of 40IU PMSG + 20IU hCG could be applied effectively for the treatment on the first delays and delayed oestrus of the common palm civets.

5. CONCLUSIONS

The use of PMSG and hCG in combination with the appropriate dosage and time is effective in stimulating ovulation, enhancing fertility in females. However, studies only assessed the effectiveness of hormone use based on reproductive performance, without considering changes in hormone dynamics before and after treatment, and did not describe clinical changes before and after treatment. On the other hand, studies on the use of sex hormones to improve reproductive performance in wildlife in captivity are quite modest.

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COMPARISON OF THE EFFECTS OF TWO POULTRY HOUSING TYPES ON REPRODUCTIVE PERFORMANCE OF TRE CHICKEN

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ABSTRACT

This study was conducted to evaluate the effects of closed and open-sided houses on the reproductive performance of Tre chickens at 36-45 weeks of age. The results showed that feed consumption of chickens between closed and open-sided houses was different (P<0.05), with an average of 55.5 g/head/day in closed and 50.0 g/head/day in open-sided houses. In addition, the laying rate of chickens in closed was higher than in open-sided houses (42.5 and 34.6%, respectively). Egg weight, fertile egg ratio, and the hatchability rate of eggs from closed houses were significantly higher than those from open-sided houses. A higher culling rate of chickens in the open-sided type (0.37%) than closed one (0.25%) was also recorded. Overall, Tre layers in the closed houses were superior in terms of reproductive performance; however, further research on economic efficiency should be taken before applying this system widely.

Keywords: *Closed house, egg production, open-sided houses, Tre chicken.*

1. INTRODUCTION

Tre chicken is one of the indigenous breeds in Vietnam with the characteristics of small size, delicious meat, high adaptability to natural conditions, and good resistance to diseases (Nguyen Thi Thu Hien and Le Thi Ngoc, 2014). Traditionally, this chicken breed was raised in open-sided houses for egg and meat production. However, with the high demands for product consumption, an intensive system for keeping these chickens are currently of interest. In closed houses, there are ventilation cooling and exhaust fans that allow easy control of temperature and humidity. This significantly contributes to preventing disease and is convenient for management, care, and mitigating the effects of the surrounding environment, thereby improving egg production. The closed system has been popular for other chicken breeds, especially in broilers and commercial egg-laying hens. Nevertheless, for local ones such as the Tre chicken breed, the information on the effectiveness of raising Tre chickens in closed houses for egg production is still limited. Therefore, the present study was undertaken to compare the two housing types on the reproductive performance of laying hens in 36-45 weeks old.

2. MATERIALS AND METHODS

2.1. Materials

The study of Tre chicken was carried out for ten weeks with the ages from 36 to 45 weeks old at a chicken farm in Phu Kiet, Tien Giang province. The implementation period was from August 2020 to October 2020.

2.2. Methods

The experiment consisted of two treatments of open-sided and closed house types to investigate the differences in the reproductive parameters of Tre chickens. The total number of experimental chickens in the closed houses was 11,896 hens arranged in 8 rows of 100 cages, with an average of 1,487. The open-sided houses contained 10,104 birds with three houses, and the number of chickens in each place being 4,487; 3,069 and 2,548, respectively. The rearing density for both types was 16 chickens/cage (ratio of 1 male/7 females).

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2.3. Collected parameters

- Temperature inside the chicken houses.

- Feed consumption during ten laying weeks.

- Indicators of reproductive performance: egg production, egg weight, fertile egg ratio, hatchability rate, and culling rate.

2.4. Statistical analysis

The data were processed using Microsoft Excel 2016, and the analysis of variance was done by General Linear Model (GLM) procedure of Minitab software version 16.0.

3. RESULTS AND DISCUSSIONS

3.1. The temperature of the two open-sided and closed houses

It is shown in Table 1 that most of the time, the temperature of the closed poultry houses was consistently lower and more stable than that of the open-sided poultry houses (P<0.05). Specifically, at 8 a.m, the temperature in closed houses ranged from 26.0°C to 26.7°C, while in open-sided houses, the temperature was higher and fluctuated in the range from 28.0-28.4°C. At 2 p.m, the temperature in both types increased, with open-sided poultry houses registering a higher temperature rise (31.8-33.3°C), while the temperature of the closed poultry houses was around 26.7-27.6°C.

Table 1. Average temperature (°C) in 2 houses

Time	Layer age (week)	Types of house		SEM	P
		Closed	Open-sided		
8a.m	36-38	26.50	28.10	0.12	0.001
	39-42	26.70	28.40	0.29	0.007
	43-45	26.00	28.00	0.11	0.001
	36-45	26.40	28.16	0.07	0.001
2p.m	36-38	27.20	33.00	0.33	0.001
	39-42	27.60	33.30	0.56	0.001
	43-45	26.70	31.80	0.44	0.001
	36-45	27.23	32.77	0.19	0.001

Climate plays a vital role in affecting poultry health. Climatic factors include temperature, relative humidity, air composition, air velocity, movement, and light (Olanrewaju *et al.*, 2006; Mendes *et al.*, 2013; Holik, 2015). Temperature is the most important environmental factor which affects the health, behavior, and reproduction of chickens. The extreme high or low temperatures can be detrimental to the growth, development, and reproductive capacity of chickens because they can cause stress and negative effects on health and reduce efficiency in poultry (Aengwanich and Simaraks, 2004). The changes in the temperature inside the house should be considered a priority when designing the poultry houses. It is optimal for the chicken health and yields in terms of egg quantity and quality, feed conversion ratio, body weight gain, and mortality rate.

3.2. Effect of poultry houses on feed consumption

Table 2. Feed consumption (g/bird/day) in houses

Layer age (week)	Types of house		SEM	P
	Closed	Open-sided		
36	58.0	51.3	1.04	0.001
37	57.1	49.9	0.81	0.001
38	57.0	51.4	0.72	0.001
39	55.2	49.5	2.05	0.070
40	48.8	49.7	1.09	0.584
41	51.7	49.9	1.08	0.264
42	53.2	50.0	0.31	0.001
43	51.0	50.2	0.39	0.196
44	61.5	49.3	1.97	0.001
45	61.7	48.6	0.91	0.001
36-45	55.6	50.0	1.02	0.003

Table 2 shows a significant difference (P<0.05) in feed consumption of Tre chickens between closed and open-sided houses with an average consumption of 55.51 g/head/day and 49.97 g/head/day in closed and open-sided houses, respectively. According to Hameed *et al.* (2012), there is a difference in the microclimate environment between closed and open-sided houses. In the closed house system, the microclimate is adjusted when necessary, while the open-sided house climate depends on the natural conditions of the surrounding environment. In industrial broiler or layer production, the control of proper farming conditions can help maximize the potential of chicken breeds. Oloyo (2018) suggested that the temperature above 26.7°C in the house, combined with the high relative humidity, adversely affected the efficiency of feed consumption,

feathers, pigmentation, and weight gain of the chickens. Furthermore, at the temperature range of 35-37.8°C, the yield of chickens was inferior regardless of changes in relative humidity. Sterling *et al.* (2003) concluded that environment temperature was highly correlated with several yield indicators, including feed and water intake, body weight, egg production, feed conversion, and egg weight.

3.3. Effect of poultry house on reproductive performance

3.3.1. Egg-laying rate

Table 3. Egg-laying rate (%) of layers in 2 houses

Layer age (week)	Types of house		SEM	P
	Closed	Open-sided		
36	46.9	36.8	0.49	0.001
37	47.5	38.5	0.64	0.001
38	46.4	37.3	0.54	0.001
39	46.5	36.1	1.05	0.001
40	45.3	33.0	0.66	0.001
41	39.4	33.5	0.57	0.001
42	40.0	33.8	0.55	0.001
43	38.2	34.5	0.53	0.001
44	36.9	32.3	1.04	0.010
45	37.5	30.2	1.02	0.001
36-45	42.5	34.6	0.62	0.001

The egg-laying rate is an evaluation criterion of laying eggs on all poultry flocks from purebred breeds, grandparent breeds, parent breeds to commercial breed flocks (Bui Huu Doan *et al.*, 2011). The laying rate was relatively low and unstable in the open-sided houses, ranging from 30.15-38.46% to 36.87-47.51% in closed houses (Table 3). This result is similar to Balnave (1998) reported that high temperature affects the performance of laying hens due to the reduction of feed consumption. According to Smith and Oliver (1972), lower egg production and poor eggshell quality are caused by low feed consumption and high temperature. When temperature ranged from 21-38°C, egg production and egg weight decreased by 40-50% at 38°C. The study of Oloyo (2018) also demonstrated that the house temperature affects the reproductive performance of Leghorn chickens, but there is no significant difference in the surveyed temperatures. In the current study, the temperature of the open-sided houses is higher than that of the closed houses, leading to lower feed consumption and chicken laying rate.

3.3.2. Egg weight

Egg weight is one of the essential criteria to evaluate egg quality, which affects hatchability. The hatchability rate is highest when the egg weight is around the average of each breed. The farther it is from the average weight, the lower the hatchability rate is (Bui Huu Doan *et al.*, 2011).

Table 4. Egg weight (g) of Tre chickens in houses

Layer age (week)	Types of house		SEM	P
	Closed	Open-sided		
36	37.6	36.5	0.78	0.362
37	37.7	36.2	0.53	0.115
38	37.6	36.8	0.33	0.176
39	37.7	36.3	0.49	0.103
40	37.8	36.2	0.35	0.033
41	37.9	36.5	0.79	0.268
42	37.7	36.6	0.58	0.278
43	38.3	35.3	0.54	0.018
44	38.0	36.5	0.40	0.057
45	38.5	37.3	0.36	0.083
36-45	37.9	36.4	0.16	0.001

The results in Table 4 show that house type had a significant effect ($P<0.05$) on the egg weight. Specifically, the average egg weight over ten weeks in the closed cage was 1.5g higher than that of the open-sided pen (37.9 and 36.4g, respectively). The difference was probably due to the influence of chicken feed consumption and heat stress in the house. According to Samara *et al.* (1996), high temperature significantly reduces eggshell weight, density, and thickness. An increase in egg breakage and a decrease in eggshell thickness due to heat stress were reported in the previous study. Additionally, Ebeid *et al.* (2012) concluded that heat stress reduced egg weight (3.24%), eggshell thickness (1.2%), eggshell weight (9.93%), and eggshell (0.66%). According to Webster and Czarick (2000), should there be a temperature change, the chicken will consume less or more of the required amount of nutrients; as a result, the egg size will vary greatly.

3.3.3. Fertile egg rate

In chicken farming, acute heat stress occurs when chickens deal with sudden changes in

temperature in a short period, while chronic heat stress happens over an extended period. Chronic stress results in deleterious effects on poultry, such as their growth, production efficiency, egg quality, meat quality, embryonic development, reproductive performance, immunity, and infected rate in broilers and laying hens (Dai *et al.*, 2012; Bhadauria *et al.*, 2013).

Table 5. The ratio of fertile eggs in different types of houses

Week old	Number of hatching eggs		Number of fertile eggs after 9 days of incubation		Ratio of fertile egg (%)		SEM	P
	Closed	Open-sided	Closed	Open-sided	Closed	Open-sided		
36-40	1,733	1,081	1,593	970	91.90	89.7	0.01	0.228
41-45	1,549	1,096	1,378	935	89.00	85.40	0.01	0.045
36-45	1,641	1,089	1,486	925	90.40	87.60	0.01	0.049

Table 5 shows that the type of house affects the proportion of fertile eggs (P<0.05) because heat directly influences the amount of feed and egg weight, hence the effect of house type on the rate of a fertile egg. Over the whole period, the percentage of fertile eggs in closed houses (90.4%) was higher than in open-sided houses (87.6%). Practically, the fertilization rate depends on the ratio of males and females, the nutritional density, and the resistance of the breed (Nguyen Van Thien, 1996).

3.3.4. Hatchability rate

Figure 1 shows that the egg hatchability rate obtained from closed and open-sided houses was significantly different (P<0.05). Over the whole period, the average hatchability rate from hens in closed houses was 6.6% higher than that in open-sided houses (82.2 and 75.6%, respectively).

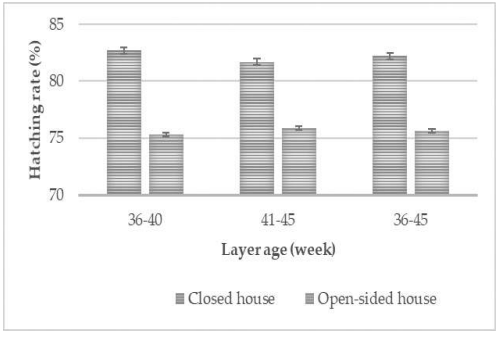

Figure 1. Hatching rate of chicken eggs in two types of houses

The high hatchability rate has great economic significance. If the hatchability results are low, the culling rate in the later nurturing period will be increased, and the breed quality is not guaranteed (Nguyen Thi Mai, 2009). Contrary to the present results, when studying the ISA Brown chicken breed, Damaziak *et al.* (2021) did not find any differences in hatchability rate between the two types of houses.

3.4. Culling rate

Figure 2 shows that, the culling rate in open-sided houses was always higher in most monitoring weeks than in closed houses (P<0.05). At the first period (36-40 weeks), the average culling rates of closed and open-sided houses were 0.25 and 0.43%, respectively. In weeks 41-45, these values were 0.24% for closed and 0.52% for open-sided houses. Over ten weeks, the average culling rate of Tre chickens in closed houses was 2.45% compared to 3.69% in open-sided houses. Causes of the loss were the poor quality of chicken, such as sickness and sick chickens that have been treated for a long time without recovering. This is also supported by similar findings of Atapattu *et al.* (2017). However, in ISA brown chickens, Damaziak *et al.* (2021) showed no difference in mortality between the two types of closed and open houses.

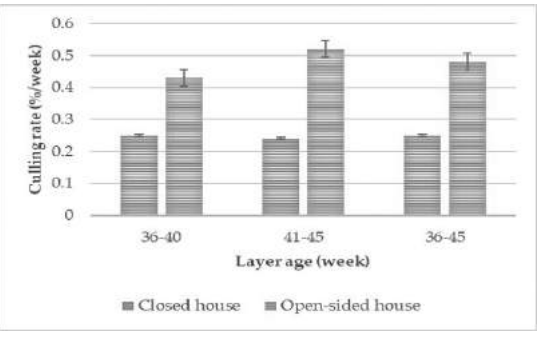

Figure 2. The culling rate of laying hens in two types of houses

4. CONCLUSIONS

During 36-45 weeks of age, Tre laying hens kept in closed houses were superior in all reproductive performance indicators. These results propose an opportunity for Tre's eggs production under the intensive system in closed houses. However, further studies are recommended for evaluating the economic efficiency before widespread application.

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EFFECT OF VEGF (VASCULAR ENDOTHELIAL GROWTH FACTOR) ON THE MATURATION OF BOVINE OOCYTES DERIVED FROM SMALL FOLLICLES

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ABSTRACT

The aims of this study were to evaluate the effect of VEGF (vascular endothelial growth factor) on the maturation of bovine oocytes derived from small follicles. Derived cumulus-oocyte complexes (COCs) from small follicles (SF; <3 mm in diameter) and medium follicles (MF; 3-7mm in diameter) were selected and subjected to culture in a medium TCM 199 plus 1% BSA, 10 IU/ml hCG and antibiotic for 22hrs at 39°C and 5% CO2. The rate of maturation was assessed by Aceto-orcein staining method. The results show that the maturation rate in the derived-COCs from MF was 67.3%, significantly higher than the group of derived-COCs from SF (37.4%; P<0.05). When derived-COCs from small oocytes were cultured in VEGF-supplemented media at different concentrations of 0, 50, 100 and 200 ng/ml, the maturation rates were 39.3, 55.4, 58.7 and 64.1%, respectively. Meanwhile, the derived- COCs from medium follicles cultured in VEGF-free medium had a maturation rate of 71.1% which was not significantly different from derived COCs from SF was exposed to VEGF at 200 ng/ml (64.1%; P>0.05). In conclusion, the meiotic resumption competence of oocytes derived from small follicles is lower than those in medium follicles of bovine oocytes. VEGF markedly improves the meiotic resumption competence of oocytes derived from small follicles, especially at a concentration of 200 ng/ml. Therefore, it is considered as a promising growth factor in improving oocyte quality obtained from small follicles, further study is required.

