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Office:	Floor 4, 73 Hoang Cau, O Cho Dua, Dong Da, Ha Noi
Telephone:	024.36290621
Fax:	024.38691511
E - mail:	tapchichannuoi@hoichannuoi.vn
Website:	www.hoichannuoi.vn
Account Name:	Hoi Chan nuoi Viet Nam
Account Number:	1300 311 0000 40
Bene bank:	Join Stock Commercial Bank for Foreign Trade of Vietnam (VietcomBank) - So Giao dich branch
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ANIMAL GENETICS AND BREEDING

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SCIENTIFIC NEWS

MOLECULAR MARKERS AND THEIR APPLICATION FOR LIVESTOCK PRODUCTION IN VIETNAM

*Tran Trung Tu[1], Le Thanh Phuong[2], Nguyen Thiet[1] and Nguyen Trong Ngu[1]**

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ABSTRACT

The advancement of molecular markers has opened new opportunities for livestock genetics and breeding. These markers significantly impacted the study of animal and poultry genomes to improve their traits of interest. The present paper provides a brief overview of some common molecular markers (microsatellites, RFLP, SNP, In/Del) employed in biodiversity determination and candidate gene identification and their uses for animal production and product quality in Vietnam.

Keywords: *In/Del, microsatellites, molecular markers, RFLP, SNP.*

TÓM TẮT

Ứng dụng của chỉ thị phân tử trên vật nuôi tại Việt Nam

Sự phát triển của các chỉ thị phân tử đã mở ra những cơ hội mới trong di truyền và chọn giống vật nuôi. Những chỉ thị này đã có ảnh hưởng đáng kể trong công tác nghiên cứu bộ gen gia súc, gia cầm nhằm cải thiện các tính trạng được quan tâm. Bài báo hiện tại giới thiệu tóm tắt một số chỉ thị phân tử thông dụng (microsatellites, RFLP, SNP, In/Del) trong xác định đa dạng di truyền, xác định những gen ứng viên và ứng dụng của chúng đối với năng suất và chất lượng sản phẩm vật nuôi tại Việt Nam.

Từ khóa: *Dấu phân tử, In/Del, microatellites, RFLP, SNP.*

1. INTRODUCTION

Genotype-based breeding narrows the generation gap by pre-selecting traits when animals are young since genes allow trait testing regardless of sex or age, increasing accuracy when selecting difficult traits, and reducing test populations or offspring via immediate genotype selection. To achieve targeted genetic improvement in livestock, the genes controlling desirable and undesirable traits must be characterized, which is not yet complete. Marker-assisted selection (MAS) in breeding helps to achieve this goal, especially in cases where pedigree data are unavailable and the targeted traits have low heritability (Dentine, 1999).

Identifying genes that control specific traits can be approached in several ways. Traits controlled by a single gene can be determined by observing variations through studying physiology and the biochemical pathways involved. For traits that are controlled by multiple genes, genetic mapping can be used. Maps with genes controlling specific traits are generated using markers of associated chromosomal regions. Identifying genes that regulate traits by genetic mapping has been done to locate the quantitative trait loci on chromosomes and identify genes in that region. Although not expected to significantly improve traits with high heritability, MAS is believed to be valuable in improving traits with low heritability such as carcass and reproduction traits.

2. SOME COMMON MOLECULAR MARKERS

2.1. Microsatellites

Microsatellites are tandemly repeated tracts of DNA composed of 1-6 base pair (bp) long units. Microsatellites loci are also known as simple sequence repeats (SSR),

[1] Can Tho University, Vietnam
[2] Emivest Feedmill Vietnam Co. Ltd
* Corresponding Author: Assoc. Prof. Nguyen Trong Ngu, College of Agriculture, Can Tho University;
Tel: (84) 989828295; Email: ntngu@ctu.edu.vn

short tandem repeats (STR), simple sequence tandem repeats (SSTR), and variable number tandem repeats (VNTR), simple sequence length polymorphisms (SSLP). They are ubiquitous in prokaryotes and eukaryotes, present even in the smallest bacterial genomes (Field and Wills, 1996). Microsatellites can be found anywhere in the genome, both in coding and non-coding regions. Because of their high mutability, microsatellites are thought to play a significant role in genome evolution by creating and maintaining quantitative genetic variation. In promoter regions, the length of SSRs may influence transcriptional activity (Kashi *et al.*, 1997). Hancock (1995) indicated that SSRs in exons are less abundant than in non-coding regions and that different taxa exhibit different preferences for SSR types (Tautz and Schlötterer, 1994).

The SSR approach offers multiple advantages, including several alleles in a locus, even distribution throughout the genome, co-dominant direction, high polymorphism and specificity, experiment reproducibility, minimal DNA usage, low cost, and ease of implementation. However, microsatellites also have the following disadvantages: high initial development expenses, lengthy development times, and high development costs. Mutations in the primer annealing sites may cause misclassification of heterozygotes as homozygotes when null alleles are present. Microsatellite markers aid in the identification of neutral biodiversity but provide no information regarding the functional characteristics of biodiversity (Teneva, 2009). Currently, SSR is the indicator of choice for forensic record studies, population genetics, and wildlife studies.

2.2. Restriction fragment length polymorphism

The RFLP marker, developed by Botstein *et al.* (1980), uses bacterial restriction enzymes to cut DNA molecules at specific recognition sequences, typically 4-6bp long. Many restriction enzymes have unique recognition sites. Variations in restriction enzyme recognition sites were discovered by cleaving genomic DNA with a restriction enzyme and electrophoresizing the fragments. Numerous segments were created to test simultaneously because of the genome's recognition sites.

The RFLP marker has a high degree of polymorphism, is co-dominant, can distinguish heterozygous and homozygous individuals, and is highly repeatable. This technique is commonly used for the detection and identification of gene polymorphisms and for recombinant DNA technology in livestock (Beuzen *et al.*, 2000). The PCR-RFLP technique is performed by restricting the PCR product of specific loci with one or more restriction enzymes and separating the cut DNA fragments on agarose or polyacrylamide gels. Primer pairs are designed based on the sequence information of the DNA or cDNA on the Genbank. These are co-dominant markers and are specific loci used to distinguish homozygotes from heterozygotes (Konieczny and Ausubel, 1993). PCR-RFLP is commonly used in diagnostic testing to determine the genotype in a known genetic mutation.

2.3. Single nucleotide polymorphisms

A single nucleotide polymorphism (SNP) is a variation in the DNA sequence that occurs at a single nucleotide in the genome (Scherf and Pilling, 2015). SNP expression sites in the genome are places where DNA sequences are distinguished by a single base when two or more individuals are compared. This difference in nucleotides can lead to changes in particular traits or phenotypes. SNPs are by far the most common form of sequence change in the genome.

Several reasons are responsible for the growing emphasis on employing SNPs as genetic analysis markers. First, they are more prevalent than other forms of polymorphisms and provide more possible markers near or at any area of interest. For example, human genomic DNA contains an SNP greater than 1,000bp (Landegren *et al.*, 1998). Secondly,

some SNPs are positioned in the coding area and influence the protein's function directly. These SNPs are directly responsible for some interindividual variances in economically significant features. In addition, SNPs are more stable than microsatellites and more dependable for genetic study than microsatellites (Lipshutz *et al.*, 1999).

Among the molecular markers, SNPs are promising for association studies. These markers are abundant and exhibit a low mutation rate, which facilitates genotyping (Chen and Sullivan, 2003). Many studies have been performed on the association between SNPs in candidate genes and metabolic pathways in several species. For example, Wong *et al.* (2004) identified 2.8 million SNPs in the chicken genome. This abundance of available SNPs may aid in the future mapping of causative polymorphisms underlying complex traits in chickens. In addition, stability and abundance made SNP popular and interesting. SNP is a co-dominant indicator; therefore, it may assess individual genotypes in a population.

2.4. Insertion/Deletion

The Insert/Delete polymorphism, abbreviated In/Del, is a type of genetic variation in which a nucleotide can be inserted or deleted in the nucleotide sequence of a gene. Although not as common as SNPs, In/Dels are common in the genome. In/Del includes a total of 3 million of the 15 million known genetic variations (1,000 Genomes Project Consortium, 2010). An In/Del in the coding region of the gene that is not a multiple of 3 nucleotides leads to a frameshift mutation. Shifting reading frames and transcriptional sequences of genes can code for a different amino acid or lead to premature codon inactivation, altering protein structure and function. An In/Del can change the DNA sequence and shift the framework, thereby altering the sequence of amino acids produced, resulting in abnormal protein production even if no protein is produced.

The In/Del mutation is a source of genetic variation, often segregated into short and long In/Del due to different approaches to longer variants. One short In/Del (less than 50bp) for 8 human SNPs (Wilson *et al.*, 2001), represents a ratio of variation. In/Del is believed to contribute more to sequence diversity than SNPs, regarding the number of distinct bases. In addition, it has been suggested that a short In/Del could be instrumental in maintaining an optimal intron size.

3. APPLICATION OF GENETIC MARKERS FOR LIVESTOCK IN VIETNAM

3.1. Biodiversity and conservation of animal genetic resources

In cattle, Pham Doan Lan *et al.* (2008) assessed the genetic diversity and genetic structure of the cattle population in Ha Giang province. A total of 23 microsatellites were used to the genotype of 530 cattle individuals. The results showed that 205 alleles were observed in the total population. The allelic diversity (average number of alleles per locus) was 8.9±2.05. The average observed and expected heterozygosity for the population was 0.67 and 0.73, respectively. The polymorphic information content (PIC) value of each locus ranged from 0.50 to 0.84. The average genetic variation between district cattle populations was very low with an FST value of 0.013.

In chickens, Le Thi Thuy *et al.* (2009) used 20 pairs of microsatellite primers to study the genetic diversity of 5 local chicken breeds in Vietnam (Ac, Choi, H'Mong, Ho, and Tre chicken). The results showed that the populations of these breeds were highly diverse and the degree of genetic dispersion among chicken populations was very large. These five chicken breeds were divided into 3 groups including (1) Tre chicken, (2) Ac chicken, and (3) others, in which the genetic distance of Choi and Ho chicken was closest. In addition, Ngo Thi Kim Cuc and Nguyen Van Ba (2019) assessed genetic diversity and genetic differentiation between Lac Son chicken and other native chicken

breeds using 20 microsatellite markers. The results indicated that microsatellite markers studied were polymorphic with an average of 6.73 alleles/locus. The genetic diversity of Lac Son chicken was on the average level with the expected heterozygosity of 0.60 and the number of alleles/locus was 6.05. The inbreeding coefficient of Lac Son chicken was very low. The lowest genetic distance was found between Lac Son chicken and Dong Tao chicken but the highest genetic distance was found between Lac Son chicken and Tai Do chicken. Sub-structuring of the Vietnamese chicken breeds was related to their geographical distribution distance. Ri, Dong Tao, and Mia chicken in the Red River Delta were in the same group, while Lac Son chicken in the northern central region was separated and different from the Tai Do chicken (Red Jungle Fowl) breed.

3.2. Identification of candidate genes associated with economic traits

In pigs, several genes are associated with weight gains such as HAL, GH, and LEP. Nguyen Ngoc Tuan and Tran Thi Dan (2005) reported that pigs carrying the HAL gene with genotype "nn" had a low growth rate but higher back fat thickness than "NN" or "Nn" pigs. For imported pig breeds, carcass ratio, carcass length, back-fat thickness, lean percentage, and fat percentage did not differ between genotypes "NN" and "Nn" but the tenderloin area group of pigs "Nn" was larger than pigs "NN" with significance ($P<0.05$). The group of pigs with the gene "nn" adversely affected reproductive parameters, while the group of pigs "NN" had the highest number of newborns per litter.

Nguyen Huu Tinh *et al.* (2020a) selected the terminal sire pig line TS3 (Duroc) performing with high production based on testing the genotype of H-FABP, MC4R, and PIT-1, and the evaluation of breeding values estimated by the BLUP procedure and genotype. In the 3rd generation, TS3 pigs were improved remarkably for tested performance traits as compared to the original generation, such as 932g for ADG, 10.8mm for BF, 63.8mm for loin depth, 144.9 days for age to 100kg, 2.45 for FCR, 62.1% for lean meat and 3.22% for intramuscular fat. Commercial crossbred pigs used TS3 as terminal sires with 921 g for ADG, 2.39 for FCR, and 61.4% for lean meat. In addition, Nguyen Huu Tinh *et al.* (2020b) selected 2 pig lines SS1 (Landrace) and SS2 (Yorkshire), and parental crossbred sows (SS12 and SS21) performing with high reproduction based on identifying FSHB and PRLR genotypes and evaluation of breeding values were estimated by BLUP procedure. Other markers associated with traits of interest in pigs are shown in Table 1.