Keywords: *Bovine oocytes, follicle, meiotic resumption, VEGF.*

1. INTRODUCTION

Up to date, many studies have been performed to increase the rate of *in vitro* maturation (IVM) in attempts to better simulate the *in vivo* micro-environment during IVM. A wide variety of oocyte maturation research has shown the addition of exogenous growth factors in culture media (Van den and Zhao, 2005; Zhang *et al.*, 2015) and application of a cell-based co-culture system that secretes various kinds of growth factors and combined the reproductive hormone such as FSH (Follicular stimulating Hormone), estrogen into the culture media (Fujita *et al.*, 2006; Nguyen *et al.*, 2011; Lee *et al.*, 2017; 2018). A large number of growth factors, such as Fibroblast Growth Factor, Epidermal Growth Factor, Transforming Growth Factor β1, and Vascular Endothelial Growth Factor (VEGF), are secreted by the female reproductive tract (Maurya *et al.*, 2013; Wang *et al.*, 2013). In female, VEGF is essential for the development of follicles, corpus luteum and for placenta establishment (Findlay, 1986). In the ovary, VEGF protein and its receptors are present in many cell types including luteal, granulosa, and theca cells and even in oocytes (Bruno *et al.*, 2009; Cao *et al.*, 2009). Einspanier *et al.* (2002) reported that VEGF concentration in bovine follicular fluid increases according to follicular development, reaching a maximum level in pre-ovulatory follicles. In bovine cumulus-oocyte-complex (COC), the expression of VEGF receptors changes remarkably in a time dependent manner during *in vitro* maturation (IVM); mRNA of VEGF receptors are enriched at the beginning of maturation (Einspanier *et al.*, 2002; Yan *et al.*, 2012). These finding suggest that VEGF is involved in the maturation of oocyte or early embryo development in mammals. So, the aims of this study were to investigate the effect of VEGF on meiotic resumption competence bovine oocyte derived from small follicles *in vitro*.

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2. MATERIAL AND METHODS

2.1. Material, chemicals and supplies

Bovine ovaries were collected from a local abattoir. All chemicals and reagents were purchased from Sigma-Aldrich (Oakville, ON, Canada), unless otherwise stated

The experiments were carried out at the Animal Embryo Technology Lab, Research Institute for Biotechnology and Environment, and Faculty of Biological Sciences, Nong Lam University in Ho Chi Minh City from Nov, 2019 to Dec, 2020.

2.2. Methods

2.2.1. Oocyte collection

Collection of ovaries and oocyte aspiration were carried out as described by Nguyen *et al*. (2012). Briefly, bovine ovaries collected from an abattoir and transported to the laboratory at approximately 25-30°C within 2h after collection. The cumulus-oocyte complexes (COCs) were manually aspirated from follicles 3-7mm in diameter using an 18-ga needle attached to a 10ml syringe (Nguyen *et al.*, 2012). Cumulus-oocyte complexes were searched under a stereo-microscope and washed (three times) in wash medium. All COCs with at least two layers of cumulus cells and uniform cytoplasm were selected for further culture.

2.2.2. In vitro maturation

Cumulus-oocyte complexes were washed (three times) in maturation media containing TCM-199 supplemented with 1% BSA (w:v), 10 IU/ml hCG, 0.05 µg/ml gentamicin. Groups of 10-20 COCs were placed in 100µl droplets of maturation media covered by mineral oil and incubated for 22h at 39°C, 5% CO_2 in air.

2.2.3. Aceto-orcein staining

Oocytes were stained and evaluated as described (Nguyen *et al*., 2019). Briefly, oocytes were mounted on glass slide (less than five oocytes per slide) under coverslip (supported with paraffin-vaseline corners) and fixed in ethanol:acetic acid (3:1, v:v) for 24h. Then, oocytes were stained in 1% orcein (w/v) in 45% acetic acid (v:v) for 20 min and differentiated by gently running differentiation solution (20% glycerol [v:v] and 20% acetic acid [v:v] in distilled water), between the slide and coverslip. The oocytes were evaluated using phase-contrast microscopy for the stage of nuclear maturation as GV, GVBD, MI, MII and Degenerated (Figure. 1).

2.3. Contents

2.3.1. Investigation the effect of COCs derived from different follicular size on bovine oocyte nuclear maturation

COCs were aspirated from medium and small follicle (3-7mm and <3mm in diameter, respectively) then subjected to culture in 100µl droplets with TCM199 + 1% BSA + antibiotic + 10 IU/ml hCG for 22hrs in 39°C, 5% CO_2 in air. After 22hrs of culture, the cumulus cells from COCs were removed and subjected to Aceto-Orcein staining for nuclear observation.

2.3.2. Evaluation of VEGF protein supplementation at different concentrations on bovine oocyte nuclear maturation

Five treatment groups were designed, COCs collected from medium follicle was used as control (T1) without VEGF and the COCs collected from small follicles were randomly divided into four groups (T2; T3; T4 and T5) and cultured in 100µl droplets with TCM199 + 1% BSA + antibiotic with different VEGF concentration (0, 50, 100 and 200 ng/ml UI/ml) for 22h in 39°C, 5% CO_2 in air.

2.4. Data analysis

All data were subjected into one-way ANOVA analysis, followed by Tukey's test using Minitab 18.1 software. The data are presented as Mean±SEM, the percentage data were transformed into arsine before ANOVA analysis.

3. RERULTS AND DISCUSSION

3.1. Investigation the effect of COCs derived from different follicular size on bovine oocyte nuclear maturation

After 22h of COCs culture, cultured COCs were removed cumulus cells, fixed and stained with Aceto-Orcein and then observed under microscope to classify the nuclear stage of oocytes. The representative image of nuclear stage and percentage of nuclear status at different stages were presented in Figure 1 and Table 1.

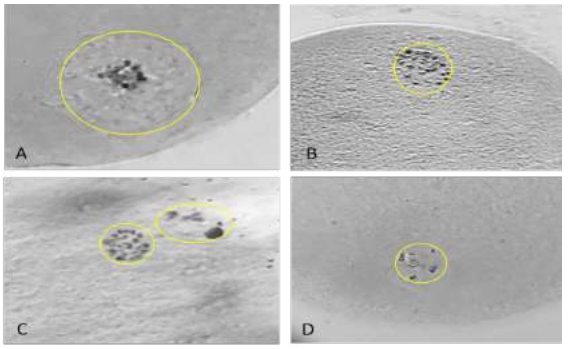

Figure 1. Classification of bovine oocytes using an Aceto-Orcein staining method

GV (Germunal Vesicle), (B): MI-metaphase I, (C): MII-metaphase II, (D): Deg-degenerated. Magnification ×100.

As showed in Table 1, the maturation rate of COCs derived from MF was significantly higher than in grouped COCs derived from SF (67.3 vs. 37.5; P<0.05). Meanwhile, the GV/GVBD was dominant in COCs derived from SF as compared to grouped COCs from MF (43.3% vs. 6.2% respectively; P<0.05). Several studies have showed that the mean number and maturation rate of ≥6mm follicles was 3 times lower than that of <6 mm follicles in the *Bos indicus* cows (Mutiga *et al.*, 1993) and higher number of follicles having <5 mm diameter (Caixeta *et al.*, 2009) in *Bos indicus* than *Bos taurus* cows. Many studies in porcine (Marchal *et al.* 2002; Bui *et al.*, 2017), bovine (Lonergan *et al.*, 1994), buffalo (Raghu *et al.*, 2002) have also demonstrated a clear relationship between follicle size and IVM and fertilization rates, it is the same trend with our current study, regard to maturation rate, is really lower than in SF as compared to those in MF.

Table 1. Classification of nuclear stage in bovine oocytes with an Aceto-Orcein staining procedure derived from different size of follicles

Group	Total	Nuclear stage (%)			
		n (GV/GVBD)	n (MI)	n (MII)	n (Degenerated)
MF (3-7mm)	110	7 (6.2^{b}±1.4)	26 (23.6^{a}±2.5)	74 (67.2^{a}±2.1)	3 (2.5±1.2)
SF (<3mm)	120	52 (43.3^{a}±2.1)	20 (16.7^{b}±1.3)	45 (37.5^{b}±2.3)	3 (2.6±1.8)

Within column, value with different superscript letters differ (P<0.05). Data are presented as Mean±SEM from 8 repetitions. GV: germinal vesicle; GVBD: germinal vesicle breakdown; MI: metaphase I; MII: metaphase II stage.

3.2. Evaluation of VEGF protein supplementation at different concentration on bovine oocyte nuclear maturation

Culture COCs in the culture medium supplemented with different concentration of VEGF protein, the nuclear stages of oocyte were observed and presented in Table 2.

Table 2. Effects of VEGF supplementation at different concentrations on bovine oocyte meiotic resumption competence

Treatment	Total (n)	Nuclear stage (%)			
		n (GV/GVBD)	n (MI)	n (MII)	n (Degenerated)
T1	68	5 (9.3^{b}±1.6)	12 (18.1±2.7)	49 (71.1^{a}±2.5)	2 (4.3±1.9)
T2	65	24 (36.7^{a}±1.1)	12 (18.2±2.5)	26 (39.9^{d}±1.1)	3 (5.2±3.6)
T3	63	10 (16.0^{b}±2.4)	16 (25.7±3.0)	35 (55.4^{c}±2.3)	2 (2.7±1.8)
T4	69	9 (13.5^{b}±1.4)	17 (27.2±2.2)	41 (58.7^{bc}±1.4)	2 (2.3±1.5)
T5	68	5 (8.4^{b}±2.7)	17 (28.7±3.1)	44 (64.1^{ab}±1.8)	2 (2.9±1.8)

Within a column, value with different superscript letter(s) differ (P< 0.05). Data are presented as mean ±SEM from eight repetitions. (T1) COC derived from MF (3-7 mm) cultured in medium without VEGF as control group; (T2) COC derived from SF (< 3 mm) cultured in medium without VEGF (Control); (T3) COC derived from SF (<3mm) cultured in medium with 50 ng/ml VEGF; (T4) COC derived from SF (<3mm) cultured in medium with 100 ng/ml VEGF; (T5) COC derived from SF (<3mm) cultured in medium with 200 ng/ml VEGF.

As shown in Table 2, the maturation rate was highest (71.1%; P<0.05) in T1 (COCs derived from MF cultured without VEGF) as compared to other treated groups. The significantly difference was found in T1 as compared to those in treated groups, except T5. When the derived-COCs from SF were exposed to VEGF at different concentration (0; 50; 100 and 200 ng/ml) during IVM, the percentages of mature oocytes was significantly higher in T3 to T5 (55.4 to 64.1%, respectively) than those in T2 (39.9%) cultured without VEGF (P<0.05). Interestingly, COCs in group T5 reached a MII stage up to 64.1% and none significant deference as compared to T1 group (P<0.05) that means supplementation of VEGF at 200 ng/ml enhanced the ability of meiotic resumption of COCs derived from SF.

With regard to COCs derived from SF treated with different concentration of VEGF protein, we found that reduced the proportion of oocyte arrested at GV/GVBD stage when increased the dose of VEGF protein supplementation. Many research found that IVM medium supplemented with VEGF at 100 and 300 ng/ml improves both bovine oocyte fertilization and subsequent embryo development, indicating that VEGF is an important growth factor for nuclear and cytoplasmic maturation (Luo *et al.*, 2002a,b). VEGF concentration is higher in pre-ovulatory follicles than in small ones in cattle (Einspanier *et al.*, 2002), in pigs (Bui *et al.*, 2017) and the lower meiotic competence of oocytes from SF may be affected by the lower concentration of VEGF secreted from COCs into the IVM medium (Bui *et al.*, 2017).

4. CONCLUSION

The meiotic competence of oocytes derived from small follicles is lower than those in medium follicles of bovine oocytes. VEGF markedly improves the meiotic resumption competence of oocytes derived from small follicles, especially at a concentration of 200 ng/ml and further study is required.

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EFFECT OF HUMAN CHORIONIC GONADOTROPIN ON THE MEIOTIC RESUMPTION OF BOVINE OOCYTE *IN VITRO*

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ABSTRACT

The aims of this study were to evaluate the effect of human Chorionic Gonadotropin (hCG) on meiotic resumption competence of bovine oocytes derived from the medium follicles (3-7mm in diameter). Cumulus- oocyte complexes (COCs) were cultured in TCM 199 medium plus 1% BSA, antibiotic and supplemented with or without the hCG or addition of hCG at different concentrations (0, 5, 10 and 15 IU/ml) for 22hrs at 39°C, 5% CO_2 in air. The nuclear maturation was assessed by Aceto-Orcine staining. The results showed that the rate of maturation in the cumulus-oocytes complexes (COCs) treated group with hCG was a significant higher than the non-treated group COCs (69.2 vs 40%, P<0.05). When the COCs were exposed to hCG in culture medium at different concentrations (0, 5, 10 and 15 IU/ml), the maturation rate was 39.5, 51.5, 70.2 and 52.7%, respectively. In conclusion, hCG alone in culture medium improve the nuclear maturation of bovine oocyte and the supplementation of hCG with a concentration at 10 IU/ml can be considered as the optimal dose to improve the nuclear maturation of bovine oocyte derived from medium follicles under *in vitro* condition.

Keywords: *Bovine oocytes, hCG, In vitro maturation, meiotic resumption.*

1. INTRODUCTION

The *in vitro*-assisted reproduction techniques have been considered an essential tool for studying oocyte maturation, early embryo development, and animal model research (Prather *et al*., 2003; Coticchio *et al*., 2015). It has been demonstrated that the intrinsic high quality of oocyte during maturation is a prerequisite condition for supporting the efficiency of early embryo development as well as fetal growth (Sagirkaya *et al*., 2007). Therefore, many studies have been performed to increase the rate of *in vitro* maturation (IVM) in attempts to better simulate the *in vivo* micro-environment during IVM. A wide variety of oocyte maturation research has shown the addition of exogenous growth factors in culture media (Van den and Zhao, 2005; Zhang *et al*., 2015) and application of a cell-based co-culture system that secretes various kinds of growth factors and combined the reproductive hormone such as FSH (Follicular Stimulating Hormone), oestrogen into the culture media (Fujita *et al*., 2006; Nguyen *et al*., 2011; Lee *et al*., 2017; 2018). Gonadotropin plays an important role in the regulation of oocyte growth and maturation *in vivo*. Physiologically, the preovulatory luteinizing hormone (LH) surge is essential to trigger the meiosis resumption and nuclear maturation of oocytes *in vivo*. The effect of gonadotropin is based on its physiological role in oocyte-cumulus cell communication and is highly beneficial to nuclear and cytoplasmic maturation of the cumulus-oocyte complex (Dumesic *et al*., 2015). In current study, we aimed to evaluate the effect of hCG supplementation alone in culture medium on the resumption meiosis of bovine oocyte *in vitro*.

2. MATERIAL AND METHODS

2.1. Material, chemicals and supplies

Bovine ovaries were collected from a local slaughter house. All chemicals and reagents were purchased from Sigma-Aldrich (Oakville, ON, Canada), unless otherwise stated.

The experiments were carried out at the Animal Embryo Technology Lab, Research Institute for Biotechnoly and Environment and Faculty of Biological Sciences, Nong lam University in Ho Chi Minh City from Nov, 2019 to Dec, 2020.

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2.2. Methods

2.2.1. Oocyte collection

Collection of ovaries and oocyte aspiration were carried out as described by Nguyen *et al.* (2012). Briefly, bovine ovaries collected from a local abattoir and transported to the laboratory at approximately 25-30°C within 2h post collection. The cumulus-oocyte complexes (COCs) were manually aspirated from follicles 3-7mm in diameter using an 18-ga needle attached to a 10-ml syringe (Nguyen *et al*., 2012). Cumulus-oocyte complexes were searched under a stereomicroscope and washed (three times) in wash medium. All COCs with more than two layers of cumulus cells and uniform cytoplasm (Grade A and B in Figure 1) were selected for culture.

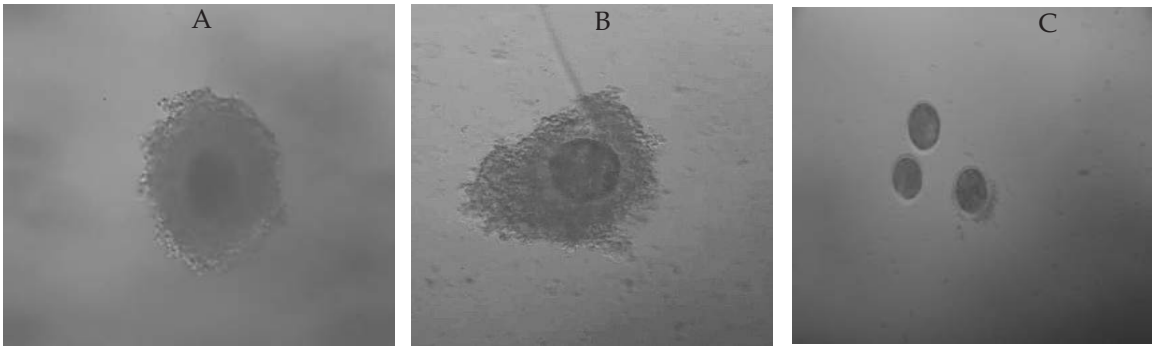

Figure 1. Classification of cumulus oocyte complexes.
A: Grade A, B: Grade B and C: Grade C. magnification X40.

2.2. *In vitro* maturation

Cumulus-oocyte complexes were washed (three times) in maturation media containing TCM-199 supplemented with 1% BSA (w:v), 0.05 µg/ml gentamicin. Groups of 10-20 COCs were placed in 100 µl droplets of maturation media under mineral oil and incubated for 22hrs at 39°C, 5% CO2 in air.

2.3. Aceto-orcein staining

Oocytes were stained and evaluated as described (Nguyen *et al*., 2019). Briefly, oocytes were mounted on glass slide (less than five oocytes per slide) under coverslip (supported with paraffin-vaseline corners) and fixed in ethanol:acetic acid (3:1, v:v) for 24hrs. Then, oocytes were stained in 1% orcein (w/v) in 45% acetic acid (v:v) for 20 min and differentiated by gently running differentiation solution (20% glycerol [v:v] and 20% acetic acid [v:v] in distilled water), between the slide and coverslip. The stained oocytes were evaluated using phase-contrast microscopy for the stage of nuclear maturation as GV, GVBD, MI, MII and Degenerated (Figure 2).