In chickens, Tran Xuan Hoan *et al.* (2000, 2001) analyzed GH gene polymorphisms in local chicken breeds and concluded that there was no association between genotypes and egg production at 36 weeks of age and body weight at 20 and 36 weeks old. Studying the influence of PIT1 gene polymorphism on performance traits of the Tau Vang chicken breed, Le Thi Thu Ha *et al.* (2015) identified 3 genotypes AA, AB, and BB in the flock of Tau Vang. The authors found only the effect of this polymorphism on the egg weight of chickens, in which hens carrying genotype AA gave the highest egg weight. Recently, Tran Thi Binh Nguyen *et al.* (2020) investigated SNP of candidate genes in Lien Minh chickens with the allele frequencies obtained were 0.97 and 0.03 for alleles A and G (GHi3), respectively; in IGFBP2, 0.47 for the A allele and 0.53 for G; in PIT1, 100% for allele B. These polymorphic loci (GHi3, PIT1, and IGFBP2) followed the Hardy-Weinberg equilibrium in the Lien Minh chicken population. These were the initial results, which could be used to analyze the association of molecular markers and grow traits in Lien Minh chickens. Other studies related to growth traits in broilers, egg producing traits in laying hens, and egg quality characteristics in quails are presented in Table 2.

Table 1. The association of some gene polymorphisms with economic traits in pigs

Genes	Polymorphisms	Traits	Population	Source
FTH1	G714A	Weight gain, tenderness of tenderloin	DP	Nguyen Trong Ngu *et al.* (2010)
	T698C, G714A	Weight gain, color and pH of tenderloin		
TNNI1	T5174C	Driploss 45 min post-slaughter	MC	Nguyen Trong Ngu&Nguyen Thi Hong Nhan (2012)
TNNC	C716G	pH 45 min post-slaughter, the toughness of the meat	MC	Nguyen Trong Ngu *et al.* (2013)
MYF5	A1205C	pH 45 min post-slaughter, tenderloin weight	MC	Nguyen Thi Kim Khang and Nguyen Trong Ngu (2013)
RYR1	C1843T	The number of white blood cells, red blood cells; crude protein (tenderloin)	YL	Do Vo Anh Khoa and Huynh Thi Phuong Loan (2013)
H-FABP	C1489T	Back-fat thickness (neck); crude protein; water holding capacity (tenderloin)	YL	Do Vo Anh Khoa *et al.* (2011)
RYR1	C1843T	Not associated with carcass performance traits, quality and chemical composition in meat	Stress-resistant P (carries CC and CT genotypes)	Ha Xuan Bo *et al.* (2013)
RYR1	C1843T	Improve reproductive performance	Stress resistant P CCxCC; P CCx D CC)	Do Duc Luc *et al.* (2013)

TNNI, TNNC: Troponin 1, C; MYF5: Myogenic Factor 5; RyR1: Ryanodine Receptor 1; H-FABP: Heart-type Fatty Acid Binding Protein; FTH1: Ferritin Heavy-Chain; MC, DL, DY, LY, PL, PY (MC: MongCai, D: Duroc; Y: Yorkshire; L: Landrace; P: Pietrain).

Table 2. Association of some SNP markers with important traits in poultry

Genes	Polymorphism	Traits	Breed	Source
NPY	I31391359D	Egg yield, egg weight (5 months of laying)	Noi chicken	Nguyen Trong Ngu *et al.* (2014)
	C31394761T	Hatching rate (5 months of laying)		
VIPR-1	C1715301T	Egg yield (5 months of laying)		
	C1704887T	Egg yield (5 months of laying)		
Insulin	C1549T	Body length, breast angle and length, thigh length&ratio, abdominal fat ratio	Tau Vang chicken	Do Vo Anh Khoa *et al.* (2014)
	C3737T	Breast deep; carcass, thigh ratio; abdominal fat weight		
GH	A3971G	Carcass ratio, breast ratio, thigh ratio	Tau Vang	Do Vo Anh Khoa (2014)
	C3199T	weight gain, FCR, thigh ratio		
	C3094	FCR	Tau Vang	Do Vo Anh Khoa *et al.* (2014)
	A662G	pH 24, 48 hours post-slaughter (chicken breast meat)	Tau Vang	Do Vo Anh Khoa (2014)
FADS1	C391A	The fatty acid composition in egg yolk	Japanese quail	Nguyen Thi Kim Khang (2006)
FADS2	C953T	The fatty acid composition in egg yolk (omega-6 and omega-3 PUFA)		
Prolactin	Indel 358 promoter (24 nucleotides)	Number of chicks hatched (5 months of laying)	Japanese quail	Nguyen Trong Ngu *et al.* (2021)

Notes: NPY (Neuropeptide Y); VIPR-1 (Vasoactive Intestinal Peptide Receptor 1); IGFBP2 (Insulin-like Growth Factor Binding Protein 2); GH (Growth Hormone); FADS (Fatty Acid Desaturase).

4. CONCLUSIONS

Practically, molecular markers are applicable to a variety of fields and animal species. Within the scope of this paper, only two topics are addressed: genetic diversity and candidate genes in the primary target species of pigs, chickens, and quails. In addition to the aforementioned topics, molecular markers have been researched in dairy cows to enhance milk yield and quality. In addition, recent research on potential genes associated with disease resistance has been discussed. When it comes to livestock production in Vietnam, the use of molecular markers in breeding is important, particularly for local breeds, in order to increase productivity while maintaining the product quality that satisfies consumer demand.

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COMPARISON OF PERFORMANCE AND MEAT QUALITY BETWEEN TWO SASSO LINES LOW-WEIGHT (SA51) AND HEIGHT-WEIGHT (SA31) REARED BY FREE RANGE SYSTEM

Nguyen Duy Hoan[1], Tran Thi Hoan[1] and Phan Thi Hong Phuc[1]*

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ABSTRACT

In chick hatcheries, males of laying hybrids are considered to be "waste" and the majority of these males are killed just after hatching. On the other hand, the interest of consumers in products from alternative systems (organic, free-range) is increasing. The idea was to evaluate the meat quality of these males when they have access to free range because there is not such a study available. This study was carried out at Hai Yen farm Song Cong town, Thai Nguyen province on 2 broiler Saso chicken lines: low-weight line (SA51) and heavy-weight lines (SA31), 90 male one-day-old chicks each line reared in captivity to 21 days with density 6 birds/m^2, then free range reared in the garden with natural grass and fruit tree with density 5m^2/bird, monitor up to 90 days of age. The aim of this study was to compare the physical and sensory quality of the meat with two lines broilers at 49 and 90 days of age when they had both access to free range to 90 days of age. The result as followed: Live weight, carcass yield, breast meat yield and the proportion of abdominal fat were significantly higher (P<0.001) in SA31 at both ages. The proportions of fat in the breast meat were significantly lower (P<0.01) in SA51 at both ages. The value of pH 24h was significantly higher in SA51 and the meat was darker (P<0.001) in these chickens. The overall acceptability was significantly better (P<0.01) in SA51 at 90 days of age. The males are acceptable for an alternative system of poultry meat production from the aspect of meat quality.

Keywords: *Chicken meat quality, free range, Sasso.*

TÓM TẮT

So sánh năng suất, chất lượng thịt của gà trống 2 dòng SA51 và SA31 nuôi chăn thả tự nhiên

Tại các cơ sở sản xuất gà giống, gà trống một ngày tuổi bị loại thải, bỏ đi nên gây lãng phí rất lớn. Trong bối cảnh người tiêu dùng ngày càng quan tâm đến chất lượng thịt gà, đặc biệt là gà được sản xuất theo hướng an toàn sinh học, nhóm tác giả đã quyết định sử dụng các con trống bị loại thải của giống gà Sasso để tiếp tục nuôi lấy thịt bằng phương thức chăn thả tự do, tiến hành đánh giá chất lượng thịt của chúng để có kết luận tính hiệu quả của ý tưởng này. Nghiên cứu được tiến hành tại tỉnh Thái Nguyên trên 2 dòng gà thịt Saso (dòng nhẹ cân: SA51) và dòng nặng cân: SA31), mỗi dòng nuôi 90 con trống1 ngày tuổi (lặp lại 3 lần), nuôi nhốt đến 21 ngày với mật độ 6 con/m^2, sau đó nuôi thả rông trong vườn có cỏ tự nhiên và cây ăn quả với mật độ 5m^2/con, kết thúc thí nghiệm lúc 90 ngày tuổi. Kết quả cho thấy khối lượng sống, năng suất thân thịt, năng suất thịt ngực và tỷ lệ mỡ bụng của dòng SA31cao hơn so với dòng SA51 ở cả 2 thời điểm 49 và 90 ngày tuổi (P<0,001). Thịt gà dòng SA51 có màu sẫm hơn, độ pH 24h cao hơn (P<0,001) và tỷ lệ lipid trong thịt ngực thấp hơn (P<0,01) so với thịt gà dòng SA31 ở cả hai lứa tuổi. Qua đó cho thấy có thể sử dụng gà trống 1 ngày tuổi của gà Sasso để nuôi lấy thịt đáp ứng cả mục đích kinh tế và chất lượng sản phẩm. Về tổng thể nếu nuôi đến 90 ngày tuổi dòng SA51 có nhiều ưu điểm hơn so với dòng SA31.

Từ khóa: Chất lượng thịt gà, chăn thả tự do, Sasso.

[1] Thai Nguyen University of Agriculture and Forestry

* Corresponding Author: Prof. Dr. Nguyen Duy Hoan, Senior lecturer Tel: (84) 913377255; Email: ndhoan@tnu.edu.vn

1. INTRODUCTION

In recent years, the interest of consumers in products from organic (free-range) systems organic is increasing mainly because these systems can be environmentally friendly, sustaining animals in good health with high welfare standards and resulting in higher quality products (IFOAN, 2014) and has a particularly attractive natural flavor (Hoan, 2014). So some assessors prefer breast fillets from a standard system to free-range or organic system (Brown *et al.,* 2008). Among others in organic production, the minimum age at slaughter shall be 70 days. In France, chickens reared under carefully specified conditions may be accorded the Label Rouge or Label Fermier quality marks. Fast- growing commercial hybrids are not suitable for these production systems, because they are slaughtered between 5 and 7 weeks and at 81 (84) days of age they are too heavy. However, in the United States organic and other specialty poultry production mostly utilizes the same fast-growing broiler genotype as in conventional production systems (Fanatico *et al.,* 2005). At Vietnam, the free-range production of chicken meat is regulated by Ministry of Agricultural and Rural Developing of Vietnam (2010) in National Technical Regulation Conditions for biosecurity of poultry farms (QCQG-01-15).

The antagonistic relationship between meat and egg production led to the separation of the meat and egg-type strains of fowl. Consequently, the day-old male layer chickens have been used in the pet foud industry as a high-quality animal protein source for predators, reptiles, falcons, hawks and zoo animals. Moreover, in hatcheries the male chickens of layer breeds have to be killed due to their poor fattening performance and consequent high fattening costs. The superiority and genetic improvement of meat-type chickens in terms of growth is well documented (Havestein *et al*., 2003; Lonergan *et al.,* 2003); however, there are only a few studies concerning the carcass composition and meat quality of commercial layer males in comparison with broilers at the same age of birds (Guni *et al.,* 2021). Fanatico *et al.* (2005) evaluated the effect of genotypes on the carcass quality, but they compared fast and slower growing broilers, but no layer males. They also compared the car¬cass quality birds at the same live weight (different age) and compared the carcass quality of slower and faster growing birds at the same carcass weight (different age and different live weight). Grashorn and Clostermann (2002) conducted a very extensive study concerning the performance and slaughter characteristics of broiler breeds for extensive production, but they also used slow-growing chickens without free range. Berri *et al.* (2005) compared the retention of protein and fat in the meat of heavy-weight and light-weight lines, but they kept the chickens in cages. Sasso chicken bred to grow under all manner of rearing systems and reach a market weight of 2kg in 3 months, so as the Sasso breed is a choice for those who want to take advantage of the in-betweens of the traditional free-range chicken and the fast-growing hybrid broiler. The difference of this study compared with previous studies is to evaluate and compare the performance and meat quality of the heavy and low weight lines of Sasso chickens raised in the grazing system.