2.4. Contents

2.4.1. Evaluation the effect of hCG supplementation in culture medium on bovine oocyte nuclear maturation

COCs were randomly divided into two groups and subjected to culture in 100 µL droplets with TCM199 + 1% BSA + antibiotic and with or without 10 IU/ml of hCG for 22hrs in 39°C, 5% CO_2 in air. After maturation, the cumulus cells from COCs were removed and transferred to Aceto-orcein staining for nuclear observation.

2.4.2. Evaluationthe effect of hCG supplementation at different concentrations on bovine oocyte nuclear maturation

COCs were randomly divided into four groups and cultured in 100µl droplets with TCM199 + 1% BSA + antibiotic with different hCG concentration (0, 5, 10 and 15 UI/ml) for 22hrs in 39°C, 5% CO_2 in air.

2.5. Data analysis

All data were subjected into one way ANOVA analysis, followed by Tukey's test using Minitab 18.1 software. The data are presented as Mean±SEM, the percentage data were transformed into arsine before ANOVA analysis.

3. RERULTS AND DISCUSSION

3.1. Effect of hCG supplementation in culture medium on nuclear maturation of bovine oocyte

After 22h of COCs culture, cultured COCs from each treatment was removed cumulus cells, fixed and stained with Aceto-Orcein and then observed under microsope to classify the nuclear stage of oocytes. The representative image of nuclear stage and percentage of nuclear status at different stages were presented in Figure 2 and Table 1.

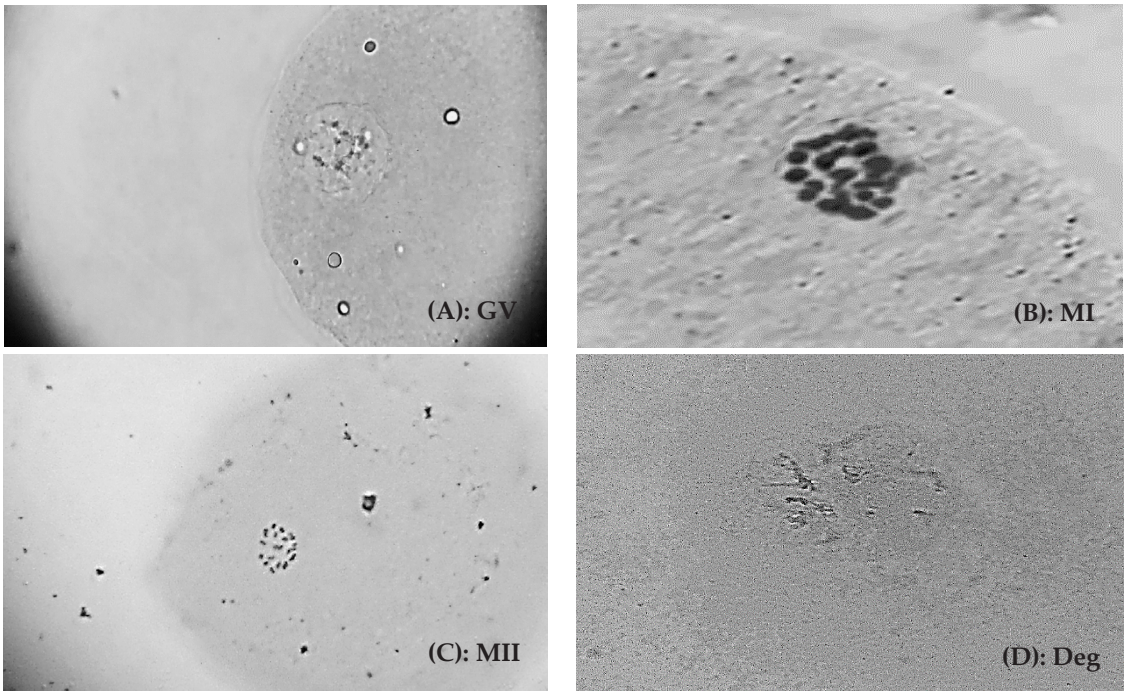

Figure 2. Classification of bovine oocyte nuclear stage using an aceto-orcein staining method

GV-Germunal Vesicle, (B): MI-metaphase I, (C): MII-metaphase II, (D): Deg-degenerated. Magnification ×100.

Table 1. Classification of nuclear stage in bovine oocytes with an Aceto-orcein staining procedure after treatment with hCG at 22h of IVM

Treament	n	Nuclear stage (%)			
		n (GV/GVBD)	n (MI)	n (MII)	Degenerated
Without hCG	129	40 (30.0^{a}±3.3)	35 (27.8^{a}±2.9)	51 (40.0^{b}±1.4)	3 (2.3±1.2)
With hCG	133	18 (13.1^{b}±3.0)	21 (15.8^{b}±1.6)	91 (69.2^{a}±2.7)	3 (1.9±1.0)

Within a column, value with different superscript letter(s) differ (P<0.05). Data are presented as Mean±SEM from nine repetitions. GV: germinal vesicle; GVBD: germinal vesicle breakdown; MI: metaphase I and MII: metaphase II stage.

As showed in Table 1, supplementation of hCG into oocyte culture medium increased the nuclear maturation rate of COCs (69.2%) as compared to none treated group COCs (40%; P<0.05). Meanwhile, the GV/GVBD or MI was dominant in none treated COCs as compared to treated COCs (30% or 27.8% vs. 13.1 or 15.8%, respectively; P<0.05). However, the effects of LH and hCG on oocyte maturation and development *in vitro* are still controversial. Chian *et al*. (2000) reported that hCG priming before oocyte retrieval not only could enhance the oocyte maturation rate but also could improve the developmental potential of oocytes *in vitro* and increase clinical pregnancy rates. However, the studies by Soderstrom-Anttila *et al*. (2005) in

humans or Junk *et al*. (2003) in mice have not obtained the same effects. The difference could be due to the different between species oocyte used, the culture media applied.

3.2. Effect of hCG at different concentration on bovine oocyte nuclear maturation

Culture COCs in the medium supplemented at different concentration of hCG, the nuclear stages of oocyte were observed and presented in Table 2.

As shown in Table 2, supplemented hCG into culture medium enhanced the nuclear maturation rate of bovine COCs in dose dependent manner. The maturation rate was highest (P<0.05) in treated group COCs with 10 IU/ml hCG, then lower in the treated groups with 5 or 15 IU/ml of hCG (51.2 or 52.7%). The lowest maturation was found in the control group COCs (39.5%; P<0.05). The data from Table 2 also revealed that the proportion of GV/GVBD and MI stage of nuclear were also dominant in control group COCs (P<0.05) as compared to those in treated group with 10 UI/ml hCG.

Ge *et al.* (2008) applied a dose of recombinant hCG at 0.5 UI/ml in culture media for patient who got POCS, it did not affect of human oocyte maturation but improved the early embryo development. On the contrary, de Lima *et al.* (2020) found that the proportion of oocytes that reached metaphase II (MII) stage was higher when eCG + hCG were added for 24h than 48h mainly at the 44h of maturation in porcine oocyte maturation.

Table 2. Effects of hCG supplementation at different concentrations on bovine oocyte nulear maturation

hCG Concentration (IU/ml)	n	Nuclear stages (%)			
		n (GV/GVBD)	n (MI)	n (MII)	n (Degenerated)
0 (Control)	86	27 (30.0^{a}±4.0)	24 (29.3^{a}±3.1)	34 (39.5^{c}±2.0)	1
5	78	18 (22.9^{a}±3.0)	21 (25.8^{ab}± 3.3)	39 (51.2^{b}±2.7)	0
10	91	10 (11.5^{b}±2.6)	15 (17.1^{b}±2.3)	65 (70.2^{a}±3.0)	1
15	94	18 (20.1^{ab}±2.0)	26 (28.1^{a}±2.1)	50 (52.7^{b}±2.7)	0

Within a column, value with different superscript letter(s) differ (P< 0.05). Data are presented as mean ±SEM from eight repetitions. GV: germinal vesicle; GVBD: germinal vesicle breakdown; MI: metaphase I; MII: metaphase II stage.

4. CONCLUSION

In this study, hCG plays an important role on meiotic resumption of bovine oocye *in vitro* and the optimal dose at 10 UI/ml in culture media is suggeted.

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A SURVEY ON GOAT FARMING IN SMALL SCALE HOUSEHOLDS OF HO CHI MINH CITY, VIETNAM

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ABSTRACT

The aim of this study was to evaluate fertility, growth, and milk yield of pure Bach Thao (BT), crossbred of Saanen × Bach Thao F_1(Sa×BT), and F_1 of Boer × BT F_1(Bo×BT) goats in 27 household farms at the suburb of Ho Chi Minh City, Vietnam from Jul 2019 to Jul 2020. Results showed that the average first farrowing age of 3 goat breeds is about 422 days. Bodyweight of 03 goat breeds range 2.13-2.87 at newborn and reach 27.8-34.6 at 9 months of age. The survival rate reflects death rate go up to 10.7% at 9 months of age with typical difficulties such as lack of feed, water, and disease. In other hands, Sa×BT as milky breed at household has just given 1.4 kg/head/day, to be lower compared with expectation. Besides that, there were no official distribution channels to support farmers in distributing goat's products to consumers at reasonable prices.

Keywords: *Bach Thao, Saanen, Boer, birth weight, milk yield.*

1. INTRODUCTION

The population of goats in Vietnam increased 30.5% annually from 1.8 million in 2015 to 2.6 million in 2017 (Van and Thu, 2018). Vietnam government is encouraging goat farming in difficult areas due to goat well-utilized agricultural by-products, saved the area of captivity, goat's milk provides a higher nutrient than cow's milk and its demand is increasing in big cities like Ho Chi Minh city and Hanoi. However, the most goat is in hands of small-scale households, therefore there are certain limitations in management, source of feed, and drinking water supply. Therefore, it is not sustainable in goat raising and productivity is lower than expectation, especially with the dairy goat. The goatherd was well developed in the suburbs of Ho Chi Minh city, close to the strong consumption of goat's products (meat and milk). In recent years, however, there has been a significant decrease in the quantity of goats leading to a shortage of supply. To better understand the difficulties of goat raising, this paper conducted a survey of goat production such as fertility and milk yield, noted the troubles in goat raising at households in suburb of Ho Chi Minh city. From that, it suggests the appropriate support to farmers to reach higher productivity, stimulating more sustainable development of goat farming.

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2. MATERIAL AND METHODOLOGY

2.1. Location and time

The survey was conducted in suburb around of Ho Chi Minh city including Binh Chanh, Hoc Mon, Cu Chi, Can Gio districts and District 9, 10 and 11 from July 2019 to July 2020. Ho Chi Minh city is in the Southeast, a subequatorial climate with two distinct seasons: the rainy season from May to November and the dry season from December to April of the following year, it has the average temperature in range 25-28^{0}C, the humidity levels average 75% throughout the year but are higher during the rainy season. The population of Ho Chi Minh City is 7,521,100 in 2011 (www.gso.gov.vn); however, uneven distribution with crowded in the city and sparsely in suburbs.

2.2. The content of survey

The scale of goat farming in Ho Chi Minh City is quite small, thus the survey would not use random sampling but rather conduct the survey on all existing households of 27 households. Small-scale households in the

suburb of Ho Chi Minh city were surveyed by a structured questionnaire that was used to collect information on primary data. The recorded information in the questionnaire was as follow:

Breeds: 100 pure Bach Thao (BT), 270 crossbred Boer and Bach Thao F_1(Bo×BT), and 440 crossbred Saanen and Bach Thao F_1(Sa×BT).

Management such as feed, water supplies, barn equipment, and barn hygiene.

Fertility.

The typical difficulties in goat farming.

For milk production, it was directly recorded on the goats in the lactation period during the survey.

2.3. Data analysis

Data were analyzed descriptive statistic such as percentages and frequency distributions in excel program.

3. RESULTS AND DISCUSSION

3.1. The bodyweight and survival rate of goat breeds in goat households

According to a survey in 27 households in the suburbs of Ho Chi Minh City, there are 3 main groups of breeds which are pure Bach Thao (BT), crossbred Boer and Bach Thao (Bo×BT), crossbred Saanen and Bach Thao (Sa×BT). Boer and Saanen goat breed were bred with Bach Thao to produce hybrids that well-adapting to feed and weather in Vietnam and improved performance. Bodyweight of Bo×BT goat at 6 and 9 months of age in the households (Table 1) showed that it was equivalent to the bodyweight of pureblood Boer reported by Doan Thi Gang *et al.* (2004), the author showed that pureblood Boer goat reached 25.9-28.1kg at 6 months and 24.7-38.5kg at 9 months.

The survival rate could be significantly affected by birth weight, litter size (Zahraddeen *et al.*, 2007) and reflected management. The average birth weight of goat (Table 1) range 2.13-2.87kg and litter size is from 1-2 kid/doe (Table 2) lead to the relatively high in survival rate in newborn. However, the death rate of goat at 9 months of age is from 7-10.7% may reflect the certain difficulties that may be faced by farmers such as disease and poor rearing condition.

Table 1. Bodyweight and survival rate (± SD)

	Newborn	3 month	6 month	9 month
Bodyweight, kg				
BT	2.13±0.47	12± 0.63	23±3.85	27.8±4.11
Bo×BT	2.73±0.38	14.7±1.90	26.2±3.54	34.6±5.22
Sa×BT	2.87±0.38	12.8±0.68	25.1±4.32	30±4.51
Survival rate, %				
BT	95	88.4	91.3	89.3
Bo×BT	95	90.1	95.6	92
Sa×BT	95.6	90	95	92.5

3.2. The fertility and milk production of 3 goat breeds at households

Table 2 showed that there was no difference in age at first farrowing (AFF) among goat breeds. However, Sa×BT was considered as milky breed longer lactation period (Table 3) compares with Bo×BT and BT. It leads to Sa×BT get highest in farrowing interval (FI) and lowest in kid/litter and litter/year.

Table 2. Fertility of goat at 27 household farms (± SD)

Traits	BT	Bo×BT	Sa×BT
AFF, day	423±7.49	422±11.3	422±11.3
FI, day	295±14.1	298±9.79	355±13.9
Kid/litter	1.90±0.7	1.70±0.47	1.60±0.50
Litter/year	1.20±0.06	1.2±0.04	1.0±0.04

There were no differences in milk yield/day but the lactation length of Sa×BT was longest lead to its total milk production/cycle is higher compare with BT and Bo×BT. Milk production of Sa×BT at household is lower compare with study of Phuong *et al.* (2020), the author reported that F_1(Sa×BT) could reach 2kg milk/head/day with average total milk yield/cycle range from 382 at litter 1 to 430kg at litter 2.

Table 3. Milk production in lactation period at households (± SD)

Traits	BT (n=100)	Bo×BT (n=256)	Sa×BT (n=67)
Milk yield, kg/head/day	1.18±0.23	1.28±0.15	1.4±0.21
Lactation length, day	146±14.1	150±9.75	205±12.2
Total milk production/cycle, kg	173±38.5	193±29.2	287±54.6

3.3. Management and typical difficulties of goat farming in small scale household of Ho Chi Minh City

Sanitation of barns and manure collection depends on the type of barn floor, the goats raised on cement floor were cleaned daily while high-floor (wood-plank floor) would be periodically cleaned. It is just 22.2% of household have equipped exhaust fan for pen design. Lack of ventilation in the goat barn would lead to high temperature in the barn on dry season and to be wet on rainy season, result in a negative effect on goat production. Zhu and Wang (2020) reported that daily milk yield of Guanzhong goat was reduced markedly, up to 16% in high temperature. Heat stressed goat have tendency of reducing feeding intake and resistance (Lu, 1989).

Table 4. Barn equipment and hygiene at small-scale household

	Waste treatment system	Barn hygiene				Water supplies		Pen		
		Daily	Periodic, days							
			15	<60	>90	Underground water	Tap water	Wood-plank floor	Cement floor	Exhaust fan
Household, n=27	No	8	3	2	14	10	17	17	8	6
Rate, %	-	29.6	11.1	7.4	51.9	37.0	63.0	70.4	29.6	22.2

Most farmers had access to tap water with 63%, mainly in Binh Chanh, district 9, 10 and 11. However, there were still households dependent on groundwater with 37% such as Cu Chi and Hoc Mon districts. For farmers in Can Gio district, with topography close to the sea, farmers had to buy tap water for raising, which gave the adverse effect of goat raising in this area.

Table 5. The typical difficulties in goat farming at small-scale household (%)

Place	Household sample, n	Lack of feed, %	Lack of water, %	Unstable price, %	Diseases, %
Binh Chanh	15	40	0	60	70
Hoc Mon	3	0	0	70	20
Can Gio	3	0	100	0	0
Cu Chi	3	0	0	100	0
District 9, 10 and 11	3	100	0	0	100

Restricted land for cultivating and goat farming due to urbanization leaded to lack of feed all year round in District 9, 10, 11 with 100% and Binh Chanh district with 40% (Table 5). Grass and leaves are common and essential feed for goats at small-scale household. Thus, when the land area is narrowed, it is necessary to have an orientation to re-distribute the goat farming area appropriately, promoting the utilization of available by-products source to reduce dependence on a single feed source. Water supply is especially important for dairy goat farming, most household in Can Gio must buy water regularly for goat farming, therefore, economic efficiency is often not high. Lack of water and feed is result diseases such as diarrhea, sore eyes, pneumonia, mastitis.

One of the difficulties mostly recorded from farmers is unstable price. Most of the distribution channels for meat and milk are through merchant. Merchant often operates with a small amount of capital, the *negotiation* is often unofficial, prices squeezing can occur when there are market fluctuations, it leads to economic risks for farmers at any time. The construction of official distribution channels is necessary to better support farmers in distributing products to consumers at reasonable prices.

The prevalence of pneumonia is the common disease occurring in households with 18.9%, followed by sore mouth with 8.24 and sore eyes with 6.68%, lowest in diarrhea with 3.53% and mastitis with 3.53% (Figure 1). However, if we consider the rate of disease in each household,

this is one of the difficulties encountered by farmers when the rate of pneumonia can reach 60%. Lack of feed, clean water, and poor ventilation in design of barn lead to reduced resistance in goats and susceptibility to common diseases.

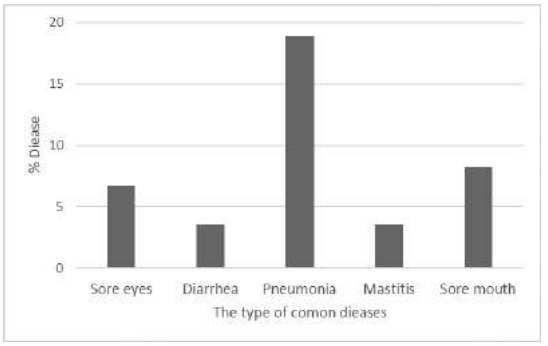

Figure 1. The rate of common diseases on total surveyed goat (n=810) at suburb of Ho Chi Minh city

The results of survey of goat farming in small-scale household in the suburbs, HCM City potentially showed unstable in the long term. Therefore, it is necessary to provide solutions for the re-distribution of goat farming areas appropriately, in addition to building a reasonable diet by utilization of available by-products such as cassava pulp, brewer's grain from factories, in order to promote productivity and decreasing dependent on the single feed. Farmers should be guided in the design of barns along with the construction of waste treatment systems to ensure the environment of goat's growth.

4. CONCLUSIONS

Survey of goat production in household at suburb of Ho Chi Minh city showed that bodyweight of goat range 2.13-2.87 at newborn and reach 27.8-34.6 at 9 months of age. The survival rate reflects death rate go up to 10% at 9 months of age with typical difficulties such as lack of feed, water, and disease. In other hand, Sa×BT as milky breed at household have just given 1.4 kg/head/day, to be lower compare with expectation depended on feed, feeding and management. Besides that, there were no official distribution channels to support farmers in distributing goat's products to consumers at reasonable prices.

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EFFECT OF TEMPERATURE-HUMIDITY INDEX ON PHYSIOLOGICAL PARAMETERS IN IMPORTED PUREBRED COWS OF RED ANGUS AND RED BRAHMAN REARED IN HOUSEHOLDS OF DONG THAP PROVINCE

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ABSTRACT

To evaluate the effect of temperature-humidity index (THI) on physiological parameters in imported purebred cows of Red Angus and Red Brahman reared in households of Dong Thap province, a study was randomly conducted on 8 cows in a household, raising 2 breeds simultaneously, about 3-4 years old, 4 cows for each breed. Cows were raised for grazing for about 1.5 months, the diet at 7:00, they were fed the *brachiaria multica*, water spinach, *pennisetum purpureum*; at 10 o'clock, going back to the cowhouse to eat dry straw and drink bran water; in the afternoon, at 14:00, cows were released to the pasture; and at 16:00, cows returned to the stable to eat dry straw and drink water. The activities of eating, sleeping, urinating, etc of each animal were recorded continuously for 24hrs. Measuring physiological indicators such as body temperature, pulse rate and respiration rate, each indicator was measured 3min, repeats 3 times. Determine atmospheric temperature, air humidity and physiological criteria in the same day, at 7, 10, 13, 16 and 19 o'clock. As a result, Angus cows spend more time eating than Brahman cows (535 vs 474min), high ruminant (61.75 vs 53.5 times/minute); the number of ruminante pieces/burps up was also higher (50.75 pieces compared to 43.25 pieces) leading to no difference in the time of sleeping, resting, taking food, drinking water and urinating. Although the THI fluctuated from 80.9 to 87.52, there was no significant physiological disturbance in the 2 breeds, but Angus cows tended to have a statistically significant increase in body temperature and pulse rate during 24hrs. Semi-grazing, ruminante activities in the two breeds both take place about 25% at noon, and about 75% at night. Thus, purebred Angus and Brahman cows can adapt quite well in well-ventilated stable at farm households in Tan Hong, Dong Thap province.

Key words: *Behaviour, Red Angus, Red Brahman, temperature-humidity index.*

1. INTRODUCTION

The demand for beef consumption has increased strongly and continuously since 2013, the domestic supply has not kept up, leading to a sudden increase in meat prices. Vietnam has to import a large amount of live cows from cold climates like Australia for slaughter. Specifically, in the year of 2015, total of 419,952 cattles were imported (Hoang Kim Giao and Le Thi Thuy, 2016; Hoang Kim Giao, 2017). It was at this time that farmers returned to invest and develop their cows, and from 2014 until now, the herd has continuously increased. In 2014, the national cow herd was 5,234,298 heads, until 2019 reached 6,060,024 heads (Vietnam Livestock Statistics, 2021).

When importing cows to the suburban area of Ho Chi Minh City, beside fresh beef supply through slaughtering, traders invest in farms to sell breeding animals. Farmers in the Southwest region in general and Dong Thap in particular, massively bought breeds, opened production farms, and traded in hybrids or purebreds of temperate origin such as Red Angus, Charolais, Red Brahman, BBB, DroughMaster. While the Southwest has a tropical monsoon climate, hot and humid all year round. Temperature-humidity index can cause heat stress on the physiological parameters of imported breeding

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cows. Meanwhile, research on assessing behavior and adaptation on this herd has not been implemented. This type of research is rarely conducted, because observations and recordings require very detailed, high accuracy; continuous recording 24/24hrs in difficult conditions such as observing all night, sitting for a long time, not communicating much, flies and mosquitoes, unhygienic environment, rain and drafts, monitoring outdoors in hot weather ect. So far, there were only a few studies on behavior in dairy and beef meat cattle by authors such as Dinh Van Cai *et al.* (2005); Vu Chi Cuong *et al.* (2007); Nguyen Thac Hoa *et al.* (2009); Van Tien Dung *et al.* (2010); Nguyen Ba Trung (2016).

Meanwhile, the influence of temperature and humidity on cow physiology is of interest in many countries. Heat stress may have a significant impact on the production and health of beef cattle. In feedlot animals heat can reduce feed intake and daily gains while in cow herds poorer reproductive performance can occur. During periods of extreme heat stress mortality can even occur. Through proper management, the effects of heat stress can be reduced, ultimately improving animal performance. Research and application of the tempertature humidity index (THI) have received great attention (Srikandakumar and Johnson, 2004; Amundson *et al.*, 2005; Khongdee and Chaiyabu, 2006). Eating, sleeping, and ruminating behavior is influenced by many factors such as atmosphere temperature, light, feed composition, cattle age and other stimuli (Sliworsky, 2009).

The combined effect of atmosphere temperature and THI was studied by Wiersama, (2006) based on the THI formula of Mader *et al*. (2006): THI=0.8xT+(RH/100)x(T-14.4)+46.4 said that when: THI<72, dairy cows will not suffer from heat stress, 72-78 will cause mild stress, 79-89 will cause quite severe stress, 89-98 will cause very heavy stress and THI>98, cows will die.

There are 3 levels of adaptation of livestock, including breeds adaptated to new living conditions, normal growth and development. The breed has not fully adapted to the new living conditions, after a few generations of purebred culture, it can live normally. The breed does not adapt to new living conditions, after a few generations, it degenerates, disease and dies. Therefore, the first step should be to choose which imported cow breed has the advantage in improving the herd in Dong Thap is necessary for research.

The objective of this study is to evaluate physiological parameters related to behavior and adaptability of imported purebred cows such as Red Angus and Red Brahman to answer the question that Angus or Brahman cows are better adapted to hot and humid living conditions in Dong Thap.

2. MATERIALS AND METHODS

The study period took place at the transition of the sunny-rainy season, from March 2016 to April 2016, at a farmer's household in Tan Hong district, Dong Thap province.

Record the cow's activities in 24hrs, including eating, sleeping, chewing, defecation, urinating... Determine the THI of the barn environment. Each breed consists of 4 breeding cows, about 3-4yrs old. Microlife infrared thermometer, wet-dry hygrometer, stopwatch, camera to capture the main activities of the animal, electric light just bright enough to take notes, flashlight with adjustable brightness to ensure no stress for animals.

Randomly selected a household in Dong Thap, raising 2 purebred Red Angus and Red Brahman cows, with the same age, nutrition, care, and without disease.

2.1. Experimental design

The experiment was carried out at a household in Tan Hong district, Dong Thap province, raising cows, satisfying the research conditions, including 4 Red Angus and Red Brahman cows in each breed, in reproductive age, without disease. Cows were raised for grazing, diet at 7.00, they were fed the *brachiaria multica*, water spinach, *pennisetum purpureum*; at 10 o'clock, going back to the cowhouse to eat dry straw and drink bran water; in the afternoon, at 14:00, cows were released to the pasture; and at 16:00, cows returned to the stable to eat dry straw and drink water

Wooden barn, double roof, mosquito net, fresh water pond all year round with 10m wide,

50m long. Around the barn planted fruit trees, helping to shade. Behind the barn is a planted grassland, with a separate area to keep the animal for eating grass (Figure 1).

Due to the staggered breeding among the rice fields, the custom of grazing cows also follows the harvest season: cows are raised for semi-grazing for about 1.5 months and grazing in the field for about 1.5 months. Arrange this observation at the semi-grazing stage. The behavior of each cow was recorded continuously for 24hrs through visual observation, counting, and recording the time the cow performed the movements. This work is done simultaneously by 2 groups, each group has 3 people and divided into 8hr shifts. Monitor one animal continuously/group each day, then move on to the next one. Thus, every day, 2 animals can be monitored, belonging to 2 different breeds. One week before monitoring, the groups had to directly take care of and feed the cows daily, to get used to them, to avoid affecting the cow's behavior and to practice making draft notes to familiarize themselves with the operation.

2.2. The method of data collection

Continuously record the cow's activity for 24hrs, then accumulate it to determine the total eating time such as get food available at the barn and in the pasture, drinking water. Total resting time, the time the animal does not rumination, does not close its eyes. Total time of rumination, frequency of rumination, number of times of rumination/minute. Number of pieces of ruminant, number of times of chewing/piece of food burped up to chew again. Number of episodes of rumination, the number of burps up to chew again /24hrs. The duration of each rumination session. Feeding frequency, number of bites/minute. Total sleep time, stool and urine excretion.

The temperature-humidity index is determined by measuring the atmosphere temperature and humidity of the environment in the barn every 3hrs in 24hrs, starting at 7.00 the day before and ending at 4.00 in the next day. The wet-dry hygrometer was placed in the cowhouse at about 0.5m above the floor of the barn. Look up the results according to TCVN 5508:2009 of the Ministry of Science and Technology.

The THI is calculated from this temperature and humidity record using the formula of Mader and Brown (2006): THI=0.8xT+(RH/100) x(T-14.4)+46.4. *Where, T is the air temperature in degrees Celsius. RH is the relative humidity of the air in %.*

The physiological parameters of cows were determined, including body temperature: using a microlife infrared thermometer close to the skin, below the midpoint of the smallest forehead width; pulse rate is measured manually at the chest area, directly measured by the farmer; determine respiration rate by observing hip concave. Each indicator measured for 3min, repeated 3 times. Time to determine atmosphere temperature, air humidity and physiological parameters of cows were measured on the same day at 7, 10, 13, 16 and 19 o'clock at the barn.

2.3. Data analysis

Using the Excel program to calculate the average values of behavioral indicators, temperature and humidity indexes and physiological indicators. Determine the linear regression equation between physiological index and THI and compare these indicators by means of pairwise comparison method of MINITAB version 16.2.

Figure 1. Draft notes in the grassland

Figure 2. Take note in the cowhouse

Figure 3. Purebred Red Angus cow

Figure 4. Purebred Red Brahman cow

3. RESULTS AND DISCUSSION

3.1 Main activities in 24 hours of cows

To assess the adaptability to hot and humid environment, in Dong Thap province, on 2 imported purebred breeds, daily behavior in cows was studied. Results in the Table 1 shows that all purebred Red Angus cows were more voracious than Red Brahman cows, the cows spend more time eating (535 vs 474.5min), this difference is statistically significant. However, the time spent on other activities such as chewing again, sleeping, resting, number of times chewing again per taking food, number of times drinking water, urinating among the two breeds were not statistically significant difference.

Table 1. Main activities in 24 hours of cows (n=4) (± SD)

Breed	Eating time (minutes)	Chewing again (minutes)	Sleeping (minutes)	Resting (minutes)	Times of chewing again/ taking food (times)	Drinking (times)	Urinating (times)	Defecating (times)
Angus	535^{a}±24.83	514.75± 10.5	72.5±6.45	317.75±38.30	13.35±3.44	3.25± 0.5	4.25±0.5	3.75^{a}±0.96
Brahman	474.5^{b}±17.21	525.5±20.27	70.75±4.34	369.3±28.3	16.78±1.94	3.25±0.5	4.75±0.96	5.5^{b}±0.58

Note: a, b: digits in the same column, with at least one different symbol, the difference is statistically significant at P<0.05.

The 24-hour feeding time of Red Angus and Red Brahman breeding cows in this study was higher than that of calves, ages 9-10 months, Sind and Angus crossbred calves of 359min, respectively (357min for males and 361min for females) and 374min (male of 351, female of 398min) in the study of Van Tien Dung *et al.* (2010). If comparing the time eating with other breeding cows, with the same age of 3-4yrs, semi-grazing, the time eating is not much different. Sind hybrids have a time eating of 380min (Nguyen Quoc Trung, 2016), Red Angus crossbred cows have 521min (Le Van Thiet, 2016), Red Brahman crossbred cows have 477min (Nguyen Huu Trong, 2016).

The results of the rumination time or chewing time, recorded in Table 1 in purebred Red Angus cattle were 514.75min, in Red Brahman cows were 525.5min. This result is higher than that of Van Tien Dung *et al.* (2010), Angus crossbred calves had 418min ruminant, Sind calves had 362min ruminant in semi-grazing environment. Compared with other breeding cow breeds, raised in this survey area, according to a study by Nguyen Quoc Trung (2016), Sind crossbred cows have 431min of ruminant, Red Angus crossbred cows have 510min. Le Van Thiet (2016) and Nguyen Huu Trong (2016) reported that Red Brahman crossbred cows have 442min of chewing again in 24hrs.

The main activities in 24hrs of experimental cows such as sleeping time, resting time, taking food, drinking water and urinating were similar, except that the eating time of Red Angus cows was significantly longer. Thus, feeding time on experimental cows is influenced by many factors such as breed, grazing regime, age and feed composition.

3.2. Cow's 24-hour feed digestion activities

To assess the adaptability of purebred cows, 24-hour forage digestion was observed in

semi-grazing mode. As a result, the frequency of grazing in the grassland of the two cow breeds was similar. However, Red Angus cows exercised more jaw muscles, resulting in better rumination frequency, better number of pieces chewing again/burps up, which was statistically significant difference.

The frequency of Red Angus cows, in Table 2, high ruminant (61.75 times compared to Red brhman is 53.5 times/min), the number of pieces for chewing again/burps up was also higher (50.75 pieces compared to 43.25 pieces). Red Angus cattle spend more time eating, have a high rumination frequency and increase the number of pieces for chewing again/burps up. As a result, in 24hrs, the sleeping and resting activities of these two breeds were not different under the same nurturing conditions.

Table 2. Cow's 24-hour feed digestion activities (n=4) (± SD)

Breed	Frequency of grazing (times/ minute)	Frequency of chewing again (times/minute)	Number of pieces chewing again/ burps up	Number of circles for chewing again (times)	Time of chewing again/circle (minute)
Angus	44.75±4.11	61.75^{a}±3.30	50.75^{a}±1.7	20.75±1.7	30.75±0.96
Brahman	45.23±3.40	53.5^{b}±3.11	43.25^{b}±3.31	19±1,83	30.25±1.71

Breeding cows and Red Angus and Red Brahman crossbreeds, after eating for about 15-30min, they burp up and chew again. However, in crossbred calves, the average duration of each rumination circle was about 18min (Nguyen Ba Trung, 2016) which was almost half lower than that of the herds in this survey (about 30min). This result, is not much different when compared with the results of the study on Red Angus crossbred cows with 30min (Le Van Thiet, 2016), Red Brahman crossbred only takes 25 min/ruminant cycle (Nguyen Huu Trong, 2016). Thus, although the frequency of grazing in the grassland, the number of circles for chewing again, and time of chewing again/circle were not different, but the Red Angus cows with 24-hour forage digestibility were superior to Red Brahman cows, statistically significant difference.