2. MATERIAL AND METHODS

2.1. Birds and diets

The formal experiment was conducted from Jul to Nov, 2021 at Hai Yen farm Song Cong town, Thai Nguyen province on 2 broiler Sasso chicken lines: low-weight line (SA51) and heavy-weight line (SA31); 60 male one-day-old chicks each breed reared in captivity to 21 days with density 6 birds/ m^2, then free range reared in the garden with natural grass and fruit tree with density 5m^2/ bird, monitor up to 90 days of age. All birds raised by the process of Vietnam MARD (2010). Temperature was maintained at 30°C

at the beginning of the experimental period, and gradually decreased to 22°C by the fourth week of age. Outdoor access to a grass paddock was provided during daylight hours. The birds were confined to indoor pens at night. The birds had free access to feed and water at all times (both outside and inside). All birds received the same diets (Table 1) adlibitum (1-14 days: starter; 15-44 days: grower; 45-90 days: finisher). Diet formulations and calculated analyses are given in Table 1. All birds were individually weighed at weekly intervals.

Table 1. Diet calculated analyses

Ingredient	Starter 1-14 days	Grower 15-44 days	Finisher 45-90 days
EM (kcal /kg)	2,694	2,807	2,714
Crude protein (%)	21.36	18.66	16.51
Methionine (g/kg)	5.12	4.23	3.90
Lysine (g/kg)	10.71	9.30	8.05
Calcium (g/kg)	8.46	9.21	8.03
Phosphorus (g/kg)	2.62	5.61	6.07

2.2. Physical and chemical analysis

At 49 and 90 days of age, 10 birds from each group were slaughtered. The birds were killed by manual exsanguinations. The plucked carcasses were eviscerated and chilled for 24h at 5°C before dissection. Boneless thighs and drumsticks with skin, breast meat and abdominal fat were weighed. The right sides of breast meat were individually wrapped in tinfoil and put to a -24°C freezer before sensory evaluation. The left side of breast meat was evaluated for colour, pH, drip loss and chemical analysis. Breast meat (4-5g) was carefully weighed, then put in a refrigerator (5°C) for 24h and then dried with filter paper and precisely weighed again. Drip loss was expressed as a percentage of the initial muscle weight.

The pH values were measured with a digital pH meter PORTAMESS 911 Ph KNICK (Knick Elektronische Messgeriite, Berlin), 1cm from the sternum in the middle part of the muscle and at a depth of 1cm at 0.5, 1.0, 1.5, 2.0 and 24h intervals. The colour parameters (L*, a*, b*) were measured on raw muscles and on the skin of thigh using a spectrophotometer (CM-2600d, Konica Minolta, Osaka). In this method, higher L* values are light, higher a* values are red, and higher b* values are yellow. Colour measurements were taken on the cross-section of the breast muscle. Chemical analyses of the breast meat were done as follows: Moisture was determined by drying at 105"C for 6h and total lipids were analysed by extraction with petroleum ether (Soxtec method).Sensory assay: 10 chickens from each genotype in both age categories were assessed by five highly trained panellists under controlled conditions of a sensory study in a sensory laboratory. Birds with average weights were chosen for the evaluation. Only the cooked breast meat was subjected to the sensory evaluation due to the lack of homogeneity of thigh muscles. The breast samples were cooked in foil in their own juice at 90°C for 1.5 h. Panellists described the colour, flavour, texture, juiciness, taste and overall acceptability. Each attribute was scored on an unstructured linescale 100 mm long. The extreme points of the linescales were as follows: colour 0-dark, 100-light, flavour 0-typical, very pleasant, 100-untypical, off-flavour, texture 0-soft, 100-tough, juiciness 0-very juicy, 100-dry, taste 0-unpleasant, aftertaste, 100-pleasant, without aftertaste, overall acceptability 0-pleasant, 100-unpleasant.

2.3. Statistical analyses

Data on live weight and sensory assays were analysed by £-test and the chemical and physical characteristics were analysed by the nonparametric Mann-Whitney U-Test using the software package Unistat 5.1, England.

3. RESULTS AND DISCUSSION

The result at table 2 show clear that due to selective breeding decisions the live weight of SA31 was significantly higher (P<0.001) than in SA51 both of 49 and 90 days of age, as it was already reported many times (Guni *et al.*, 2021; Lonergan *et al.*, 2003). Surviral rate till 90 days of age was 92.38% in SA51 and 90.75% in

SA31 (P<0.05). The feed conversion ratio till 90 days of age was 3.12 kg/kg in SA31 and 3.56 kg/kg in SA51 (P<0.05).

The carcass characteristics and meat quality are shown in Table 3. As expected, carcass weight and carcass yield percentages were also significantly higher (P<0.001) in SA31. Regardless of the age, breast yield was significantly higher (P<0.001) in heavy-weight SA31 than in low-weight SA51, as a lot of authors have shown (Fanatico *et al*., 2005). This is the result of intensive selective breeding for this characteristic in broilers. The heavier weight of SA31 resulted in all their components being heavier than those of SA51. But there were no significant differences between the genotypes in the percentage of leg muscle plus skin (thigh and drumstick). Horsed *et al.* (2005) found that the proportion of the less valuable parts and the percentage of leg tended to be higher in egg-type males than in broilers. Fanatico *et al.* (2005) observed a significant effect of the genotype (fast vs. slow) on the percentage of both breast and leg meat to the total weight of the carcass. In their experiment with slow-growing chickens, the percentage of breast meat was lower, but the percentage of leg meat was higher in comparison with fast-growing broilers. They did not note a significant difference in the breast, thigh, or total meat production. At both ages, the amount of abdominal fat was significantly lower (P < 0.001) in SA31 than in SA51.

Table 2. Survival rate, growth and feed conversion

Targets	Day of age	SA51	SA31	P
Survival rate (%)	49	94.79	93. 84	ns
	90	92.38[a]	90.75[b]	*
Average body weight (g)	49	1224.12[a]	1423.45[b]	***
	90	2019.01[a]	2218.10[b]	***
Feed conversion ratio (kg/kg)	49	3.94	3.59	*
	90	3.56	3.12	*

*Where: *, * *, ** * indicates significance level at 0.05, 0.01 and 0.001, respectively*

On the basis of his scientific works Havenstein *et al.* (2003) reported that the proportion of abdominal fat was higher in SA31 at 43 days of age (1.40%) than in Athens-Canadian Randombred Control at 85 days of age (1.21%). On the other hand, Grashorn and Clostermann (2002) found a partly higher proportion of abdominal fat in slow-growing breeds (SA51) than in the SA31 line

Table 3. Slaughter trait, chemical and physical characteristics of breast meat

Carcass quality		Age (days)	SA51 (n=10)	SA31 (n=10)	P
Live weight (g)		49	1,224.12[a]	1,423.45[b]	***
		90	2,019 .01[a]	2,218.10[b]	***
Carcass weight (g)		49	751.97[a]	980.75 [b]	***
		90	1,285.50[a]	1,584.83[b]	***
Carcass yield (%)		49	61.43[a]	68.90[b]	**
		90	63.67[a]	71.45[b]	***
Breast yield (%)		49	15.23[a]	17.94 [b]	***
		90	16.68[a]	19.24 [b]	***
Leg muscle+skin yield (%)		49	25.12	25.48	ns
		90	26.44	26.65	ns
Abdominal fat (%)		49	0.12[a]	2.02 [b]	***
		90	0.73[a]	2.77 [b]	***
Dry matter - breast (%)		49	25.14	25.41	ns
		90	27.64[a]	25.75[b]	***
Fat - breast (%)		49	0.42[a]	2.08 [b]	**
		90	0.65[a]	1.43 [b]	**
Drip loss - breast (%)		49	3.13	3.45	ns
		90	1.51[a]	0.70[b]	**
pH 30 min		49	6.12	6.14	ns
		90	6.14	6.29	ns
pH 24h		49	5.75[a]	5.57[b]	**
		90	5.73[a]	5.64[b]	**
Skin colour 24h	L*	49	71.43	71.53	ns
		90	68.15	71.06	ns
	a*	49	6.54	6.15	ns
		90	7.18	8.69	ns
	b*	49	27.43[a]	20.54[b]	*
		90	31.63[a]	26.65[b]	**
Breast colour 24h	L*	49	54.16[a]	58.13[b]	*
		90	50.33[a]	54.31[b]	***
	a*	49	2.78[a]	1.27[b]	*
		90	0.05	0.17	ns
	b*	49	17.72[a]	15.45[b]	**
		90	12.82[a]	10.79[b]	**

The chemical characteristics of breast meat (Table 3) showed almost the same values

of dry matter at 49 days but significantly higher (P<0.001) in SA31 at 90 days. Holcman *et al.* (2003) reported also a higher content of dry matter in the breast meat of SA51 broilers than in SA31. Fanatico *et al.* (2005) showed significantly higher dry matter in fast-growing hybrids but they compared birds of the same weight but at different ages.

However, age (maturity) significantly affects the content of dry matter in breast meat. At both ages, the content of fat was significantly higher (P<0.01) in SA31, which corresponds with the findings of Castellini *et al.* (2002) and Cong *et al.* (2022). According to Lonergan *et al.* (2003), the breast meat of modern fast-growing broilers also contained a higher percentage of lipids and a lower percentage of proteins compared with the slow-growing line. Havenstein *et al.* (2003); Sanka *et al.* (2021) suggested that the selection of birds based on their body weight concomitantly promoted fat accretion. On the other hand, Robert (2010) did not observe any increase in breast fat content in fast-growing broilers depending on their age, but they found a significant increase in breast fat content in slow-growing chickens (P<0.01) depending on their age.

There was no significant difference between samples regarding drip losses at 49 days. But at 90 days the drip loss was significantly higher (P<0.001) in SA51 as Debut *et al*. (2003) and Fanatico *et al*. (2005) also reported. Regardless of the age, the genotype had no significant effect on pH 0.5h but pH 24h was significantly higher (P<0.01) in SA51 for both ages. Castellini *et al.* (2003) and Alvarado *et al.* (2005) also reported higher pH in slow-growing chickens. But Debut *et al.* (2003) and Lonergan *et al.* (2003) did not find a significant effect of genotype on pH, these authors did not observe a significant difference between slow and fast-growing chickens in L*, a*, b*, either. In this experiment the meat colour as an indicator of meat quality was also affected by genotype. The L* values of the breast were significantly higher at both ages in SA31 (49 days P<0.05; 90 days P<0.001). The same effect of genotype on L* was reported by Debut *et al*. (2003). Grashorn and Clostermann (2002) observed the significantly lowest L* in broilers with the significantly lowest live weight, but only at 84 days of age (not at 70 days). The SA51 had higher redness (a*) at 49 days (P<0.05) but at 90 days the difference was not significant. Debut *et al.* (2003) did not observe a significant difference between slow and fast-growing lines in a* values, either. Significantly higher (P<0.01) b* values were found at both ages in SA51, which confirmed the effect of genotype on this characteristic (Debut *et al*., 2003; Lonergan *et al*., 2003; Fanantico *et al*., 2005). The colour difference was apparent not only by instrumental means but was also visible and confirmed by sensory evaluation. The b* values of skin were also significantly higher in SA51 (49 days P<0.05; 90 days P<0.01). The yellowness of the Sasso birds may be related to the increased foraging of plant material.

Table 4. Sensory quality of breast meat

Breast meat quality	Day of age	SA51	SA31	P
Colour	49	35.69[a]	50.04[b]	***
	90	52.78[a]	63.09[b]	***
Flavour	49	49.28	54.17	ns
	90	33.50	38 .11	ns
Texture	49	54.22[a]	44.53[b]	**
	90	47.97	55. 17	ns
Juiciness	49	64.00	60.84	ns
	90	38.89	47.23	ns
Taste	49	51.05	50.88	ns
	90	39.43[a]	48.00[b]	**
Overall acceptability	49	56.38	53.32	ns
	90	47.98[a]	56.19[b]	**

The results of sensory quality are also shown in Table 4. At both ages of 49 days and 90 days, breast meat was significantly darker (P<0.001) in SA51. The breast meat of SA51 was tougher (P<0.01) at 49 days, but at 90 days there was no significant difference in the texture of breast meat between SA31 and SA51. Brown *at al*. (2008) reported significantly less tough (P<0.01) breast meat from ISA 657 than Ross. The two genotypes delivered no significant difference in flavour. The

intensity of flavour increased with age in both genotypes, which was reviewed by Horsed *et al.* (2005). There were no significant differences between genotypes in juiciness at both ages. The overall acceptability was significantly better (P<0.01) in SA51 at 90 days of age, but at 49 days there was no difference between genotypes. Castellini *et al.* (2003) also showed an overall preference for slow-growing birds in com parison with fast-growing ones.

Some authors (Berri *et al.*, 2005; Fanatico *et al.*, 2005) drew a different conclusion concerning the effect of genotypes on meat quality, but they compared slow and fast-growing chicken at different ages but at the same weight. Increasing the age of slaughter affects the meat quality (Horsted *et al.*, 2005; Sanka *et al.*, 2021). Alvarado *et al.* (2005) also reported some similar results (pH, L*, b*), but they compared different genotypes bred in different conditions (diets, age at slaughter). In addition to genotypes, both the diet and the age also have an effect on sensory attributes, mainly on texture and appearance.

In organic, free-range or Label Rouge systems there is no advantage in improved growth rate, since birds cannot be slaughtered before a specified age and the body weight of fast-growing hybrids at these ages exceeded the requirements of the market. Males seem to offer utility for an alternative system of poultry meat production. Of course, the rate of growth is lower in comparison with slow-growing chickens and the meat yield would also be lower, but the meat quality of Sasso males is higher mainly due to the fat content. Colour, taste and overall acceptability seem to be influenced by genotype to the greatest extent, while the Sasso males demonstrate superior attributes. The quality of meat was comparable or even higher in comparison with fast-growing chickens. This is completely consistent with the conclusion of Sosnówka *et al.* (2017) that the organically raised chickens were characterised by higher body weight, better feed conversion and more favourable fatty acid profile of the muscles compared to the conventionally reared birds

4. CONCLUSIONS

Hight-weight line (SA31) had significantly higher parameters such as live weight, carcass percentage, breast meat percentage and belly fat percentage at both time points (49 and 90 days old) compared to low-weight line (SA51). However, the meat quality of SA51 at 90 days of age was significantly better than that at 49 days of age and compared with SA31.

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POLYMORPHISMS OF CANDIDATE GENES RELATED TO GROWTH RATE AND MEAT QUALITY IN VIETNAMESE NATIVE FATTY PIG BREED "I"

*Phan Thi Tuoi[1], Nguyen Thai Anh[2], Ha Xuan Bo[2], Tran Xuan Manh[3], Nguyen Van Phu[3], Nguyen Van Hung[3], Nguyen Hoang Thinh[2] and Do Duc Luc[2]**

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ABSTRACT

The aim of the study was to investigate the polymorphisms in the *PIT1, H-FABP, CAST, PIK3C3, MYOG* and *GHRH* genes in I pigs. Ear tissue samples were collected from 277 individuals raised at Phu Tho Dabaco Breeding Pig farm in Phu Tho province, Vietnam. The interested polymorphisms of *PIT1* (*RSaI*), *H-FABP* (*HinfI*), *CAST (RSaI, HinfI, MspI), PIK3C3 (Hpy8I) MYOG* (*MspI*) and *GHRH (AluI)* were identified by PCR-RFLP technique. Three genotypes AA, AB, BB were observed in *PIT1* with high frequencies of AA and AB genotypes (0.434 and 0.520, respectively). For *H-FABP*, the frequencies of HH, Hh and hh genotypes were 0.240, 0.383 and 0.377, respectively. Three genotypes at each polymorphism of *CAST* were detected including CC, CD, DD *(MspI)*, AA, AB, BB (*HinfI*) and EE, EF FF (*RsaI*) with frequencies of 2 alleles ranged from 0.442 to 0.558. *PIK3C3* appeared with 3 genotype CC, CT, and TT with frequencies of 0.140, 0.587 and 0.273, respectively. No individual with BB genotype of *MYOG* and *GHRH* genes was found in the population. The HWE were existed in *MYOG* and *GHRH* (P>0.599), while the genotypic frequencies of *PIT1, H-FABP, CAST* and *PIK3C3* were deviated. The same trend was observed in female population. For male pigs, genotypic frequencies of all studies genes were consistent with HWE, except for *MYOG*. The polymorphisms of *PIT1, H-FABP, CAST, PIK3C3, MYOG* and *GHRH* genes were various in I pig population, 4 of 6 genes were inconsistent with HWE, that indicated inbreeding in the population.

Keywords: *Native pigs, polymorphisms, Hardy–Weinberg equilibrium.*

TÓM TẮT

Đa hình của một số gen ứng viên liên quan đến sinh trưởng và chất lượng thịt của lợn Ỉ Việt Nam

Thí nghiệm được tiến hành nhằm khảo sát đa hình đối với gen *PIT1, H-FABP, CAST, PIK3C3, MYOG* và *GHRH* ở lợn Ỉ. Mẫu mô tai được thu thập tử 277 cá thể lợn Ỉ nuôi tại công ty TNHH lợn giống Dabaco Phú Thọ. Đa hình của *PIT1* (*RSaI*), *H-FABP* (*HinfI*), *CAST (RSaI, HinfI, MspI), PIK3C3 (Hpy8I) MYOG* (*MspI*) và *GHRH (AluI)* được xác định bằng kỹ thuật PCR-RFLP. Ba đa hình kiểu gen được phát hiện ở gen *PIT1* là AA, AB và BB, trong đó kiểu gen AA và AB có tần số vượt trội (0,434 and 0,520). *H-FABP* ở lợn Ỉ xuất hiện 3 kiểu gen HH, Hh và hh với tần số lần lượt là 0,240; 0,383 and 0,377. Cả 3 kiểu gen trên mỗi vị trí đa hình của gen *CAST* bao gồm CC, CD, DD (*MspI*), AA, AB, BB (*HinfI*) and EE, EF FF (*RsaI*) đều xuất hiện trên quần thể lợn Ỉ với tần số của 2 allen trong khoảng từ 0,442 đến 0,558. *PIK3C3* xuất hiện 3 đa hình CC, CT and TT với tần số lần lượt là 0,140; 0,587 and 0,273. Không có cá thể nào mang kiểu gen BB của gen *MYOG* và *GHRH* được tìm thấy trên lợn Ỉ trong nghiên cứu này. Tần số kiểu gen trên các gen *MYOG* and *GHRH* tuân theo định luật Hardy–Weinberg (P>0.599), nhưng không duy trì đối với các gen *PIT1, H-FABP, CAST* và *PIK3C3*. Xu hướng này cũng được phát hiện trên quần thể lợn cái. Đối với lợn đực, trừ gen *MYOG*, đa hình

[1] Hong Duc University
[2] Vietnam National University of Agriculture
[3] Dabaco Nucleus Breeding Pig Company
* Corresponding Author: Assoc. Prof. Dr. Do Duc Luc, Department of Animal Breeding and Genetics, Faculty of Animal Science, Vietnam National University of Agriculture, Tel.: (84) 912370193. Email: ddluc@vnua.edu.vn

của tất cả các gen còn lại trong nghiên cứu này đều tuân theo định luật Hardy–Weinberg. Đa hình các gen *PIT1, H-FABP, CAST, PIK3C3, MYOG* và *GHRH* xuất hiện đa dạng trên quần thể lợn Ỉ, trong đó 4 trên 6 gen nghiên cứu có đa hình không tuân theo định luật Hardy–Weinberg, điều này cho thấy có cận huyết trong quần thể lợn Ỉ.

Từ khóa: *Lợn bản địa, đa hình, cân bằng Hardy-Weinberg.*

1. INTRODUCTION

From biodiversity conservation point of view, selection based on high performance, lean meat percentage and low feed conversion ratio is also known as a reason of genetic diversity erosion in pig breeds. In Vietnam, of 26 native pig breeds known to exist, five indigenous pig breeds have become extinct, and nine are severely endangered by extinction (Jica and Nafro, 2020). "I" pig, a Vietnamese native pig breed, is at high risk of extinction (Le Viet Ly, 1999). They have excellent adaptability to ecological and husbandry conditions, poor-quality feeds, and disease resistance. In addition, meat of I pigs is tasty and flavorful, that tends to be in favor by local customers (Jica and Nafro, 2020). Nevertheless, due to the disadvantages of reproductive and growth performance, the number of I pigs is rapidly decreasing, only a few individuals of I pigs is continuously reared nowadays (Dang-Nguyen *et al.*, 2010). To prevent the extinction of indigenous pig breeds and genetic erosion, conservation of I pig breed is imperative need. To select the individuals and improve productive performance of I pigs for sustainable conservation program, integration of marker assisted selection into traditional breeding techniques is a necessary.

Single nucleotide polymorphisms (SNPs) have been used as DNA markers in pigs for breeding selection. Various candidate genes related to growth rate and meat quality in pigs has been identified (Zsolnai *et al.*, 2013) such as Pituitary-specific transcription factor (*PIT1*), Heart fatty acid-binding protein (*H-FABP*), Calpastatin (*CAST*), Porcine phosphoinositide-3-kinase, class 3 (*PIK3C3*), Myogenin (*MYOG*), Growth hormone-releasing hormone (*GHRH*).

The polymorphisms of favorable candidate genes including *PIT1*, *H-FABP*, *CAST*, P*IK3C3*, *MYOG* and *GHRH* were detected in many pig breeds and confirmed the association with economic traits. However, no information about the status of above genes and their polymorphisms in I pig is available. The objective of this study was to investigate the single nucleotide polymorphisms present in the *PIT1*, *H-FABP*, *CAST*, *PIK3C3*, *MYOG* and *GHRH* genes in I pigs.

2. MATERIAL AND METHODS

The "I" pigs used in this work were raised in Phu Tho Dabaco Breeding Pig farm in Phu Tho province. The ear tissue samples of 227 individuals were collected, then kept in collection tube and transported to the laboratory and stored at -20^{0}C for DNA extraction.

Genomic DNA isolation from ear tissue was performed following standard procedures of Green and Sambrook (2012) with slight modifications for suitable of laboratory condition**.** The determination of concentration and purity of DNA was test by agarose gel electrophoresis and measured using NanoDrop 2000 *Spectrophotometer device (Thermo fisher scientific company)*, then DNA working solution (concentration of 50 ng/µl) was prepared by dilution of DNA stock solution with dd H_2O for PCR reaction.

The genotype of *PIT1, H-FABP, CAST, PIK3C3, MYOG* and *GHRH* genes with interested SNPs were identified by PCR-RFLP method of Yu *et al.* (1994), Gerbens *et al.* (1998), Ernst *et al.* (1998), Kim *et al.* (2005), Soumillion *et al.* (1997) and Baskin and Pomp (1997) respectively. Information on primer sequences, PCR product size, restriction enzymes and allele sizes are shown in Table 1.

Table 1. Primer sequences, PCR product size, restriction enzymes and allele sizes

Genes	Primer sequence	PCR product size (bp)	Restriction enzyme	Allele size (bp)
PIT1	F-5′-AGTGTAGCCAGAGCATCT-3′ R-5′-ACCACATCTGCACACTCA-3′	1746	*RSaI*	E: 710 F: 388, 322
H-FABP	F-5′GGACCCAAGATGCCTACGCCG-3′ R-5′CTGCATCTTTGACCAAGAGG-3′	693	*HinfI*	H: 339, 172, 98, 59, 25 H: 339, 231, 98, 25
CAST	F-5′GCGTGCTCATAAAGAAAAAGC-3′ R-5′TGCAGATACACCAGTAACAG-3′	1423	*HinfI*	A: 646, 372, 200, 174 B: 503, 372, 200, 174
			RSaI	E: 649, 240, 183, 162, 89 F: 649, 370, 183, 162, 89
			MspI	C: 646, 502, 275 D: 502, 370, 275
PIK3C3	F-5′-ATTTCGTCTAGAC CTGTCCG-3′ R-5′-TGAATCTGTTCTACCACCGC-3′	102	*Hpy8I*	C: 67, 35 T: 102
MYOG	F-5′-TCAGGAAGAACTGAAGGCTG-3′ R-5′-GTTTCCTGGGGTGTTGC 3′	353	*MspI*	A: 353 B: 219, 134
GHRH	F-5′ GTAAGGATGC(C/T)(A/G)CTCTGGGT 3′ R-5′ TGCCTGCTCATGATGTCCTGGA 3′	445	*AluI*	A: 250, 100 B: 230, 100

Hardy-Weinberg equilibrium (HWE) was performed using χ^2 with one of degree of freedom or Fisher exact test when at least one expected count less than 5. P-values >0.05 were considered as consistent with HWE. The data were analyzed using SAS 9.0 (SAS, 1989).