3.3. Cow's rumination time in 24 hours

To evaluate the effect of heat and humidity of rearing environment on imported purebred cows of Red Angus and Red Brahman, a 24-hour rumination time was recorded. As shown in Table 3, the rumination time between the two groups of cows was not statistically significant difference (P>0.05). Cows burp up, ruminating at noon, about 25%, and at night, about 75%. Thus, purebred Red Angus, Red Brahman cows have ruminant activity mainly in the evening, when the temperature is cool and when the animal is at rest.

Table 3. Cow's rumination time in 24 hours (± SD)

Breed	At noon		At night		Total	
	Minutes	Rate (%)	Minutes	Rate (%)	Minutes	Rate (%)
Angus	130.50±9.54	25.34±1.34	384.25±8.22	74.66±3,8	514±10,5	100
Brahman	127.50±11.90	24.31±2.77	398±23.76	75.69±2.76	525.5±20.27	100

This result is not significantly different from the study of Van Tien Dung *et al.* (2010), in semi-grazing, Angus and Sind crossbred cattle only ruminant at noon, about 20% and evening, about 80%. With the method of complete captivity, cows do not take time to find food, so eating time is shortened, cows rest a lot, rumination takes place both morning 20%, and noon 20%, and evening 60% (Nguyen Ba Trung, 2016). Thus, the purebred Red Angus and Red Brahman cattle initially had normal ruminant activities in the semi-grazing habitat in Dong Thap province.

Table 4. Regression between physiological parameters of cows and THI (± SD)

	Variable	7 o'clock	10 o'clock	13 o'clock	16 o'clock	19 o'clock
THI		81.45	80.90	86.0	87.52	86.26
Red Angus	Body temperature (°C)	37.95^{a}±0.41	38.55^{a}±0.60	39.10^{a}±0.42	39.18^{a}±0.48	38.53^{a}±0.53
	Pulse rate (times/minute)	67.90^{a}±1.13	69.25^{a}±1.70	70.00±1.83	69.50±1.29	67.25±2.50
	Respiration (times/minute)	57.50±4.12	59.05±2.65	62.00±4.08	59.75±2.06	58.25^{a}±0.96
Red Brahman	Body temperature (°C)	36.70^{b}±0.43	37.08 ^{b}±0.36	37.58^{b}±0.39	37.68^{b}±0.48	36.78^{b}±0.74
	Pulse rate(times/minute)	64.50^{b}±0.58	66.25^{b}±0.96	68.75±0.96	68.00±0.82	65.00±1.41
	Respiration (times/minute)	55.6±1.45	57.2±0.91	59.13±1.03	56.63±1.38	55.25^{b}±1.26

3.4. Regression between physiological parameters of cows and THI

The effects of atmosphere temperature, air humidity and their correlation at the change of sunny to rainy seasons on body temperature, pulse rate and respiration rate of purebred Red Angus and Red Brahman cows were studied from March to April in Tan Hong district, Dong Thap province. As a result, the THI ranged from 80.9 to 87.52, Red Angus cows had higher body temperature and pulse rate than Red Brahman, which was statistically significant difference, at most of the time measured in 24hrs (Table 4). However, between the two cow breeds, respiration rate was completely unchanged during 24hrs. Demonstrating a cool grazing regime in the morning at 7 o'clock, returning to the barn to rest at noon, and continuing to go to the pasture in the afternoon when the temperature was reduced at 14 o'clock; the combination of cool barn, shady trees, covered ponds and lakes helps the cows somewhat quickly adapt to the hot and humid environment.

During periods of extreme heat stress mortality can even occur. Through proper management, the effects of heat stress can be reduced, ultimately improving animal performance (Dennis, 2021).

Mader *et al.* (2006) revealed that a THI of 79-89 would be quite stressful for cows, especially dairy cows. In this study, the THI was very high, ranging from 80.9 to 87.52. Through observations in purebred Red Angus cattle, they did not show obvious heat stress. Compared with the study of Nguyen Ba Trung, (2016), the Red Angus crossbreed calf's respiration rate peaked at 13 hours (32 times/minute) which was statistically different from the Brahman hybrid calf (29 times/minute); Angus calves are breathing heavily, clearly seen in the afternoon, especially in households with poorly ventilated stables and lack of shade trees. The Table 4 clearly demonstrate that physiological indicators such as body temperature, pulse rate and respiration rate of all animals tend to increase gradually from 7.00 to 13 and 16 o'clock, then the trend gradually decreases to the normal state at 19.00. So, at the time of transition of the sunny to rainy season, although purebred Red Angus cows show signs of heat stress, in general both breeds tend to adapt to semi-grazing environments, with good barn conditions, such as the households in Tan Hong, Dong Thap.

The graph shows the relationship between cow physiology such as body temperature, pulse rate, respiration rate and THI has a positive correlation, the larger the THI value, the higher body temperature, pulse rate, and respiration rate. The results of correlation analysis and determination of linear regression equations between cow physiological parameters and THI were shown in Table 5. These equations have not high coefficients of determination (R^2<75%) and were not reliable (P>0.05). Therefore, although the high THI, from 80.9 to 87.52 caused heat stress in Red Angus cows, only at the level of increased on body temperature, pulse rate; while respiration rate was not significantly affected in both breeds, thanks to airy barn.

Table 5. The regression equations between physiological parameters and THI (± SD)

Variable	The regression equations		Results of statistics			
			Red Angus		Red Brahman	
	Red Angus	Red Brahman	P	R^2 (%)	P	R^2 (%)
Body temperature (°C)	Y = 0.124x + 28.16	Y = 0.087x + 29.8	0.14	56.9	0.29	34.88
Pulse rate (times/minute)	Y = 0.063x + 63.5	Y = 0.33x + 38.3	0.79	2.7	0.34	30.08
Respiration rate (times/minute)	Y = 0.271x+ 36.4	Y = 0.07x + 51.2	0.42	22.7	0.83	1.71

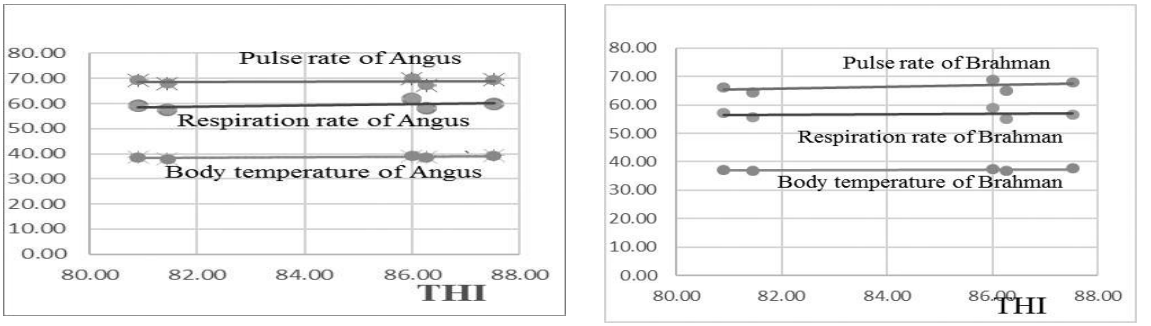

Figure 5. Linear regression correlation between physiological parameters of cows and THI

4. CONCLUSION

Spring-Summer season, change from sun to rain, March-April, Red Angus and Red Brahman purebred cows can adapt to the environment of small farms. However, it should pay attention to ventilation and coolness when raising these breeds.

Dong Thap can import purebred cows of Red Angus and Red Brahman.

The THI ranged from 80.9 to 87.52 without causing significant physiological disturbance in the 2 breeds, but pure Red Angus cows tended to have a statistically significant increase in body temperature and pulse rate throughout 24hrs.

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A STUDY OF CRYOPRESERVATION OF PHUQUOC DOG SPERMS AFTER SEPARATED THROUGH BOVINE SERUM ALBUMIN MEDIUM COLUMN

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ABSTRACT

This study to assess the effectiveness of cryopreservation of Phuquoc dog sperms collected from lower layer after separated bovine serum albumin medium columns. The sex separation method of dog sperms was conducted with two types of separation columns including the continuous column consisted of one layer of 10,5% bovine serum albumin meidum and the discontinuous column consisted of two layers of 15% bovine serum albumin in concentration in lower layer and 6% bovine serum albumin in concentration in upper layer. The result showed the separated effectiveness in discontinuous column better than in continuous column based on evaluating sperm concentration and motility of the separated sperm samples. The cryopreservation effectiveness of the collected sperms from separation through discontinuous column was conducted in two cryo-volume with the result that thawed sperm motility of the cryopreserved sperm samples in 5ml cryo-tubes was 55.3±0.6% higher than 42.1±0.3% in 0.5ml cryo-straws. The ratio of F-body bearing sperms (Y bearing chromosome sperms) of the cryopreserved samples was 66.9±0.1%.

Key words: *Phuquoc Dog, Cryopreservation, Bovine Serum Albumin Medium Column, Sperm Gender Separation.*

1. INTRODUCTION

Over the world, gender pre-selection by several sperm sex separation techniques is very useful in breeding, studying and producing. The selection of new generation's sex has been a quest of couples for a very long time. Since the 1600's scientific efforts were made to sway the chances of having a chosen sex by a variety of methods achieving pregnancy (Ericsson *et al.*, 1973; Dmowski *et al.*, 1979; Hoppe and Koo, 1984; Iizuka *et al.*, 1987; Johnson *et al.*, 1989; 1992; Beernink *et al.*, 1993; Windsor *et al.*, 1993; Samura *et al.*, 1997; Chen *et al.*, 1997; Mohammad *et al.*, 2007; Chaudhary *et al.*, 2014; You *et al.*, 2017). The ratio of male:female births have been reported to be slightly in favour of males, namely 106 males for every 100 females. The basis of this imbalance is unclear, but theoretical possibilities include unequal numbers of X and Y chromosome bearing spermatozoa in an ejaculate and spermatozoa selection during fertilization. Reproductive biologists have attempted to develop accurate methods of safely separating spermatozoa to allow only those spermatozoa capable of producing the desired sex to be exposed to the female egg. While a variety of methods have been reported and studied, in reality, very few of these methods had withstood scientific scrutiny.

Sperm sex separation to select gender in new generations in pig is a new approach and techniques to support some specially purposes. The most technically effective techniques in sperm sex separation at the present is sorting sperm via flow cytometry sorter. But disadvantage to limit application of this technique in fact production is complicated handling, expensive machine system, low productivity, low quality and low fertilization rate of sorted sperms. Also some authors reported that sperm sex separation method by using bovine serum albumin concentration gradient medium column is one of feasible, simple and economic techniques. The

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biological albumin separation method requires that the spermatozoa are placed on the top of an albumin gradient media. Differential separation of X and Y spermatozoa during centrifugation depends on their sedimentation velocities. The X chromosome bearing spermatozoa contains 2-8% more DNA than the Y chromosome bearing spermatozoa. Theoretically this should result in Y bearing spermatozoa being lighter and having greater swimming ability than the X bearing spermatozoa, which is believed to be heavier. The use of an albumin separation method in patients on clomphinecit rate stimulation drugs seems to yield a higher success rate for conceiving a male baby after in vitro fertilisation. This indicates that the albumin separation procedure enrich the proportion of Y chromosome bearing spermatozoa. Ericsson *et al.* (1973) reported that albumin enriches Y bearing spermatozoa by approximately 85%. However, a recent study that attempted to repeat their study, found that the albumin separation method do not enrich Y spermatozoa. In other studies, spermatozoa separation techniques using albumin for selecting Y bearing spermatozoa have been reported to alter the sex ratio to a degree that is clinically relevant.

Currently, artificial insemination techniques have been studied and widely applied in the worldwide in breeding in cattles, pigs and and also in dogs. To have materials supplied for artificial insemination, we must first carry out the preservation process to storage and maintain animal sperms in good quality outside the body. The process includes techniques such as sperm dilution techniques, sperm short-term and long-term liquid preservation techniques and sperm cryopreservation techniques.

Over the years, the most commonly used extenders for semen cryopreservation contain egg yolk (EY) as cryo-protectant (Pugliesi *et al.*, 2012; Stănescu and Bîrțoiu, 2012; Diaz *et al.*, 2013; Şen *et al.*, 2015; Buranaamnuay *et al.*, 2017). Egg yolk contains phospholipids and low density lipoproteins (LDL) which ensures efficient protection of the sperm membrane and nutritional support for these cells. The low-density lipoproteins (LDLs) of EY protect the sperm against damage during storage, cooling, and freezing. Most diluents for canine semen contain 10-20% EY (Silva *et al.*, 2003). Because of complicated processing of EY before added into extender and unpredictable proteins concentration in EY component, sperm survival rates after cryopreservation could be influenced.

The aims of this study were (i) assessing the effectiveness and comparing the effectiveness of dog sperm separation through two types of the bovine serum albumin concentration gradient columns: the continuous column consisted of 1 layer of 10.5% bovine serum albumin in concentration and the discontinuous column consisted of 2 layers of 15% bovine serum albumin in concentration in lower layer and 6% bovine serum albumin in concentration in upper layer; and (ii) assessing the effectiveness of cryopreservation process of sperm population obtained from lower layer of bovine serum albumin concentration gradient columns after separating process.

2. MATERIALS AND METHODS

2.1. Animals and semen collection

6 male dogs belong to Phu Quoc dog breed from 15 to 24 months of age were used in this experiment.

2.2. Experiment design

Experiment 1 - Assessment of dog native semen quality: collecting successfully 6 individual male dogs of Phu Quoc breed with in good state of semen production to collect the good quality semen (semen was collected by digital manipulation each 5 days with replicating 10 times per experiment; all of the semen volume of the ejaculates was collected for the experiments). The native semen samples were assessed volume of semen (ml); sperm motility (%); sperm concentration (billions of sperms/ml) and sperm abnormal morphology (%).

Experiment 2. Assessment of sperm separation efficiency through BSA continuous column and discontinuous column: assessing the effectiveness and comparing the effectiveness of dog sperm separation through two types of the bovine serum albumin concentration gradient columns: the continuous column consisted of 1 layer of 10.5% bovine serum albumin in

concentration and the discontinuous column consisted of 2 layers of 15% bovine serum albumin in concentration in lower layer and 6% bovine serum albumin in concentration in upper layer; exprement replication: 6 males, 6 semen collecting times/dog; comparing the effectiveness of dog sperm separation through two types of the bovine serum albumin concentration gradient columns via comparing obtained results of two sperm parameters including sperm motility (points) and sperm concentration in the obtained sperm layer after separating process; based on the comparation results, chose one of two separating columns which we can obtain higher number of sperm in collected sperm layer after separating process to achieve the experiment 3.

Experiment 3 - Assessment of cryopreservating effeciency and the ratio of F-body bearing sperms of selected sperms after separating process: carrying out the sperm cryopreservation process with the separated sperm samples in 0.5ml straws and 5ml cryo-tubes via quickly cryopreservation procedure and also with the corresponding native semen samples as the control samples; comparing the 30th cryopreserved day cryo-sperm motility thawed at 37°C; each sperm sample collected from separating procedure through the bovine serum albumin concentration gradient column are centrifugated, sperm pellets are dilluted into cryo-extender then pipeted into five 0.5ml cryo-straws and five 5ml cryo-tubes. Then sperm samples are put into the quick sperm cryopreservation procedure according to Zorinkimi *et al.* (2017).

2.3. Methods

2.3.1. Methods in experiment 1

Dog semen collecting method: Applying the massage method according to Thomassen *et al.* (2009). Validating god semen quality parameters including semen volume, sperm concentration, sperm motility, abnormal morphology sperm according to Thomassen *et al.* (2009).

2.3.2. Methods in experiment 2

Methods to separating dog sperms through bovine serum albumin medium column according to Ericsson *et al.* (1973): (i) preparing Tyrode medium, (ii) preparing 6, 10.5 and 15% bovine serum albumin medium, (iii) preaparing discontinuous bovine serum albumin medium column (6 and 15% bovine serum albumin medium) and (iv) preaparing continuous bovine serum albumin medium column (10.5% bovine serum albumin medium).

2.3.3. Methods in experiment 3

Method to detect F-body of Y chromosome bearing sperms using quinacrine dye according to Ogawa *et al.* (1988). Dog semen freezing procedure according to Zorinkimi *et al.* (2017): Semen samples were centrifuged at 600-700g for 10min (Schäfer-Somi *et al.,* 2006). Purpose of the centrifugation was to remove the excess of prostatic fluid which has a negative effect on motility and vitality of frozen spermatozoa after thawing (Sirivaidyapong *et al.,* 2001; Rota *et al.,* 2001). The supernatants were discarded and the sperm pellets were diluted and mixed well in cryo-media then sucked into four 0,5ml cryo-straws per each cryo-medium and 5ml cryo-tubes. The semen straws were incubated for 1hr at room temperature with light avoidance then continuously incubated at 5°C for 2hrs. The straws were placed 4cm above liquid nitrogen for 20min then dipped in liquid nitrogen for second 20min. Finally, samples were stored in liquid nitrogen tanks (-196°C). After 30 days of storage time in liquid nitrogen tanks, frozen semen samples were thawed at 37°C for 1min then evaluated sperm motility and abnormal sperm morphology.