3. RESULTS AND DISCUSSION

Table 2. Genotypic and allelic frequencies of *PIT1, H-FABP, CAST, PIK3C3, MYOG* and *GHRH* genes

Genes	n	Genotype			Allele		χ^2	P
		AA	AB	BB	A	B		
PIT1	196	85	102	9	272	120	9.923	0.002
		0.434	0.520	0.046	0.694	0.306		
		HH	Hh	hh	H	h		
H-FABP	167	40	64	63	144	190	7.989	0.005
		0.240	0.383	0.377	0.431	0.569		
		CC	CD	DD	C	D		
CAST/MspI	220	52	93	75	197	243	4.638	0.031
		0.236	0.423	0.341	0.448	0.552		
		AA	AB	BB	A	B		
CAST/HinfI	227	65	95	67	225	229	6.026	0.014
		0.286	0.419	0.295	0.496	0.504		
		EE	EF	FF	E	F		
CAST/RsaI	163	58	66	39	182	144	5.224	0.022
		0.356	0.405	0.239	0.558	0.442		
		CC	CT	TT	C	T		
PIK3C3	172	24	101	47	149	195	6.594	0.010
		0.140	0.587	0.273	0.433	0.567		
		AA	AB	BB	A	B		
MYOG	151	148	3	0	299	3	-	1.000
		0.980	0.020	0	0.990	0.010		
		AA	AB	BB	A	B		
GHRH	210	175	35	0	385	35	-	0.424
		0.833	0.167	0	0.917	0.083		

Contents in each cell: (1st line) genotype, (2nd line) observed count, (3rd line) genotypic or allelic frequency; the symbol "–" in the column χ^2 indicated using Fisher exact test

The genotypic and allelic frequencies of PIT1, H-FABP, CAST, PIK3C3, MYOG and GHRH genes for all studied I pigs are shown in Table 2. These frequencies are presented according to the gender (female and male) in Table 3.

The polymorphisms were observed for all studied genes; however, BB genotype were not found for *MYOG* and *GHRH* for the I population (Table 2). The same trend was observed for the female while only AA genotype was detected in *MYOG* (Table 3).

HWE test were performed for all pigs (Table 2) and separately for females and males (Table 2). HWE was existed for *MYOG* (P=1.0), *GHRH* (P=0.424) for I population. Considering female and male populations, the genotypic frequencies of all studied genes were found to be in HWE (P>0.599) except *MYOG* with only AA genotype (Table 3). The deviation from HWE indicated inbreeding in the population (Wigginton *et al.*, 2005).

For *PIT1*, AA and AB frequencies were represented the main part of 0.954. The allelic frequencies of A and B were 0.694 and 0.306, respectively. These frequencies were similar for female and male (Table 3). This result was consistent with study of Hà Bích Hồng *et al.* (2021) on black pigs in Vietnam. The study of Yu *et al.* (1994) confirmed that allele B was not found in Chinese pig breeds (Fengjing, Meishan and Minzhu) while this allele was obtained in other exotic breeds (Duroc, Landrace, Hampshire and Duroc).

Alleles H and h of *H-FABP* appeared with frequencies of 0.40 and 0.6 respectively for female, and 0.5 for each allele for male (Table 3). HWE was observed in the male population (P=0.267) while not in the female (P=0.010). The similar result was obtained in native złotnicka spotted pig in Poland (Jankowiak *et al.*, 2010). Chen *et al.* (2014) found both alleles (H and h) in local pigs (Yanan and Jinhua) and exotic pigs (Duroc, Landrace and Yorkshire) in China.

Table 3. Genotypic and allelic frequencies of different genes according to genders

Genes	Female								Male							
	n	Genotype			Allele		χ^2	P	n	Genotype			Allele		χ^2	P
		AA	AB	BB	A	B				AA	AB	BB	A	B		
PIT1	144	63	75	6	201	87	7.966	0.005	52	22	27	3	71	33	2.048	0.152
		0.438	0.521	0.041	0.698	0.302				0.423	0.519	0.058	0.683	0.317		
		HH	Hh	hh	H	h				HH	Hh	hh	H	h		
H-FABP	115	25	42	48	92	138	6.576	0.010	52	15	22	15	52	52	1.231	0.267
		0.217	0.365	0.418	0.400	0.600				0.289	0.423	0.288	0.500	0.500		
		CC	CD	DD	C	D				CC	CD	DD	C	D		
CAST/MspI	141	34	55	52	123	159	6.038	0.014	79	18	38	23	74	84	0.092	0.762
		0.241	0.390	0.369	0.436	0.564				0.228	0.481	0.291	0.468	0.532		
		AA	AB	BB	A	B				AA	AB	BB	A	B		
CAST/HinfI	143	37	59	47	133	153	4.170	0.041	84	28	36	20	92	76	1.531	0.216
		0.259	0.413	0.328	0.465	0.535				0.333	0.429	0.238	0.548	0.452		
		EE	EF	FF	E	F				EE	EF	FF	E	F		
CAST/RsaI	111	39	42	30	120	102	6.300	0.012	52	19	24	9	62	42	0.089	0.765
		0.351	0.378	0.271	0.541	0.459				0.365	0.462	0.173	0.596	0.404		
		CC	CT	TT	C	T				CC	CT	TT	C	T		
PIK3C3	106	11	66	29	88	124	8.445	0.004	66	13	35	18	61	71	0.294	0.588
		0.104	0.623	0.273	0.415	0.585				0.197	0.530	0.273	0.462	0.538		
		AA	AB	BB	A	B				AA	AB	BB	A	B		
MYOG	104	101	3	0	205	3	0.022	1.000	47	47	0	0	94	0		NA
		0.971	0.029	0	0.986	0.014				1.000	0	0	1.000	0.000		
		AA	AB	BB	A	B				AA	AB	BB	A	B		
GHRH	160	135	25	0	295	25	-	0.599	50	40	10	0	90	10	-	0.868
		0.844	0.156	0	0.922	0.078				0.800	0.200	0	0.900	0.100		

NA: not available to test

Three restriction enzymes *MspI*, *HinfI* and *RsaI* were used for the *CAST* at 3 polymorphisms including *CAST/MspI*, *CAST/HinfI* and *CAST/RsaI*. At each polymorphism, 3 genotypes were identified for male and female (Table 3); 2 alleles were detected and ranged from 0.442 to 0.558 (Table 1). HWE was found in male population (P>0.216) but not in female (P<0.041). Two polymorphisms *CAST/HinfI* and *CAST/MspI* was studied in Mong Cai pig and two alleles at each site were detected (Nguyen, 2012). Wang *et al.* (2007) found only DD genotype at *CAST/HinfI* and FF genotype at *CAST/MspI*) in Meishan pig.

Genotypic, allelic frequencies and HWE of *PIK3C3* had a similar tendency as for *CAST* (Tables 2, 3). Genotypic and allelic frequencies of *PIK3C3* in Duroc were published by Hirose *et al.* (2011). The authors confirm that genotype TT was low from 0.44 to 7.6 according to generations.

Beside BB genotype of *MYOG* and *GHRH* not presented in the studied population (Table 2) nevertheless the genotypic frequencies of these genes were found to be in HWE (P>0.424). Additionally, only allele A of *MYOG* was found in the males (Table 3) and therefore HWE could not be tested. Inversely, both alleles A and B of *GHRH* were found in Landrace, Large White and Piétrain pigs (Piórkowska *et al.*, 2013; Balatsky *et al.*, 2016). In addition, 3 genotypes of *MYOG* were found in Yorkshire pig (Do Vo Anh Khoa and Nguyen Thi Dieu Thuy, 2012) and in hybrid pigs between Piétrain, Duroc, Landrace and Large White (Stupka *et al.*, 2012).

Based on the above reviewed studies, the genotypic and allelic frequencies of *PIT1*, *H-FABP*, *CAST*, *PIK3C3*, *MYOG* and *GHRH* genes were largely varied according to breeds, strategy of selection. Inbreeding is a reason of deviation from HWE. In our study, the small population of I pigs in the beginning might be led to inbreeding and therefore HWE was not detected for the almost studied genes.

4. CONCLUSIONS

All polymorphisms of investigated genes (*PIT1, H-FABP, CAST, PIK3C3, MYOG* and *GHRH*) were found in I pig population, except for MYOG and GHRH genes with only BB genotype. The HWE equilibrium were existed in *MYOG* and *GHRH*, while the genotypic frequencies of *PIT1, H-FABP, CAST* and *PIK3C3* were deviated. The same trend was observed in female population. For male pigs, genotypic frequencies of all studies genes were consistent with HWE, except for *MYOG*. HWE was not detected for the almost studied genes indicated inbreeding in the I pig population.

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THE MORPHOLOGICAL CHARACTERISTICS AND REPRODUCTIVE PERFORMANCE OF LANG DONG KHE PIG IN CAO BANG PROVINCE

*Bui Huy Doanh[1], Nguyen Thi Huyen[2], Hoang Thi Hieu[2], Nguyen Van Trung[3], Do Duc Luc[1] and Nguyen Hoang Thinh[1]**

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ABSTRACT

Lang Dong Khe pig is one of the indigenous pig breeds with the excellent meat quality, sweet taste, and delicious. The animals are located in Dong Khe district, Cao Bang province. These pigs adapt to local natural conditions in the mountainous area. This study aimed to assess the morphological characteristics and reproductive performance of the Lang Dong Khe pigs raised in the condition of households in Cao Bang province. The results showed that the hairs of Lang Dong Khe pig are relatively thick and coarse, with a black-and-white color. The hair and skin on the head and ears are black, with white patches shaped from the forehead to the snout. Lang Dong Khe pigs have a straight face shape, accounting for 100%, and have mainly a long, pointed snout accounting for 74.44%. The pigs have small and straight ears, similar to other indigenous pig breeds. The reproductive traits were varied from the first to the fourth parity (P<0.05) such as number born alive from 7.35 to 9.45 pigs, number weaning from 6.24 to 8.17 pigs. Individual body weight at birth and weaning were approximately 500g and 5kg, respectively. The intervals between parities were from 162.88 to 171.83 days.

Keywords: *Lang Dong Khe, indigenous pig, reproduction performance.*

TÓM TẮT

Đặc điểm hình thái và năng suất sinh sản của lợn Lang Đông Khê tại tỉnh Cao Bằng

Lợn Lang Đông Khê là một trong những giống lợn bản địa có chất lượng thịt tốt, vị ngọt và thơm, ngon. Giống lợn này được nuôi chủ yếu ở huyện Đông Khê, tỉnh Cao Bằng. Lợn Lang Đông Khê thích nghi với điều kiện tự nhiên địa phương ở khu vực miền núi. Nghiên cứu này nhằm đánh giá đặc điểm hình thái và năng suất sinh sản của lợn Lang Đông Khê được nuôi trong điều kiện chăn nuôi nông hộ tại tỉnh Cao Bằng. Kết quả cho thấy, lông của lợn Lang Đông Khê tương đối dày và thô, có màu đen và trắng. Lông và da trên đầu và tai có màu đen, với các mảng trắng trải dài từ trán đến mõm. Lợn Lang Đông Khê có kiểu hình mặt thẳng chiếm 100%, chủ yếu là mõm dài và nhọn chiếm 74,44%. Lợn có tai nhỏ và thẳng, giống với các giống lợn bản địa khác. Các đặc điểm sinh sản thay đổi từ lứa đẻ thứ nhất đến lứa đẻ thứ tư (P<0,05) cụ thể: số lợn sinh ra còn sống trên ổ từ 7,35 đến 9,45 con và số lợn cai sữa từ 6,24 đến 8,17. Khối lượng lợn con lúc mới sinh và cai sữa lần lượt khoảng 500g và 5kg. Khoảng cách giữa các lứa đẻ từ 162,88 đến 171,83 ngày.

Từ khóa: *Lang Đông Khê, lợn bản địa, khả năng sinh sản.*

1. INTRODUCTION

In Vietnam, there are numerous extraordinary indigenous pig breeds such as Mong Cai, Muong Khuong, Hung, Lung Pu. Black, Huong, Meo, Van Pa, Ba Xuyen, Tap Na, Co, Ha Lang... (Nguyen Van Duc, 2012). However, the proportion of indigenous pigs has decreased from 72% of the total herd in 1994 to only 12% in 2006 (Lemke *et al*., 2008). Many local pig breeds were vulnerable or endangered, and especially endangered (Pham *et al*., 2014). The Lang Dong Khe is an indigenous black and white pig with delicious

[1] Vietnam National University of Agriculture
[2] Crop Production and Livestock Production Unit, Cao Bang Province
[3] National Institute of Animal Science
*Corresponding Author: Assoc Prof. Nguyen Hoang Thinh, Faculty of Animal Science, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi. Tel: (84) 968643535; Email: nhthinh@vnua.edu.vn

meat quality. These pigs have mainly been raised in the mountainous area Thach An district, Cao Bang province, Vietnam. Lang Dong Khe pigs have many advantages that exotic pigs do not have such as adaptation to natural local conditions, disease resistance, low cost, and good meat quality (Bui Thi Thom *et al*., 2021). However, in the past 20 years, due to the massive development of hybrid pigs, Lang Dong Khe with its small body size and low growth rate has gradually declined. Many original characteristics of purebred indigenous pigs are being gradually lost due to genetic erosion because of crossbreeding and endangered. Therefore, raising Lang Dong Khe pig as well as other indigenous pig breeds is considered one of the important factors to protect biodiversity and genetic diversity, contributing to the sustainable development of the livestock industry farming in Vietnam. The objective of this study is to determine the morphological characteristics and reproductive performance of Lang Dong Khe pigs in Cao Bang province to contribute more information about indigenous pigs in general and Dong Khe village pigs in particular.

2. MATERIALS AND METHODS

2.1. Materials and methods

Lang Dong Khe pigs raised in small householders in Thach An district, Cao Bang province were used in this study. We carried out interviews with farmers and ascertained the visually observable characteristics of Lang Dong Khe pigs. The morphological characterization was conducted on 90 pigs at age of 6 to 24 months based on hair color, texture, and appearance characteristics. Reproduction performances were collected from 46 sows in 4 litters from September 2020 to April 2022. The sows and piglets were always kept in an individual pen (before mating, during pregnancy and lactation). The reproduction traits were age at first farrowing (day), body weight at first mating (kg), age at first farrowing (day), pregnancy duration (day), number of pigs born (piglet), number of pigs born alive (piglet), number of pigs weaned (piglet), survival rate at birth (%), survival rate to weaning (%), weaning time (day), litter weight at birth (kg), individual body weight at birth (kg), litter weight at weaning (kg), individual body weight at weaning (kg) and duration of cycle (day).

2.2. Statistical analyses

The data was performed using Minitab 16 software. The values in the table are number of observation (n), arithmetic mean (Mean) and standard error (SE). The fowling statistical model was used to evaluate effect of parity on reproduction traits: $y_{ij} = \mu + parity_i + \varepsilon_{ij}$, *where:* y_{ij} *is reproductive traits of* j^{th} *sow at* i^{th} *parity, Parity* i^{th} *is effect of* i^{th} *parity,* ε_{ij} *is residual error*. Pairwise comparison was made using Duncan test.

3. RESULTS AND DISCUSSION

3.1. Morphological characteristic of the Lang Dong Khe pig

The hair of Lang Dong Khe pig is relatively thick and straight with a black-and-white color. On the head, the white patches are from the forehead to the nose. The distribution of the white and back are divided into three groups: In the group one, white patches are covering the entire abdomen and 4 legs, with black from the head to back (Figure 1a). A total of 54 pigs are in this group equivalent of 60.00% (Table 1). In the group two, white patches are extending all the way through the abdomen, with black spots on the back (Figure 1b) with 32.22% (Table 1). For the group three, white is extending from the back to the abdomen and 4 legs, black spots on the buttocks (Figure 1c), accounted 7.78% (Table 1).

Our result is consistent with the study on Dong Khe pigs reported by Bui Thi Thom *et al*. (2021). In comparison with other indigenous pig breeds, Lang Dong Khe pigs have certain difference. For Mong Cai pig, head is black with small and upright ears. Patches in the black color are elsewhere on the body with a white band running from one side of the abdomen over the shoulder to other side of the abdomen, making a black saddle over the middle of its concave back (Nguyen Van Duc *et al*., 2004).

Table 1. The morphological characteristics of Dong Khe pigs

Index	Character	Number	Ratio (%)
General	The white patches covering the entire abdomen and 4 legs, with black from the head to back	54	60
	The white extending all the way through the abdomen, with black spots on the back	29	32.22
	The white extending from the back to the abdomen and 4 legs, black spots on the buttocks	7	7.78
Head	The hair and skin of the head, ears are black, with white streaks shaped from forehead to snout	90	100
Snout	White, pointed and long	90	100
Abdomen	White color	90	100
Leg	Small white legs	90	100
Tail	Black color	90	100

Lang Dong Khe pig has a straight face shape, accounting for 100%, and has mainly a long, pointed snout accounting for 74.44% (Table 1). It is similar to other indigenous pig breeds. The results of our study on Lang Dong Khe pigs are lower. The pig has a small and straight ear, like to other indigenous pig breeds. Lang Dong Khe breed is closely related to the Mong Cai breed (Bui Thi Thom *et al*., 2021). The proportion of pigs with 12 and 14 teats were 57.78% and 23.33% respectively. According to Nguyen Van Trung *et al*., 2021, the Hung and Meo pigs with 10 teats were 83.80 and 65.22%, respectively.

Table 2. The morphological of Dong Khe pigs

Item	Character	Number	%
Hair	straight, thick	90	100
Face	straight	90	100
Snout types	long	67	74.44
	short	23	25.56
Ear types	small, erectile	90	100
Abdomen	big	82	91.11
	slim	8	8.89
Back	straight	3	3.33
	hammock	87	96.67
Walking style	nail-walking	90	100
Teat number	10	15	16.67
	11	2	2,22
	12	52	57.78
	14	21	23.33

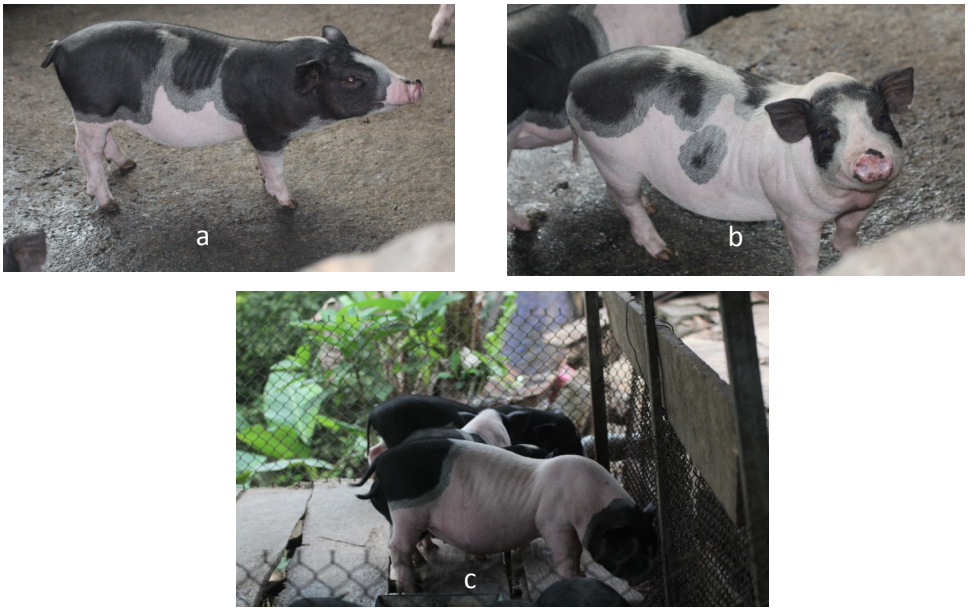

Figure 1. Morphological characteristics of Dong Khe pigs

3.2. Reproductive performance of the Lang Dong Khe pig

The results of reproductive physiology of Lang Dong Khe pig are showed in Table 2. The age and the first mating of Lang Dong Khe pig was 212.36 days of age. The age at the first farrowing of Lang Dong Khe pig were at 326.26 days of age. In comparison with other Vietnamese pig breeds, the age at first farrowing Dong Khe pig was earlier. The age at first farrowing of Co pig were 388.2 days of age (Phan Thi Tuong Vy, 2014), the age at first farrowing of Mong Cai pig and Van Pa pig were 354 days of age and 376.34 days of age, respectively (Nguyen Van Duc *et al*., 200); Tran Van Do, 2006). Xayalath *et al*. (2021) reported that the age of first mating and first farrowing of indigenous Lao pig were 7.42 months and 11.22 months, respectively. The age of first farrowing in Dong Khe pig was longer than that of Huong pig (283.30 days) (Nguyen Hoang Thinh *et al*., 2019), Tap Na pig (313.95 days) (Nguyen Van Duc, 2013).

Table 3. Reproductive physiology of Lang Dong Khe pig

Parameters	Unit	n	Mean±SE
Age at first mating	day	50	212.36±3.91
BW at first mating	kg	50	44.60±2.32
Age at first farrowing	day	46	326.26±4.10
Pregnancy duration	day	46	114.00±0.76

The reproductive traits of Lang Dong Khe are presented on the Table 4. The results of this study showed that the number of pigs born per liter of Lang Dong Khe was lowest in the first parity (7.35 piglets) and highest in the fourth parity (9.45 piglets) (P<0.05) and no difference between the second and third parity (P>0.05). This trend was present in the survival piglets at birth.

Table 4. Reproductive traits of Lang Dong Khe pigs (Mean±SE)

Variable	Unit	1st parity (n=46)	2nd parity (n=42)	3rd parity (n=42)	4th parity (n= 42)
Number born/litter	pig	7.35^{c}±1.2	8.52^{b}±1.6	8.95^{ab}±1.71	9.45^{a}±1.73
Number born alive/litter	pig	6.65^{c}±1.08	7.79^{b}±1.09	8.26^{ab}±1.19	8.79^{a}±1.26
Number weaned/litter	pig	6.24^{c}±0.9	7.29^{b}±0.81	7.71^{ab}±0.89	8.17^{a}±1.12
Survival rate at birth	%	91.72±10.34	92.53±9.76	93.34±8.54	93.48±8.33
Survival rate to weaning	%	93.77±7.45	93.9±7.51	94.22±7.48	94.2±8.35
Weaning time	day	39.61^{a}±3,59	32.86^{b}±3.14	31.88^{bc}±2.85	30.93^{c}±2.61
Litter weight at birth	kg	4.02^{b}±4.69	4.07^{b}±0.58	4.33^{a}±0.6	4.64^{a}±0.7
Individual body weight at birth	g	505^{b}±40.21	523.57^{ab}±36.68	526.67^{a}±34.12	530.95^{a}±36.01
Litter weight at weaning	kg	32.6^{c}±4.43	39.21^{b}±4.3	41.76^{ab}±4.92	43.45^{a}±8.86
Individual body weight at weaning	kg	5.25^{b}±0.38	5.39^{ab}±0.34	5.42^{ab}±0.32	5.46^{a}±0.33
Duration of cycle	day		171.83^{a}±4.11	164.17^{b}±3.24	162.88^{b}±2.83

Number weaned pigs was highest in parity 3 and 4 (7.71 and 8.17 piglets) and lowest in parity 1 (6.24 piglets) (P<0.05). Compared to the study on Dong Khe pigs by Bui Thi Thom *et al*. (2021), the result of present study was higher in first parity (6.98 piglets) but lower in second parity (7.79 piglets) and third parity (8.55 piglets). According to Vu Dinh Ton *et al*. (2012), the Number born, survival piglets after 24 hours/litter and number of weaned pigs per litter of the Ban pig were 7.33, 6.67 and 5.80 piglets, respectively. The Number of pigs born of Tap Na pig was 6.85 piglets (Nguyen Van Duc *et al*., 2002). Number born and number weaned per litter of Van Pa pig were 7.64 and 7.4 piglets, respectively while number weaned per litter of Khua pig was 6.5 piglets (Nguyen Ngoc Phuc *et al*., 2009). That liter size at birth of Huong pig breed was 9.49 piglets while the weaning pigs/litter were 8.59 piglets (Nguyen Hoang Thinh *et al*., 2019).