2.3.4. Statistical analysis

Data was statistically analysed using Excel program and SAS software version 9.1 with P-value 0.05.

3. RESULTS AND DISSCUSION

3.1. Results of vaidating dog semen quality parameters

The quality of native semen decides effectiveness of any sperm treatment procedure and effectiveness of fertilization rate using treated sperms. After evaluation, check semen quality may allow continued use of frozen or be eliminated. The parameters evaluated to assess the quality of native semen including semen

color, semen volume, the pH value of semen, sperm concentration, sperm motility, sperm abnormal morphology. The test results of quality parameters of native semen samples 3 individual Phuquoc male dogs are presented in Table 1.

Table 1. Quality parameters of the native semen samples collected from 6 individual Phuquoc dogs (n=36)

Native semen quality parameters	Mean±SD
Color of semen	Milky-white
Semen volume (ml)	7.3±0.8
pH value of semen	6.4±0.2
Sperm concentration (millions/ml)	218.6±3.7
Sperm motility (%)	82.3±3.2
Abnormal morphology sperm rate (%)	15.1±1.2

About sperm concentration, compared with the results of Rensselaer *et al.* (2002) showed that the male dogs in this study achieved sperm concentration parameter was very good. According to Batista *et al.* (2010), sperm progressive motility must achieve at least 70% to be enough to used in sperm freezing experimen, then the average of sperm motility is relatively high, reached 82.3% and also higher than the results obtained by Rijsselaere *et al.* (2002). The average of abnormal morphology sperm rate obtained from 6 experiment male dogs were 15.1±1.2%, compared with the results of Rijsselaere *et al.* (2002), was slightly higher but still within the permitted range of technical requirements for sperm quality of dog for artificial insemination.

3.2. Quality parameters of dog sperms after separated through bovine serum albumin medium column

To separate sperms in order to obtain layers containing high percentage of sperm bearing desired sex chromosome to serve manufacturing, firstly the sperms needed to be ensure the certain quantity and good quality to reach the requirements to success of fertilization process. There are some parameters for evaluating sperm quality as the integrity of the plasma membrane, sperm agglutination level, total of progressive sperms, sperm concentration, sperm morphology... In this experiment, evaluated two parameters are sperm concentration and sperm motility. In which, from sperm concentration of obtained dog sperms after separated through two types of BSA concentration gradient medium columns we can comparing the total number of separated sperms to initial sperm numbers then calculate the productivity of separating process and predict the sex sperm separating efficiency. Of course, to know the exact F-body bearing sperm ratio or X/Y chromosome bearing sperm ratio in any sperm population we have to perform fluorescent staining method.

The results (Table 2) showed that the sperm motility values of obtained dog sperms from lower layer after separated are different statistical significantly between two types of bovine serum albumin medium column ($P<0.05$). The average sperm motility of the sperm population collected from the lower layer of discontinuous bovine serum albumin medium column is 92.3±2% is higher than that of continuous bovine serum albumin medium column (81.1±3%). This result is consistent with research by Ericsson (1973) on human sperms. The reason is: in the continuous bovine serum albumin medium column, dog sperms from the initial medium (Tyrode solution with a little amount of bovine serum albumin 2g/l mixed with semen in 1:1 ratio) immediately swimed down to 10,5% bovine serum albumin in the upper layer of bovine serum albumin medium (means that 10.5g/l bovine serum albumin) where the viscocity is much higher than in initial solution. This takes more energy for sperms to swimed down continuously. It is different in discontinuous bovine serum albumin medium column, where sperms have times to adapt to the new environment with the not too high viscocity in the upper layer of 6% bovine serum albumin medium.

Table 2. Sperm progressive motility after separated through bovine serum albumin medium columns

Types of columns	Layer 1 (upper layer)	Layer 2 (lower layer)
Continuous column (10.5% BSA)	73.6±3^{a}	81.1±3^{a}
Discontinuous column (6 and 15% BSA)	86.2±2^{b}	92.3±2^{b}

Note: on the same column, difference of upper letters indicates significant difference ($P<0.05$) between treatments

As showed on Table 3, sperm concentration in the lower layer of the bovine serum albumin medium column after separating was 4.4±2.4 millions of sperms/ml is too low, if appling this method to proceed in the fact production will be required to use the large amount of chemicals and tools, more sperm leading to low production efficiency. For the lower layer collected from the bovine serum albumin medium column after separation, sperm concentration was 9.7±4.0 millions of sperms/ml, higher than collected from lower layer continuous column. In experiment 2, we used 1ml native semen mixed with 1ml of Tyrode (ratio 1: 1). The collected sperms from the lower layer after separation of the discontinuous bovine serum albumin medium column were in 4 ml in volume. Means that from 1ml native semen we obtain 4ml in volume of separated sperm containing medium. With the collected sperm concentrations as shown in table 3, in the continuous bovine serum albumin medium column, after separation we had 4ml x 4,4 millions of sperms = 17.6 millions of separated sperms in total. Means that we have to perform 6 columns using 6ml of native semen (half of sperm rich volume collected each ejaculation) to produce 1 dose of separated sperms to use for AI once (100 millions/dose according to Thomassen *et al.*, 2009). In contrast, in the discontinuous bovine serum albumin medium column, with the collected sperm concentrations was 9.7 millions of sperms/ml, means we had 4ml x 9.7 millions of sperms = 38.8 millions of separated sperms in total. That means we just have to perform 3 columns using 3ml of native semen to produce 1 dose of separated sperms to use for AI once. Hence, separation effectiveness through discontinuous bovine serum albumin medium column is higher. Therefore, we can conclude that using the bovine serum albumin medium column process was more suitable because of the higher collected sperm amount with high motility, more stability and the implementation process was not too complicated to be applied to fact production. Simultaneously, we chose the discontinuous bovine serum albumin medium column consisted of 2 layers of 15% bovine serum albumin in concentration in lower layer and 6% bovine serum albumin in concentration in upper layer to perform the experiment 3.

Table 3. Sperm concentration (millions of sperms/ml) **of collected dog sperms after separated through two types of bovine serum albumin medium columns** (n=36)

Types of columns	Layer 1 (upper layer)	Layer 2 (lower layer)
Continuous column (10.5% BSA)	31.8±7.4[a]	4.4±2.4[a]
Discontinuous column (6 and 15% BSA)	23.0±6.7[b]	9.7±4.0[b]

3.3. Assessing the cryopreservation effectiveness and the F-body ratio of collected sperms after separated through bovine serum albumin medium column

By performing the experiment 2 again using the discontinuous bovine serum albumin medium column to collect sperms to perform the cryopreservation experiment. After separation, we conduct the sperm cryopreservation procedure following the protcol of Zorinkimi *et al.* (2017) in 2 types of cryo-volume: 0,5ml cryo-straws and 5ml cryo-tubes. After storage in liquid nitrogen for 30 days, straws and tubes are thawed following two methods: method 1 - incubation at 37°C for 1min for 0,5ml cryo-straws and method 2 - incubation at 37°C for 5min for 5ml cryo-tubes. The thawed sperms are evaluated their motility and stained with quinacrine in procedure described in chapter 3 to assess the quality of cryo-sperm samples and sex separating effectiveness through discontinuous bovine serum albumin medium column. The cryopreservation and thawing procedure are also performed with the corresponding native semen samples to be used as control samples.

The sperm progressive motility after thawing of the cryopreserved samples from separated sperms in 5ml cryo-tubes was 55.3±0.6%, is higher than in 0.5ml cryo-straws (42.1±0.3%). This result is very valuable to support for the whole techniques experimented in this thesis to apply to fact manufacture because in as larger volume means that sperms supplied more nutrient and protectants from cryopreservation medium as better quality of cryopreserved sperms. The results also showed that sperm progressive motility after thawing of the cryopreserved samples from separated

sperms is lower than control in the same volume. This shows that, pressure of separating process affects to sperm quality and then reduces sperm motility after thawing of the cryopreserved samples from separated sperms.

To assess the effetiveness of any sperm sex separation method in any mammalia animal in general and particularly in dog first of all we have to evaluate the X/Y chromosome bearing sperm ratio before test new generation's sex ratio *in vivo*. There are some methods applied in the world to perform this, the first method of which was applied as Giemsa staining to determine sex chromosomes in cells but just available to soma cells. Some others are more sensitive and more specific but not economic. So, in this experiment, quinacrine staining method is used to determine the X/Y chromosome bearing sperm ratio. The quinacrine dye is not so expensive. The staining procedure is not so complicate to specifically stain the F-body of the Y chromosome bearing sperms and expose them with the brighten dot in the head of sperm when observed on the fluorescent microscope. The results of determining and comparing the F-body bearing sperm ratio between cryopreserved samples of cryopreserved samples of collected sperms were 66.9±0.1%. It can be concluded that the sperm sex separation process by discontinuous BSA concentration gradient medium column help to enrich Y chromosome bearing sperms in separated layer, adapted to sperm quality parameters as sperm motility and concentration in artificial insemination. The ratio of F-body bearing sperms (Y bearing chromosome sperms) of the cryopreserved samples of collected sperms is approximately 66.9%. According to Ericsson *et al.* (1973), human sperm sex separation in a concentration of 6% bovine serum albumin gaved the percentage of 66% Y chromosome bearing sperms. Thus, following to the the results of this experiment are presented as previous part, with bovine serum albumin concentration of 15% obtained the percentage of F-body bearing sperm is approximately 66.9%. As the results above, the separated sperm samples completely adapt to requirements to artificial insemination.

4. CONCLUSION

(i) The sperm dog sex separation method through bovine serum albumin medium column was conducted successfully. Based on tested in two types of separation columns including the continuous column consisted of 1 layer of 10.5% bovine serum albumin in concentration and the discontinuous column consisted of 2 layers of 15% bovine serum albumin in concentration in lower layer and 6% bovine serum albumin in concentration in upper layer, the result showed the separated effectiveness in discontinuous column better than in continuous column based on evaluating sperm concentration and motility of the separated sperm samples. (ii) The cryopreservation effectiveness of the collected sperms from separation through discontinuous column was conducted successfully in two cryo-volume with the result that thawed sperm motility of the cryopreserved sperm samples in 5ml cryo-tubes was 55.3±0.6% higher than 42.1±0.3 in 0.5ml cryo-straws; the ratio of F-body bearing sperms (Y-bearing chromosome sperms) of the cryopreserved samples of collected sperms was 66.9±0.1%.

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THE RESEARCH RESULTS ABOUT BEEF CATTLE AT RUMINANT RESEARCH AND DEVELOPMENT CENTER

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1. HISTORY AND MAIN ACTIVITY OF RUMINANT RESEARCH & DEVELOPMENT CENTER[1]

Ruminant Reasearch and Development Center (RRDC) had been Song Be Buffalo and Forage Research Center when it was established under Decision No.211-NN-TCCB/QĐ dated July 20, 1977. The Center had belonged National Institute of Animal Sciences with functions of Research on adaptation and domestication, crossbreeding Murrah buffalo, research on adaptation of imported grass groups, establishing on pasture, inspecializing with grass or intercropping to feed buffaloes, calves and grass seed. On April 8, 1994 Minister of Agriculture and Food Industy signed the Decision No.204-NN-TCCB/QĐ which changed from Song Be Buffalo and Forage Research Center to Song Be RRDC and added more functions. Beside functions at Decision No.211-NN-TCCB/QĐ dated July 20, 1977, the Center had more functions such as research on beef cattle, dairy cattle, meat and dairy goat and more investment such as infrastructure, research, transferring technology, beef cattle. Research results about beef cattle of RRDC from 1994 to present are below:

From 1994 to present, the Center has been carrying out on beef cattle: Research on imported, crossbred of beef cattle, dairy cattle in tropical condition of Vietnam (State branch theme 1997-1999); Research on development of beef cattle in the Southern provinces by 3-4 breed rotation crossbreeding system (Ministerial theme, 2000-2001); Research on selection and crossbred to improve beef production in Vietnam (Ministerial theme, 2002-2005); Research on high quality pure and cross-bred beef cattle in Vietnam (Ministerial branch theme, 2006-2010); Research on feeding condition to improve productivity and quality of Brahman (Br) cattle at RRDC (base theme, 2016); Research on adaptation of imported pure Red Angus (RA) cattle (base theme, 2017-2018); Evaluation of productivity and economic efficiency of crossbred between BBB and Wagyu with Zebu crossbred cows (base theme, 2019-2022); Research on the growth performance of the first generation RA calves at RRDC (base theme, 2021). Evaluation on productivity, quality and economic efficiency of crossbred between RA, Droughtmaster (DrM), Charolais (Cha) bulls with crossbred Br and Br cows (Ministerial branch theme, 2018-2022); Research on crossbred beef cattle between Senepol with crossbred Zebu and Br cows (Ministerial branch theme, 2020-2024).

2. RESEARCHING RESUTS ON CROSSBRED BEEF CATTLE

2.1. Fertility of Lai Sind cows

In 1997-2000, the average conception rate of Lai Sind (LS) cows at first insemination was 63.75%. It was 75% in using LS bulls and 55-65% in using Cha, Hereford (Her), Simmental (Sim) semen. Percentage of cows calving to cows pregnant was 98.6% (Pham Van Quyen *et al*., 2001).

In 2000-2007, the average conception rate of LS cows at first insemination with LS bulls was 76%. The conception rate at first insemination among groups of LS cows with artificial insemination was 52.94-56.00%. The conception rate of three inseminations with DrM, Br, and Cha at Center was 92.00, 89.87, 100%, respectively. The number of semen per concept was 1.65-1.86 (Dinh Van Cai and Pham Van Quyen, 2007).

Gestation length of LS cows at RRDC, at farmers and the average was 276.24-282.15, 281.65-282.94 days and 281.71-282.36 days

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respectively. There was no significant effect (P>0.05) of foreign semen breed for gestation length (Pham Van Quyen *et al*., 2001; Dinh Van Cai and Pham Van Quyen, 2009).

2.2. Appearance of diferent first crossbred calves

2.2.1. F_1(Cha x LS) calves

F_1 Cha calves had short head, deep chest, long body, wide and flat back, round butt, big and medium heigh thighs, wheaten in hair colour (92%) and creamy white in hair colour (8%), thick, long and slightly curly hair. Forehead hair was long and curly. They had white eyes, pinkish nose, pinkish edges of eyes.

2.2.2. F_1(Her x LS) calves

The calves had short head, wide shoulder, straight and wipe back, long and wipe butt, wide chest, short leg. Most of them (78%) had brown or light yellow with white in forehead, dewlap, underline and tail. The remaining amount (28%) had a striped black with pale yellow and they had white in forehead, dewlap, underline and tail. Nose and eyes were pink.

2.2.3. F_1(Sim x LS) calves

The calves had wipe and light short head, deep and wipe chest. As the crossbred Sind, their colour was dark brown (84%) or yellow brown (16%). They had black eyes and eyeliner.

2.2.4. F_1(DrM x LS) calves

The calves had small hump, long neck, medium ears, large bib and navel. They had dark yellow or brown red colour, pink eyeliner and pink nose but some on them had black eyeliner and black nose.

2.2.5. F_1(Br x LS) calves

The calves had hump, long neck, large and pendulous ears, deep chest, flat back, long leg, large bib under neck. They had brown red colour but some of them had white patches under neck and bib. They had black nose and black toenail.

2.2.6. The withers height, heart girth, and body length of F_1 crossbred calves

The withers height, heart girth, and body length of crossbred calves from birth to 24 months were different but the rate of increase was similar. Withers height, and body length increased fastly from birth to 9 months and increased slowly from 9 month to 24 months. Heart girth increased fastly from 12 to 24 months. The size of F_1 Cha, F_1 Her, F_1 Sim, F_1 DrM and F_1 Br crossbred calve were bigger than LS calves from birth to 18 and 24 months.

The dimension of F_1 Cha, F_1 Her, F_1 Sim and LS calves at 18 months was 112.13, 120.25, 106.27, 104.50cm in withers height; 152.06, 149.94, 146.47, 144.06cm in heart girth; 123.06, 120.13, 117.20, 115.19cm in body length, respectively (Pham Van Quyen *et al.*, 2001).

The size of F_1 Cha, F_1 DrM, F_1 Br and LS calves at 24 month was 143.55, 134.59, 126.51, 117.44cm in withers height respectively; 180.02, 175.06, 163.70, 155.87cm in heart girth respectively; 151.02, 144.98, 136.61, 130.68cm in body length, respectively (Dinh Van Cai and Pham Van Quyen, 2009).

2.3. The growth performance of crossbred calves

The results from 1997-2000 showed that at 18 months, the average weight of male and female was the highets in F_1 Cha (308.81kg), following in F_1 Her (291.63kg), in F_1 Sim (220.27kg) and the lowest in LS calves (205.50kg), (P<0.01). At 12 months, if the weight of LS calves was taken as 100%, the weight of F_1 Cha calves was 132.54%, F_1 Her was 124.63%, F_1 Sim was 121.38%. At 18 months, the weight of F_1 Cha calves (150.27%), F_1 Cha calves (141.91%), F_1 Sim calves (107.19%) compared with the weight of LS calves (100%) determined by Pham Van Quyen *et al*. (2001). The average daily gain (ADG) of crossbred calves groups was diffirent between heifer and steer in each period but ADG from birth to 18 month was the highest in F_1 Cha (533.31 g/head/day), F_1 Her (500.95 g/head/day), F_1 Sim (370.37 g/head/day) and the lowest in LS (344.79 g/head/day).