The litter weight and individual bodyweight at birth of Lang Dong Khe pig were lower in parity 1 and higher in parity 3

and 4 (P<0.05). The individual body weight at weaning was from 5.25kg in the first parity to 5.46kg in the fourth parity. There was no significant difference in these values (P>0.05). While the litter size and birth weight of indigenous Lao pigs were 7.72 piglets and 0.70kg, respectively (Xayalath *et al*., 2021). The observed results in this study showed that reproductive performance of Lang Dong Khe pig is higher than those of Tap Na, Van Pa and Khua pigs but lower than Lao pigs. The duration of cycle was highest in the second parity (171.83 days) and lower in third and fourth parity due to longer weaning time at the first parity than other. The reproductive performance of Lang Dong Khe were higher than the standard of TCVN 9713-2013 in comparison with Mong Cai, Muong Khuong.

4. CONCLUSIONS

The morphological characteristics of Lang Dong Khe pig were determined with black and white color. The general characteristics were white patches extending from the forehead to the snout. The age at the first mating and the first farrowing were 212.36 days and 326.26 days, respectively. The number of piglets per litter at birth increased from 7.35 piglets in the first parity to 9.45 piglets in the fourth parity. The number of weaning pigs per litter were from 6.24 to 8.17 piglets. The body weight at birth was more than 500g and weaning weight was 5.46kg at 31 days of age.

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EXPRESSION OF GROWTH DIFFERENTIATION FACTOR 9 GENE IN PORCINE OVARIAN TISSUE AND CUMULUS OOCYTE COMPLEXES AT DIFFERENT STAGES OF MATUATION *IN VITRO*

*Thi Kim Thao Nguyen[1] and Ngoc Tan Nguyen[1]**

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ABSTRACT

This study aimed to evaluate the mRNA expression of Growth Differentiation Factor 9 (GDF9) gene in ovarian tissue according to the developmental stages of follicle, cumulus oocyte complexes (COCs), cumulus cells (CC) and denuded oocytes (DO) in different stages of porcine oocyte maturation *in vitro*. Samples were collected from ovarian tissue derived from small follicles (SF; <3mm in diameter), medium follicles (MF; 3-7mm) and large follicles (LF; >7mm), COCs at 0, 22 and 44h post maturation culture or CC and DO separately at 0h post maturation culture. Extracted RNA was used to amplify the fragment product of GDF9 with 269bp by one-step RT-PCR, GAPDH gene with 187bp, using as internal control, was also amplified as parallel with GDF9. Semi-quantitative was applied to measure the relative density of bands of target gene in agarose (1.5%) by ImageJ software. The result showed that the expression of GDF9 at mRNA level was lowest in the ovarian tissue derived from small follicles (1,03±0,26), reached to highest level in medium follicles (2.09±0.18) and then declined in large follicles (1.70±0.43). The significant difference was found between the ovarian tissue derived from small follicles and medium follicles (P<0.05). According to maturation process, the expression of GDF9 at mRNA was highest at 0h (1.46±0.36), which was significantly different compared to the other two groups, at 22h (0.51±0.10) and at 44h (0.60±0.10). At the 0h post culture, the expression of GDF9 in the DO was higher than in CC (1.39±0.40 vs. 0.55±0.26, respectively, P<0.05). In conclusion, expression of GDF9 gene is in relation to oocyte maturation in the manner and can be considered as a candidate marker gene for oocyte quality evaluation *in vitro*.

Keywords: *Pig, oocyte, ovarian tissue, cumulus oocyte complexes, Growth Differentiation Factor 9.*

TÓM TẮT

Biểu hiện gen GDF9 ở mô buồng trứng và phức hợp cumulus-tế bào trứng heo theo các giai đoạn phát triển khác nhau trong điều kiện *in vitro*

Mục tiêu của nghiên cứu nhằm đánh giá sự biểu hiện mRNA của gen Growth Differentiation Factor 9 (GDF9) trong mẫu mô buồng trứng theo các giai đoạn phát triển của nang noãn, trên phức hợp tế bào trứng (COC), tế bào cumulus (CC) và tế bào trứng (DO) theo các thời điểm của quá trình nuôi cấy thành thục nhân tế bào trứng heo *in vitro*. Mẫu mô thu nhận chứa nang noãn nhỏ (<3mm), trung bình (3-7mm) và lớn (>7mm), hoặc phức hợp COC thu nhận theo thời điểm: 0, 22 và 44 giờ sau nuôi cấy hoặc tế bào trứng và tế bào cumulus thu nhận theo thời điểm 0 giờ. Ly trích ARN và áp dụng kỹ thuật one-step RT-PCR để khuếch đại đoạn gen mục tiêu của GDF9 với kích thước 269bp, sử dụng đoạn gen GAPDH với kích thước 187bp như là đối chứng nội. Sử dụng kỹ thuật bán định lượng mức độ biểu hiện bằng phần mềm ImageJ để xác định mật độ điểm ảnh cho band biểu hiện mRNA của gen GDF9 và GAPDH trên ảnh điện di. Kết quả cho thấy rằng sự biểu hiện của gen GDF9 ở mức độ mRNA thấp nhất trong mô buồng trứng chứa nang noãn nhỏ (1,03±0,26), cao nhất ở mẫu mô chứa nang noãn trung bình (2,09±0,18) và sau đó giảm ở các nang lớn (1,70±0,43), sự khác biệt có ý nghĩa giữa mô buồng trứng chứa nang noãn nhỏ và trung bình (P<0,05). Theo quá trình nuôi cấy thành thục của tế bào trứng, sự biểu hiện của gen GDF9 ở mức độ mRNA cao nhất ở 0h (1,46±0,36), khác biệt có ý nghĩa so với hai nhóm còn lại ở thời điểm 22h (0,51±0,10) và 44h (0,60±0,10). Tại thời điểm 0h, sự biểu hiện của GDF9 ở tế bào trứng cao hơn ở tế

[1] Nong Lam University in Ho Chi Minh City

* Corresponding Author: Dr. Nguyen Ngoc Tan, senior lecturer. Faculty of Biological Sciences - Nong lam University in HCMc; Tel: (84) 948 993 338; Email: nntan@hcmuaf.edu.vn.

bào cumulus (1,39±0,40 và 0,55±0,26; P<0,05). Sự biểu hiện của gen GDF9 có liên quan đến quá trình thành thục của tế bào trứng và có thể được coi là gen ứng cử để đánh giá chất lượng tế bào trứng trong quá trình nuôi cấy *in vitro*.

Từ khóa: *Heo, tế bào trứng, mẫu mô buồng trứng, phức hợp tế bào trứng, Growth Differentiation Factor 9.*

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). The communication of oocytes and granulosa cells is a bidirectional process; that is, oocytes affect different functions of granulosa cells around oocyte (Pablo *et al*., 2018). The oocyte secreted factors regulate the production of steroid hormones by the expression of different genes in granulosa cells, including the genes encoding the LH receptor (Eppig *et al*., 1997). The first oocyte-specific factor influencing the function of granulosa cells was identified and characterized in mice with targeted deletion of the growth differentiation factor 9 (GDF9) gene, primordial and primary onelayer follicles could be formed, but follicular development beyond the one-layer follicle stage was blocked. And, GDF9 mRNA was synthesized only in oocytes from the primary stage until after ovulation and not in somatic follicular cells in wild-type mouse (Carabatsos *et al*., 1998; Elvin *et al*., 1999).The extended expression of GDF9 throughout the oocyte development suggests that GDF9 affects processes in later stages of follicular development (Elvin *et al*., 2000). The expression of *GDF9* have been reported in rodents, goats, sheeps, buffalo, cattle, pig and humans in oocyte, cumulus cells and ganulosa cells and there is convincing evidence that they are important for ovarian function (Prochzka *et al*., 2004; Paradis *et al*., 2008; Hosoe *et al*., 2009; Li *et al*., 2014; Mester *et al*., 2015; Pan *et al*., 2015; Chang *et al*., 2016; Haas *et al*., 2016; Mishara *et al*., 2016; Silva *et al*., 2016; Kona *et al*., 2018; Pablo *et al*., 2018). In current study, we aimed to evaluate the expression of GDF9 at the mRNA level in porcine ovarian tissue, COC, CC and DO at different stages of maturation *in vitro*.

2. MATERIAL AND METHODS

2.1. Material, chemicals and supplies

Pig 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 Biotechnology and Environment and Faculty of Biological Sciences, Nong Lam University in Ho Chi Minh City from Nov, 2021 to May, 2022.

2.2. Methods

2.2.1. Ovary and Oocyte collection

Collection of ovaries and oocyte aspiration were carried out as described by Nguyen *et al.* (2012). Briefly, porcine ovaries collected from a local abattoir and transported to the laboratory at approximately 30-35°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 were selected to culture for further use.

2.2. *In vitro* maturation

Cumulus-oocyte complexes were washed (three times) in maturation media containing TCM-199 containing Earl's salts, L-glutamine and Sodium bicarbonate (Sigma M4530, USA), supplemented with 10% follicular fluid, 0.8% BSA (Bovine serum albumin), 100 IU/mL Penicillin G sodium salt and 100 IU/mL Streptomycin sulfate salt. Add 10 IU/mL of hCG hormone (human Chorionic Gonadotropin for the first 22h of culture (TCM^+) and no hormone for 22h of culture after (TCM^-). Groups of 20 COCs were placed in 100 µl droplets of maturation media under mineral oil and incubated for 44h at 39°C, 5% CO_2 in air.

2.3. Detection of GDF9 mRNA by Reverse Transcription-Polymerase Chain Reaction

Extraction of mRNA: Total RNA was extracted from samples of ovarian tissue derived from (A) small follicle (SF), (B) medium follicles (MF) and (C) large follicles (LF) or COCs at the different time points of maturation: (D) 0h, (E) 22h and (F) 44h poat culture or (G): cumulus cell (CC) or (H) denuded oocyte (DO) as showed in Figure 1. The extraction mRNA following the TRIsure-Chloroform (Méndez *et al*., 2011).

Primer design: Taking into account the sequences recently published by the National Center for Biotechnology Information (NCBI) and the data base of the porcine genome sequence, specific primers were designed in order to amplify porcine gene coding regions *GDF9* using the online software Primer-BLAST. Additionally, specific primers *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) were also used as an internal control. The primer sequence for GDF9 (NM_001001909.1) with F 5′-GGGTGTACCATCCTCTCGTC-3′ and R 5′- TGTGAACCGGAGAGCCATAC-3′; for GAPDH (AF017079) with F 5′-AGCAATGCCTCCTGTACCAC-3′ and R 5′-AAGCAGGGATGATGTTCTGG-3′. These primers were expected to generate a 269 and 187bp cDNA fragment for GDF9 and GAPDH, respectively.

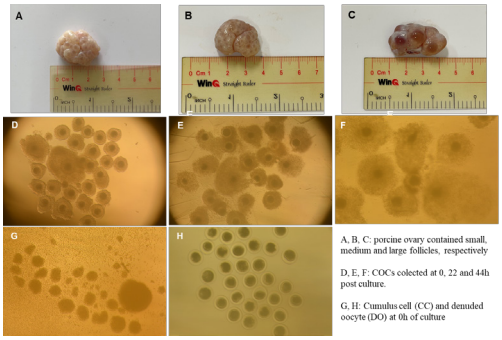

Figure 1. Representative sampled images

Ampplification by the reverse transcription-polymerase chain reaction (RT-PCR): The RT-PCR was carried out by MyTaq One-Step RT-PCR Kit (Bioline). For each sample, the amplification of each gene was run in a separate tube. The reaction conditions were as follows: cDNA synthesis at 45°C for 20 min, predenaturation at 95°C for 1 min, and then 35 numbers of PCR cycles consisting of denaturation (95°C for 10 sec), annealing (59°C for 20 sec), extension (72°C for 30 sec), and final extension (72°C for 7 min).