The research results from 2000-2007 showed that: at 24 months, the average weight was the highest in F_1 Cha calves (394.95kg), then F_1 DrM calves (355.81kg), F_1 Br calves (318.09kg) and the lowest in LS calves (276.50kg), (P<0.05). The ADG from birth to 12 month of crossbred male anh femal calves was 425.22-653.25 g/head/day, from 13-24 month period was 304.44-379.86 g/head/day, from birth to 24 month period was

364.83-516.56 g/head/day. The gain weight was the highest in F_1 Cha calves, following F_1 DrM and the lowest in LS calves. The ADG from birth to 24 months of steer was 1.14 times greater than that that of F_1 DrM, 1.06 times of F_1 Br, 1.05 times of F_1 Cha and LS), 1.08 times of all groups. At 12 months, if the weight of LS calves was taken as 100%, the weight of F_1 Cha calves was 154.70%, of F_1 DrM was 148.59%, of F_1 Br was 117.56%. At 24 months, the weight of F_1 Cha calves (142.84%), F_1 DrM (128.68%), F_1 Br (115.02%) determined by Đinh Van Cai and Pham Van Quyen (2009).

2.4. Physiological parameters of F_1 DrM

Body physiological parameters at 12-13h in dry season with 30-33^{0}C temperature and 64-70% humidity, respiration rate of F_1 DrM cattle was 36.81 breaths/min that was similar to crossbred cattle other and LS cattle (36.60 breaths/min). Rectal temperature of F_1 DrM cattle was 38.72^{0}C. In general, physiological parameters of crossbred cattle were normal so that F_1 DrM cattle was not affected by heat stress in the hot and humid of Sourthern Vietnam. Heamatological blood parameters: Blood composition of F_1 DrM cattle and the other crossbred cattle was normal: RBC 5.87x10^{6}mm^3, Hemoglobin 9.75%, Hematocrit 26.54%, WBC 11.34x10^{3}mm^3 (Dinh Van Cai and Pham Van Quyen, 2009).

2.5. Tick infectation

The research results from 1997 to 2000 showed the rate of tick infection of crossberd calves from 0-18 months was 30-42%. It was the highest in Her crossberd calves (41.46%), next Cha crossberd calves (39.02%), Sim crossberd calves (35%), and the lowest in LS calves (30.43%). The average number of ticks on calf was 29 ticks (Pham Van Quyen *et al*., 2001).

The research results from 2000-2007 showed the rate of tick infection of crossberd calves from 0-24 months was 26.67-38.24%. It was the highest in Cha crossberd calves (38.24%), next Br crossberd calves (28.81%), LS calves (27.78%), and the lowest in Droghtmaster crossberd calves (26.67%). The average number of ticks on a calf of F_1 DrM, F_1 Br, F_1 Cha, Sind crossbred was 13.43; 15.20; 19.83, and 13.85 sticks respectively (Dinh Van Cai and Pham Van Quyen, 2007).

2.6. Intestinal parasite infection

Crossberd calves at RRDC were infected *Neoascaris vituloum*, *Hemonchus* sp., *Strongyloides parallorus*, *Oesophagostomum* sp., *Monieza* sp., and *Coscidia* sp. The rate of intestinal parasite infection of crossbred calves from 0-18 months was the highest in *Neoascaris vituloum, Hemonchus* sp., and *Coscidia* sp. The rate of infection *Neoascaris vituloum* was the highest in Cha crossbred calves (34.15%), the lowest in LS calves (21.74%). The rate of infection *Hemonchus* sp was not different between crossbred calves groups (27.50-34.15%). The rate of infection *Coscidia* sp. was the highest in Cha crossbred calves (31.71%) and the lowest in Her crossbred calves (26.83%) (Pham Van Quyen *et al*., 2001).

The rate of infection *Neoascaris vituloum, Monieza* sp. and *Coscidia* sp. tended to decrease with age. The reason was that in 0-6 month stage, calves suckled and grazed so that they could contact to pathogens on the floor, in the soil, in the manure. Futhermore, in this stage the resistance to pathogens of crossbred calves was not high so the infection rate was high.

2.7. Survival rate of crossbred calves

From 1997-2000, the average of survival rate of crossbred calves 0-18 months was 90.14% (88.24-94.12%) (Pham Van Quyen *et al*., 2001). From 2000-2007, the survival rate of crossbred calves from 2-3 months was 92.86-100.00%. The survival rate of crossbred calves from 4-24 months was 100% (Dinh Van Cai and Pham Van Quyen, 2007).

2.8. Meat production

In 1997-2000, the research results of Pham Van Quyen *et al*. on nonfattened F_1 cattle showed that the carcass percentage, lean meat percentage, and rib-eye area were highest in Cha crossbred, following Her crossbred, Sim crossbred, and the lowest in LS cattle. At 18 months, the carcass percentage was 56.32% for Cha crossbred, 54.74% for Her crossbred, 48.28% for Sim crossbred and 44.62% for LS. The lean meat percentage was 44.83, 43.23, 38.40 and 35.60%, respectively.

In 2000-2007, the research results of Dinh Van Cai and Pham Van Quyen on fattened F_1 crossbred cattle showed that the carcass

percentage, lean meat percentage, and rib-eye area were the highest in F_1 Cha, then DrM, F_1 DrM, Br crossbred cattle and the lowest in LS cattle. The carcass percentage was 53.13% for F_1 Cha, 53.06% for DrM, 50.76% for F_1 DrM, 49,06 for F_1 Br, and 46.78% for LS. The lean meat percentage was 42.96% for F_1 Cha, 42.71% for DrM was higher than F_1 DrM (40.96%), F_1 Br (39.95%) and LS (37.44%).

2.9. Chemical composition of meat

In 1997-2000, the research results on nonfattened F_1 crossbred cattle showed that chemical composition of meat was not different between crossbred cattle groups. The average percentage composition was 77.21-78.10 in water, 20.00-20.35 in protein, 0.70-0.85 in lipid (Pham Van Quyen *et al*., 2001).

In 2000-2007, the research results on fattened F_1 cattle showed that chemical composition of cattle meat was different not much between crossbred cattle groups. Water percentage was 71.55-72.50; protein percentage was from 20.20 (LS) to 22.45 (F_1 Cha) according to the meat sample in its natural state. Lipid percentage in LS meat (6.22) was higher than other crossbred meat (DrM 4.26, F_1 DrM 4.11, F_1 Br 4.98, F_1 Cha 6.22) (Dinh Van Cai and Pham Van Quyen, 2009).

It, therefore, the F_1 crossbred cattle between LS dam with Cha, Her, Sim, DrM, Br sire grew well. The weight, gain weight, and meat production were higher than LS cattle.

3. PUREBRED BEEF CATTLE

3.1. DrM cattle

3.1.1. Appearance and growth pẻomance

DrM cattle had brown red to dark yellow colour, low hump, long bib, roud body, deep chest, short leg, deep eyes, obvious muscle. They had brown nose, brown eye-mucosa, sparse and short hairs. The hump in bull was higher than in cow that it was dark red in some one. Cows had fairly developed udders, large nipples, and vulva with many wrinkles.

Withers height, heart girth, and body length of imported DrM at 24 months were 138.19cm, 172.86cm, and 148.69cm, respectively. The size of male calves which were born from imported DrM was higher than female. At 24 months, the average of withers height, heart girth, and body length were 138.73, 177.14 and 149.77cm, respectively. The size of female calves was lower than their mother as the same age. The size of Droughmaster cows was higher than LS cows, F_1 Droughmaster, F_1 Br but it was similar to F_1 Cha (Dinh Van Cai and Pham Van Quyen, 2009).

The average weight of imported DrM at 24 months was 338.64kg. The weight at first insemination was 330-340kg (22-23 months). The weight at first calving was 380-420kg (32 months). The average weight of cow was 452.25kg in 46 months of the second calving. The gain weight of DrM was good in 15-18 month stage but it was slow in 19-24 months of age. The gain weight in 25-32 month stage was higher than in 19-24 months of age because cow had pregnant. The gain weight in 33-46 month sate was slow. The gain weight in 15-24 and in 24-46 month of age was 217.33 and 172.14 g/head/day, respectively (Dinh Van Cai and Pham Van Quyen, 2009).

The birth weight of DrM calves from their imported mother was 23.73kg, 12 months was 236.14kg, 24 months was 376.28kg. The weight of female was lower than their mother as the same age. The gain weight was fast from 0-6 months of age. The ADG from birth to 12 months was 590.03 g/head/day, but was slowly in 13-24 month stage (389.28 g/head/day). The ADG from birth to 24 months was 489.65 g/head/day.

Growth rate and weight of DrM calves was higher than LS calves with the same feeding regime in RRDC. If the weight of LS calves was taken as 100%, the weight of F_1 Cha was 141.49% at 12 months, and 136.09% at 24 months determined by Dinh Van Cai and Pham Van Quyen (2009).

3.1.2. Reproduction of imported

The average first age of insemination of heifer in Ben Cat was 22.17 months. The number of semen per conception was 1.59 straws. The percentage pregnant of first insemination was 62.30. The percentage pregnant of first three inseminations was 96.10. Phi Nhu Lieu (2009) reported that the age of first heat, age of first calving of DrM at RRDC were 22.86 months and 33.71 months respectively. The interval

from calving to insemination was 144.22 days. Calving interval was 421.24 days that was higher than calving interval of DrM in Australia. The heat duration of DrM was 21.58hrs (heifer), 22.76hrs (cow). The duration of standing heat was 8.91-9.33hrs. There were some signs of heat: mucus discharge (93.94%), mouting other cows (96.97%), standing to be mouted (100%), and decreased feed intake (45.45%). The gestation length was 284.03-283.46 days. The interval from calving to insemination was 118.5 days and calving interval was 421.24 days (Dinh Van Cai and Pham Van Quyen, 2009).

The best time to breed was from 4hr of duration of stading heat to 12hr after duration of stading heat that percentage of pregnant was 63.64-54.55%. At the same breed, percentage of pregnant in cows (71.43) was higher than in heifer (50%). Progesteron quantitative ELISA technique could be used to identify early non-pregnant cows 21 days afer insemination. The correct diagnosis rate for non-pregnant cows was 100% and for pregnant cows was 76%.

3.1.3. Body physiological parameters, tick infectation, popular diseases, culling and survival rate

Body physiological parameters at 12-13h in dry season with 30-33^{0}C and 64-70% humidity, respiration rate of DrM was 42.75-49.80 breaths/min that it was higher than LS (36.60 breaths/min) but it was lower than Holstein Friesian (78.3 breaths/min). Rectal temperature of DrM was 38.50-38.54^{0}C. So that DrM was not affected by heat stress in the hot and humid of Sourtheast (Dinh Van Cai and Pham Van Quyen, 2009).

The rate of tick infection of DrM was 28.17-36.6%. The average number of ticks on cattle was 15.28-18.24 ticks. The rate of tick infection and the average number of ticks of DrM were higher than LS in the same feeding regime (Dinh Van Cai and Pham Van Quyen, 2009).

Test results for imported Droughmaster showed that they were not infected with spirochete disease, infectious causes of abortion, blood parasitic disease, bluetongue disease. DrM cattle had low in percentage of disease, well in growth, low culling rate. There were some common diseases: Diarrhea, umbilical abcess with 7.99 and 3.48% per month, respectively. The culling rate of cows for 3 years was 10% (Dinh Van Cai and Pham Van Quyen, 2009).

The survival rate of calves from 0-3 months was 84.09%. The survival rate of calves from 4-24 month was 100.00% (Dinh Van Cai and Pham Van Quyen, 2009).

It, therefore, DrM cattle could grow well under the climate and husbandry condition of Southeast Vietnam: Physiological parameters were within normal physiological range; proportions of tick infection, routine morbidity and culling were low; passable good reproduction; survival rate was high of calves; the body weight, gain weight per month of age and meat productivity were same with F_1 Cha but higher than F_1 DrM, F_1 Br and LS.

3.2. Br cattle

The resaerch results on purebred Br at RRDC were not much. There was not detail report about growth, reproduction as DrM. Nguyen Ngoc Hai (2016) reported that: The first age of heat of Br that was imported from Australia at RRDC was 23.93 months. The weight of first heat was 286.94kg. The first age of pregnant was 24.7 months and the number insemination per conception was 1.61 times. The interval from calving to insemination was 110 days. The interval from calving to conception was 131.9 day. The calving interval was 417.1 days.

3.3. Red Angus cattle

Red Angus was famous cattle with high carcass yield and nicely marbled meat increasing soft and fat level of meat. RA had the ability to produce quicky meat which meeting requirement of market and a low price. Beside, RA was mature extremely early and high reproduction.

In 2016, RRDC was the first center in Vietnam had imported RA heifers from Australia. The research results from 2016 on adaption, reproduction of imported RA cows and growth of calves showed that:

Hematological and physiological parameters of RA were normal so that they were able to adapt to climatic and feeding condition in Southeast. Age of first insemination and

weight at first insemination were 21.76 months and 345.16 kg respectively. Age of first calving and weight at first calving were 32.26 months and 432.27kg, respectively. Calving interval of RA was 410.38-417.65 days. The number of semination per concept was 1.87 times and gestation length was 281.75 days. Birth weight of RA calves that were born in Vietnam was 24.87kg.

RRDC have been researching on growth and reproduction of next generation RA in Vietnam.

3.4. Senepol cattle

Senepol cattle are a result of crossbreeding between N'Dama cattle from Senegal and Red Poll cattle. The breed was naturally polled, good meat production. Average birth weight of calves was 34kg. The gain weight from 0-6 month was over 1,600 g/head/day. The average weight of adult bulls was 830kg (800-900kg). The average mature weight of cows was 550-650kg (500-700kg). Cows had 2,500kg milk/lactation (270 days), easy calving and vigour calf. The calving interval was 12-14 months. They stayed in production upward 15-20 years. Senepol cattle had excellent immunity to disease and insect resistance. The carcass rate was 60-62%.

In 2021, RRDC imported 40 Senepol from Australia (38 heifers and 2 bulls). First of all, research on adaptation, growth, reproduction of imported Senepol will be studied.

4. ON FEEDLOT CATTLE

The research results on feedlot DrM steer and F_1 DrM steer as the most successfully in RRDC.

4.1. Diet and nutrition of feedlot diet

The nutrition of diet for feedlot period was 2,470-2,494Kcal ME/kg DM and 129.4-136.7g CP/kg DM. The percentage of concentrate was 67-71%.

4.2. Gain weight and feed conversion rate

The ADG in 3 months feedlot was in LS (833.3 g/head/day), F_1 DrM (911.1 g/head/day), DrM (1,037 g/head/day), F_1 Br (1,103.7 g/head/day) and the highest in F_1 Cha (1,148.0 g/head/day).

The FCR on dry matter for was 6.2 kg/kg in F_1 Cha, 6.5 kg/kg in F_1 Br, 7.03 kg/kg in DrM, 7.8 kg/kg in LS, and 8.0 kg/kg in F_1 DrM.

The FCR on metabolic energy was the highest in F_1 DrM (19.8 Mcal/kg) and the lowest in F_1 Cha (15.6 Mcal/kg).

Feed cost in feedlot showed that feed efficiency, ADG, and economic efficience of F_1 Cha and F_1 Br was higher than DrM and F_1 DrM. Feed cost at experiment time was 11,387 VN dong/kg (F_1 Cha), 11,900 dong/kg (F_1 Br), 12,598 dong/kg (DrM), 14,249 dong/kg (LS), and 14,620 dong/kg (F_1 DrM) (Dinh Van Cai and Pham Van Quyen, 2009).

5. FUTURE RESEARCH

5.1. Breeding

In the future, the most important matters will be studied at RRDC, such as:

Researching on purebred, creating new generations of Br, DrM, RA and Senepol.-

Researching on crossbreeding between imported cattle and Zebu crossbred cattle or between imported cattle.

5.2. Nutrition requirement and feedstuff

Researching on nutrition, feeding regim will be suitable for imported cattle and their crossbred cattle. It will research on feeding menthod for reproduction and feedlot to improve the quantity and quality of breed.

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GOAT PRODUCTION IN VIETNAM

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1. INTRODUCTION

Goats (*Capra hircus*) are seen as critical multifunctional animals in socio-economic and ecological terms. Due to their great adaptability to different environmental conditions and versatile diets, goats can be raised in almost all areas, especially in harsh conditions and managed by resource-poor, landless smallhodler farmers. Goats have always been considered beneficial animals because they are easy to handle, have good productivity, and they do not compete with humans for food and can consume cheap feeds. In developing countries, the contribution of goats is highly valued and has a vital role in feeding the populations. Goat production is also considered more sustainable compared with the other livestock in terms of price and disease constraints. For these reasons, goats have always been considered beneficial animals for rural development programs in Vietnam and similar countries like Laos, Indonesia.

The growing demand for goat meat in Vietnam provides an opportunity for smallholder crop-livestock farmers to increase household income by supplying to this demand. However, they must improve their goat production systems and marketing to be sustainable. Up to 90% of goats produced in surveyed regions of Lao were being exported to Vietnam and on average the price received was 30% higher than Vietnamese crossbreed goats (Gray and Walkden, 2019). Besides that, the survey results also confirmed the lack of inputs to the mainly smallholder farmers who supply the market, and farmers are facing many constraints like high mortality, disease control, poor animal husbandry. This paper aims to provide some information on goat production to enhance income-generating opportunities for goat-raising households in Vietnam.

2. AN OVERVIEW OF GOAT PRODUCTION IN THE WORLD

The world goat population has been steadily increasing during the last six decades. The world goat population, which was 348.727 million in 1961, increased to 1045.916 million in 2018, resulting in a 200% increase over 60 years (FAOSTAT, 2020). More than 94% of the global population of goats in 2018 were from Asia (52.48%) and Africa (41.88%). The goat population in Asia had increased by 176.65% over the last six decades. This reflects that goats were in greater demand in the developing countries of Asia and Africa. Based on the goat population in 2018, China ranked first, with 138.383 million goats, followed by India with 132.750 million. Goat development in certain regions is related to religious beliefs impacting food consumptions. For instance, in all Islamic countries, pork is not consumed, while beef is not traditionally consumed in India. This may partly explain why the majority of the world's goat population is raised in these regions.

Goat raising in the world is mainly for meat and milk production and these products have very high nutritive value for human beings. This contributes to the price of goat meat and milk being consistently higher than other livestock products. Of the approximate 280 million tons of meat consumed per year globally, goat meat accounts for only 2% of this total amount at about 4.9 million tons (Miller and Lu, 2019). Developing countries produced approximately 97% of goat meat, reflecting its great importance to feed these populations. China leads the world production of goat meat, accounting for 38%. Most of this meat is not commercialized, but is produced and consumed locally (Mazhangara *et al.*, 2019).

3. AN OVERVIEW OF GOAT PRODUCTION IN VIETNAM

In recent years, goat production in Vietnam has developed very fast. Between 2008 and 2018, the total number of goats and sheep in Vietnam increased from 1.2 million to 2.8 million heads, with an average annual increase of 8.2% (Department of Livestock Production - DLP,

2019). This demonstrates that goat and sheep production have increased in popularity in Vietnam recently. However, the 2018 goat and sheep population (2.8 million head) was lower than what was planned by the Vietnamese government, at 3.7 million heads. Vietnam has imported goat meat (1059 tons in 2015, FAOSTAT, 2020) mainly from Lao PDR due to the high demand for goat meat for consumption (Nguyen Van Thu and Do Thi Thanh Van, 2018). Research under SRA LPS/2016/027 project (Gray and Walkden-Brown, 2019) found that up to 90% of goats produced in surveyed regions of Laos were exported to Vietnam, and on average, the price is 30% higher than Vietnamese crossbreed goats.

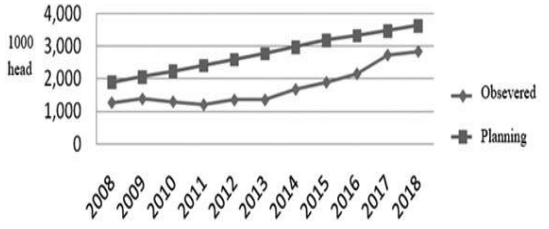

Fig 1. The Vietnamese population of goat and sheep in period 2008-2018 (DLP, 2019)

In general, goats are primarily raised in some provinces in each region, closely related to the goat consumer's tradition and the ecological characteristics (Figure 3). Most goats are found in the hilly and mountainous areas. Goat can be raised well in poorly vegetated, bare soils, and relatively dry regions. Ninh Thuan province is an example of this. Some provinces in Vietnam have a high number of goats, they are: Ha Giang, Nghe An, Son La, Thanh Hoa, Ninh Thuan, Dong Nai, Ben Tre, Gia Lai. Meanwhile, goat production is suitable for small households in the central and high-land areas. The goats are kept mainly in family farms with 5 to 7 head. In forested mountainous areas or hilly regions, the family herd can reach 10 to 20 head or more. In recent years, some commercial goat farms for milk and meat have been established. For example, Mang Den milk goat farm was established some years ago and has more than 5,000 heads. Three goat managing systems have been applied in Vietnam. They are: intensive, extensive, and semi-intensive management systems. About 70% of goat farmers practiced semi-intensive systems, which means goats are grazed and supplied feed at the goat house at night time. This system is easily applied to existing small ruminant production systems in Vietnam. Very few farmers/enterprises raising high-yielding goats for milk and meat applied intensive systems. In recent years, there was a trend of moving from goat keepers to goat producers.

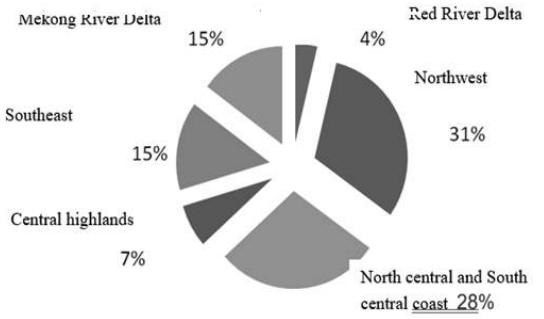

Fig 2. Distribution of goats and sheep in regions and sub regions in 2018 (DLP, 2019)

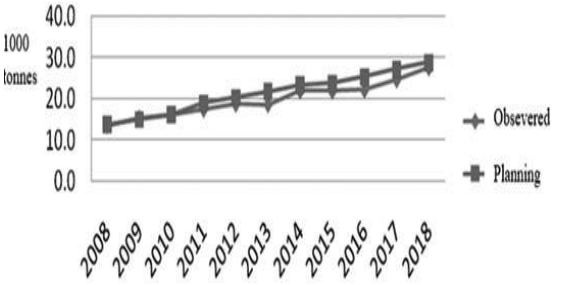

Fig 3. Production of goats and sheep meat in period 2008-2018 (DLP, 2019)

In the 2008-2018 period, goat and sheep meat production increased from 13.5 to 27.5 thousand tonnes, with an average annual increase of 5.5% (DLP, 2019). After a period of restructuring animal breeding, some provinces selected goats and sheep as dominant livestock species for their areas. Hence, meat production almost reached government development objectives (Figure 4). The Vietnam Ministry of Agriculture and Rural Development aimed to increase the number of goats and sheep by about 4-4.5 million head,

in which 90% of this number are goats and crossbreed sheep, which were mainly raised at the large-scale farms with a combination of in-stall feeding systems and controlled grazing systems. However, there have been quite a lot of challenges to reaching the target, so there might be a chance for goats from Laos to be exported to Vietnam.

4. OPPORTUNITIES AND CHALLENGES IN DEVELOPING GOAT IN VIETNAM

The growing demand for goat meat in Vietnam provides an opportunity for smallholder crop-livestock farmers in Vietnam to increase household income through more productive goat production. Farmers in Vietnam have responded positively to this opportunity by rapidly increasing goat numbers and changing goat production systems from free-grazing to semi-intensive and intensive systems. While demand for goat meat is increasing, Vietnam government have strong support policies for developing goat production. In Vietnam, there are some Government decrees and decisions, including Decision No 10/2008/QD-TTG on an animal breeding and development strategy (7% annual growth in goat and sheep population), Circular No 14/2014/TT-BNNPTNT on promulgation of specified high-yielding livestock breeds and Decision No. 1684/QD-TTG (2015) on international economic integration in agriculture. The Ministry of Agriculture and Rural Development is oriented to increase goat and sheep production by about 4-4.5 million heads; 90% of them is goats will be mainly raised at the large-scale farms with a combination of in-stall feeding systems and controlled grazing systems (DLP, 2019). Vietnam has invested in goat production and marketing. Poor farmers in Vietnam will have a chance to access low-interest-rate loans, innovative knowledge, key inputs (forage seeds), and input services (advance veterinary and technical assistance) which may accelerate their transition from market-oriented smallholder farmers to small-scale commercial goat producers.

Goat production has excellent potential for Vietnam farmers. In Vietnam, goat enterprises are regarded as solid opportunities for farmers in poor areas to access high-value markets. However, the sustainability of goat developments in both countries are facing significant challenges that need to be overcome. Potential risks include a collapse of the export market to Vietnam and/or premiums for Lao goat, low productivity and efficiency, disease, overexploitation of communal forage resources, and social costs. While the demand for goat meat has been increasing, a majority of the small goat flock owners cannot improve the productivity of their goats. With a steady increase in the goat population and increasing threats of global warming, the feed availability on community land has been reducing significantly. There are some important technical limitations that farmers cannot easily overcome by themselves. Kid mortality can be a severe problem if grazing and housing are not well managed. Only a small portion of goat keepers provide supplementary feed, even for pregnant does and weaning kids, and a large majority of them are incapable of providing the required nutrition. Parasite control, feed and feeding systems, and better housing will need to be considered to help farmers improve production and become more market-oriented.

5. SUGGESTED COMPONENTS FOR SUSTAINABLE GOAT DEVELOPMENT PROGRAMEE IN VIETNAM

To ensure goat enterprises in Vietnam can be both profitable and sustainable, research is needed to understand the role of goats in smallholder farming systems, understand goat production systems in the regions, and the opportunity and risks associated with the high demand in Vietnam. The sustainability of goat trade requires an improved understanding of the price premium and the risks associated with the export trade. There is a need to develop sustainable production systems that can meet this demand and position the sector for a long productive future. Key research questions to address in order to meet this need include: (1) What technologies and management practices can improve the efficiency and profitability of smallholder goat production in Vietnam? (2) What goat value chain and marketing changes can allow participants in each stage of the chain to maximise productivity and become more

engaged in the market, thus enhancing, in particular, the profitability of smallholder goat producers? (3) What mechanisms for knowledge transfer and innovation can most successfully lead to expanded impacts for smallholder goat producers?

6. CONCLUSION

Goat production plays a critical role and has excellent potential for Vietnam farmers. The growing demand for goat meat in Vietnam provides an opportunity for smallholder crop-livestock farmers in Vietnam to increase household income through improving goat production systems and marketing. In both countries, goat enterprises are regarded as solid opportunities for farmers in poor areas to access high-value markets. The sustainability of goat development in both countries is facing significant challenges that need to be overcome. Potential risks include a collapse of the export market to Vietnam and/or premiums for Lao goat, low productivity and efficiency, disease, overexploitation of communal forage resources, and social costs. To ensure goat enterprises in Vietnam can be both profitable and sustainable, research is needed to understand the role of goats in smallholder farming systems, understand goat production systems in the regions, and the opportunity and risks associated with the high demand in Vietnam. There is a need to develop sustainable production systems that can meet this demand and position the sector for a long productive future.

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SUCCESSFUL CLONED I PIG BREED FROM AN EAR TISSUE SOMATIC CELL

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The successful generation of cloned animals by somatic cell nuclear transfer (SCNT) opens up many potential applications in basic research, medicine, and agriculture. Mammal cloning using SCNT has many advantages over cloning using embryos derived from embryonic cells. The SCNT can be applied to animals with known phenotypes, an abundant and easy-to-use source of cells, leading to an increase in the number of embryos and clones produced. The SCNT technology has generated new directions for both basic research and applied research, such as genetically modified animals, endangered animal species conservation, cloned animals with compatible organs for human transplantation. Somatic cell nuclear transfer is currently being studied and applied to high-quality animals breeding and endangered species conservation.

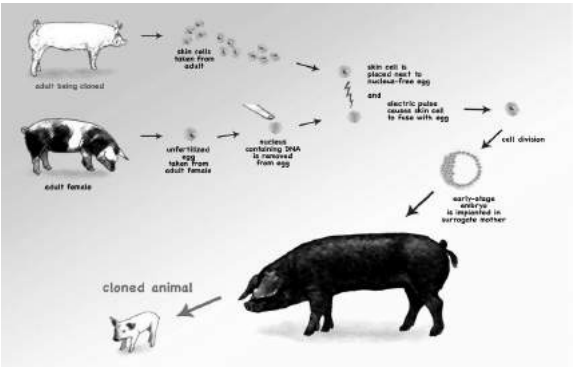

Fig 1. Basic steps of the animal cloning process

In Vietnam, Ỉ pig is derived from the fat pig breed in Nam Dinh. After a long time of growth and development, now there are two main breeds of pigs: Ỉ-mo (also known as Fatty Ỉ, the typical small short-legged pig, with small upward-pointing ears and a short snout) and Ỉ-goc pig (also known as Ỉ-pha, Large Ỉ have longer legs and a longer snout, with bigger ears held horizontally). Before the 70s, Ỉ pigs were raised throughout the Northern Delta provinces such as Nam Dinh, Ha Nam, Ha Tay, Hung Yen, Vinh Phuc, Hai Duong, Thai Binh, Quang Ninh, Ninh Binh, Thanh Hoa and Hai Phong. According to the statistics of 1969, both of these pig breeds still had 2 million heads. However, the number of Ỉ pigs is dwindling closer to the point of extinction; now, only boars are left and raised in Thanh Hoa. From 2001 to 2003, fifty Ỉ female pigs and four boars were preserved in this area; so far, only thirty female pigs and four boars left. Although Ỉ pig has delicious meat and is easy to raise, it has little meat and lots of fat (lean rate is only 36%); growing Ỉ pigs the whole year is only 40-50kg. Therefore, pigs cannot compete with other breeds of pigs and are in danger of extinction. Therefore, cloned pigs created by somatic cell nuclear transfer helps preserve pig genetic resources and opens up the direction of conservation and development of other valuable domestic animals in Vietnam.

From July 2017 to December 2020, researchers from The Key Laboratory of Animal Cell Biotechnology (KLACB) carried out the project "*Research on creating Ỉ pigs by somatic cell nuclear transfer*" under the "*Key program on the application of biotechnology in the field of agriculture and rural development until 2020*" with the goal: to successfully research and apply the technology of creating cloned animals by somatic cell nuclear transfer technique in Vietnam, towards serving the conservation and development of rare and precious livestock species. The staff of the KLACB has developed processes in pig cloning technology Ỉ such as the process of creating cell lines from pure pig ear tissue used for the operation of transplanting donor cells and creating cloned pig embryos; the process of creating a recipient cell line with or without zona pellucida membrane used for the process

of transplanting donor cells and creating cloned pig embryos; the process of transplanting cell nucleus and creating cloned pig embryos; cloned pig embryo transfer process.

During the project's implementation, the KLACB researchers have studied, standardized, and applied new methods such as creating Ỉ pig embryos cloning without zona pellucida instead of having zona pellucida like most previously published in the world. It is a simple, effective, and easy-to-operate method compared with the traditional way (with zona pellucida) with a higher number of cloned pig blastocysts generated than with the zona pellucida method. With this method, the current rate of Ỉ cloned pig blastocyst of the KLACB is >24%. In addition, during the implementation of the project, researchers from the KLACB found that the transfer of pig embryos cloned without zona pellucida at the blastocyst stage will improve the conception rate. These results have been published in domestic journals (Journal of Biotechnology, Journal of Animal Husbandry Sciences and Technics) and internationally (Journal of Theriogenology). And on March 10, 2021, 04 Ỉ pigs cloned were born from somatic cell nuclear transfer technique. These are the first cloned animals in Vietnam. For the first time in Vietnam, it has been successfully cloned in mammals in general and in pigs, particularly somatic (adult) cells. So far, these cloned pigs are still growing and developing healthy, with body weights ranging from 20-26kg. The success of cloned pig creation has confirmed the mastery of animal cloning technology and concurrently enhanced the position of researchers at the KLACB, National Institute of Animal Science, in terms of international scientific standards.

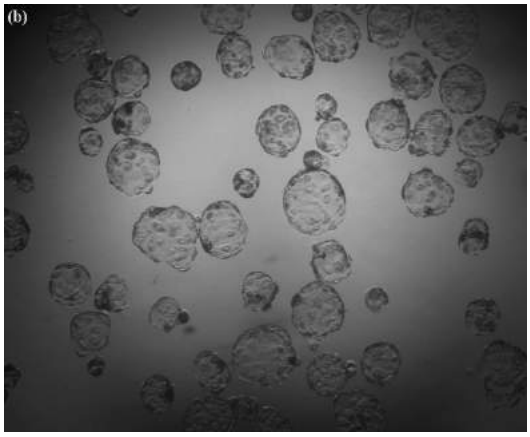

Fig 2. Cloned Ỉ pig blastocyst without zona pellucida

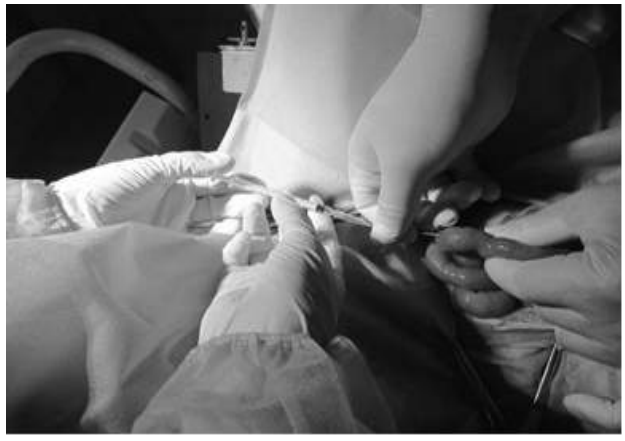

Fig 3. Transplant blastocysts into the uterine horns of recipient pigs

Fig 4. Cloned Ỉ pigs (4 months old) from ear tissue somatic cell