For semiquantitative RT-PCR: Products of the RT-PCR were separated by electrophoresis on 1.5% agarose gel and visualized by Gelred. The intensity of the objective bands was determined by scanning densitometry using Image J Version 1.29 free software (National Institute of Mental Health, Bethesda, MD). The relative abundance of GDF9 mRNA was expressed as the ratio of GDF9 to GAPDH.

2.4. Contents

2.4.1. Determination of GDF9 expression at the mRNA level in ovarian tissue

Ovarian tissue from SF, MF and SF (Figure 1A, 1B, 1C) was collected separately for extraction of mRNA.

2.4.2. Determination of GDF9 expression at the mRNA level in COCs at different stages of matuarion in vitro

COCs were cultured in 39°C, 5% CO_2 in air and COCs were collected at 0, 22 and 44 h post culture as showed in the Figure 1D, 1E, 1F and subjected to extract mRNA.

2.4.3. Determination of GDF9 expression at the mRNA level in CC and DO at 0h post culture

Right after collection of COCs, the COCs were removed cumulus cell to collect denuded oocyte without cumulus cells (DO) and cumulus cells (Figure 1G and H) then subjected to extract mRNA.

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 at least from three replicates.

3. RERULTS AND DISCUSSION

3.1. Determination of GDF9 expression at the mRNA level in ovarian tissue derived from small folliles (SF), medium follicles (MF) and large follicles (LF)

After electrophoresis of RT-PCR product of target gene using mRNA extracted from avarian tissue samples, the relative abundance of mRNA in GDF9, GAPDH bands was measured and presented in Figure 2.

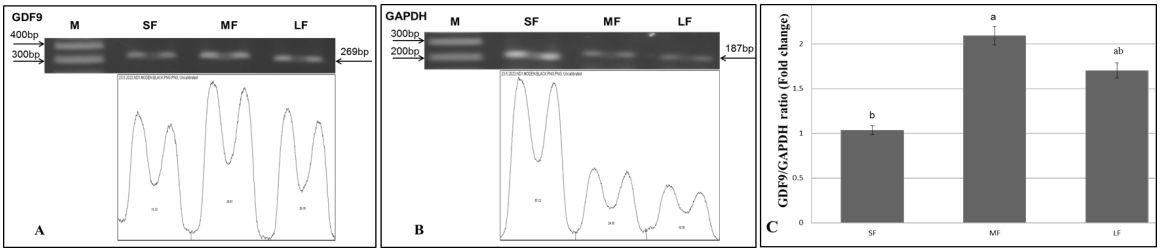

Figure 2. Representative image of GDF9 and GAHPD expression in the ovarian tisuue derived from deffirent stages of follicle development (A, B) and the relative abundance of GDF9 mRNA (C)

** Ovarian tissue derived from SF, MF and LF, data presented as mean ± SEM from three replicates (P<0.05)*

Data from Figure 2 showed that the mRNA of GDF9 was detected in ovarian tissue derived from SF, MF and LF (Figure 2A). GAPDH gene was also applified in all kinds of ovarian tissued (Figure 2B). The relative abundance of GDF9 mRNA (Figure 2C) was lowest (1.03±0.26) in ovarian tissue derived from SF, highest (2.09±0.18) in MF significant difference compared to SF (P<0.05) then declined in ovarian tissue derived LF (1.70±0.43). No significant difference between SF and LF or LF and MF was found (P>0.05). In sheep, Pan *et al.* (2018) documented that the GDF9 mRNA levels in the ovary were significantly higher (P<0.01) than in other tissues, with lower levels being found in the pituitary, liver, hypothalamus, spleen, cerebellum, uterus, lung, oviduct, and heart. In current study, it is the first report on expreesion of GDF9 at the mRNA level in porcine ovarian tissue derived from different stages of follicle development.

3.2. Determination of GDF9 expression at the mRNA level in COCs at different stages of matuaration *in vitro*

Using extracted RNA from COCs collected at 0, 22 and 44h of maturation culture conducted RT-PCR, after electrophoresis of RT-PCR product, the relative abundance of mRNA in GDF9, GAPDH bands was measured and presented in Figure 3.

Results from Figure 3 indicated that the mRNA of GDF9 was detected in COCs at 0, 22 and 44h post culture *in vitro* (Figure 3A). GAPDH gene was also applified in all treatments (Figure 3B). The relative abundance of GDF9 mRNA (Figure 3C) was highest (1.46±0.36) in COCs collected at 0h of maturation culture and significant difference compared to grouped COCs at 22h (0.51±0.10) and 44h (0.60±0.10). Significant differences were found in COC obtained at 0h compared with COC obtained at 22 and 44h post culture

in vitro (P<0.05). Several studies documented that expression of mRNA GDF9 was highly expressed in the COCs at the beginning of maturation culture and slowly declined throughout the process (Lee *et al.*, 2008; Li *et al.*, 2008). The level of mRNA GDF9 in GVBD and MI stages of nuclear maturation was significantly higher than GV stage and then declined to MII stage of porcine oocyte (Lin *et al.*, 2013) and human oocytes maturation *in vitro (*Zhao *et al.*, 2010)

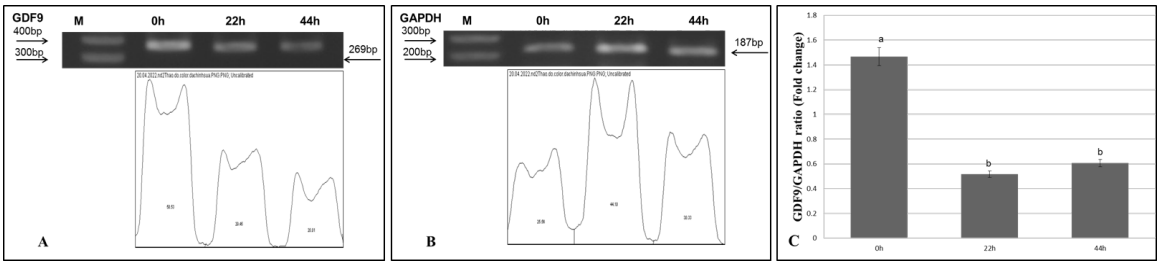

Figure 3. Representative image of GDF9 and GAHPD expression in the COCs at different time points of oocyte maturation *in vitro* (A, B) and the relative abundance of GDF9 mRNA (C).

** In the COCs at 0, 22 and 44h post culture, data presented as mean ± SEM from three replicates (P<0.05)*

3.3. Determination of GDF9 expression at the mRNA level in CC and DO at 0h post culture

Taken the results from Figure 3, using extracted RNA from CCs and DOs collected at 0h of maturation culture performed RT-PCR, after electrophoresis of RT-PCR product, the relative abundance of mRNA in GDF9, GAPDH bands was measured and presented in Figure 4.

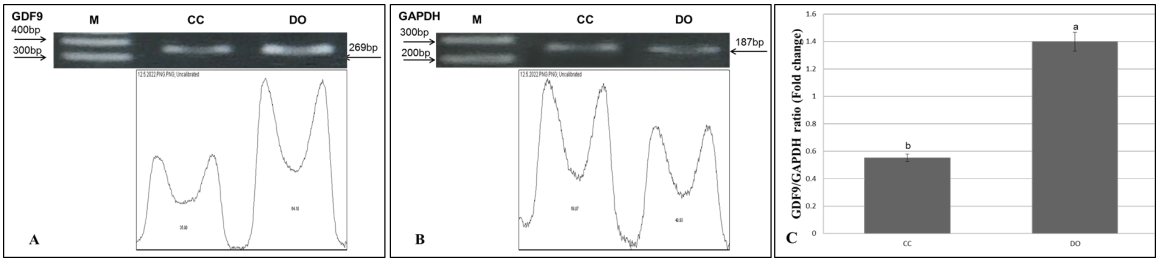

Figure 4. Representative image of GDF9 and GAHPD expression in the CC and DO (A, B) and the relative abundance of GDF9 mRNA (C).

** In cumulus cell (CC), denuded oocyte (DO), data presented as Mean±SEM from three replicates (P<0.05)*

Results from Figure 4 showed that the mRNA of GDF9 was detected in CC and DO at 0h post culture *in vitro* (Figure 4A). GAPDH gene was also applied in all treatments (Figure 4B). The relative abundance of GDF9 mRNA (Figure 4C) in the DO was higher than CC group (1.39±0.40 and 0.55±0.26, respectively, P<0.05). Lee *et al.* (2008) reported that the GDF9 mRNA was strongly expressed in the oocyte as compared to those in cumulus and granulosa cells of female domestic animal. In pig, Prochazka *et al.* (2004) also documented the expression of mRNA of GDF9 gene was dominant expressed in COC as compared to CC, it is the same trend with our current study.

4. CONCLUSION

The expression of GDF9 gene is in relation to oocyte maturation in the manner and can be considered as a candidate marker gene for oocyte quality evaluation *in vitro*

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MOLECULAR ANALYSIS USING MITOCHONDRIAL DNA TO INFER THE FORMATION PROCESS OF KAZAKH KUSHUM HORSE POPULATIONS

Nguyen Ba Trung[1]*

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ABSTRACT

The Kazakhstan Kushum is a new breed of horses that confirmed in the 1950[S] through a cross between stallions of Thoroughbred, Trotter, Russian Don breeds and mares of Kazakhstan local horse to provide military horses. In order to discover the genetic characteristics of this breed, a 247bp mitochondrial D-loop sequence was analyzed by PCR and Sanger sequencing techniques. The statistical quantities for these sequences, including number of haplotypes (Nh), haplotype diversity (Hd), and nucleotide diversity (p), were calculated using DnaSP 5.1 software. A median-joining network was constructed to detect haplotype differentiation in this population with the NETWORK 4.6 software, based on 247bp of 22 Kushum D-loop sequences and 87 sequences reported by Cieslak et al. (2010). Result, we detected 19 polymorphism sites, 10 mtDNA haplotypes that dropped into 8 of the 17 major haplogroups of horse mtDNA, showing unique haplotype composition with high genetic diversity including haplotype diversity of 0.9221; nucleotide diversity by 0.02365. The high genetic diversity of Kushum horses suggested that the founder of this breed was not from limited maternal lineages. Since the Kushum breed was established by cross-breeding between mares of Kazakhstan local horse and stallions of exotic breeds, the mtDNA haplotypes of Kushum horses must have originated from the population of local horses in the early 20th century. Consequently, the result of this study regarding the mtDNA haplotype is concordant with the recorded maternal origins of the Kushum horses in Kazakhstan.

Key Words: *Kazathstan Kushum Horses, Mitochondrial DNA D-loop, Genetic Diversity, Maternal Origin.*

TÓM TẮT

Phân tích gen ty thể để tìm hiểu Quá trình thành lập quần thể ngựa Kazakh Kushum

Kazakhstan Kushum là một giống ngựa mới được xác lập vào thập niên 1950, thông qua sự lai tạo giữa ngựa đực giống Thoroughbred, Trotter, Russian Don và ngựa cái địa phương ở Kazakhstan để cung cấp ngựa cho quân đội. Nhằm tìm hiểu đặc điểm di truyền của giống này, chúng tôi đã phân tích trình tự vòng lặp của gen ty thể - 247bp bằng kỹ thuật PCR và giải trình tự Sanger. Phân tích thống kê số lượng các trình tự này, bao gồm số lượng haplotype (Nh), đa dạng haplotype (Hd), đa dạng nucleotit (p) được tính toán bằng cách sử dụng phần mềm DnaSP 5.1. Một hệ thống tính toán kết hợp giá trị trung bình phát sinh chủng loài được xây dựng để phát hiện sự khác biệt haplotype trong quần thể này bằng phần mềm NETWORK 4.6, dựa trên 247bp của 22 trình tự vòng lặp ở ngựa Kushum và 87 trình tự của Cieslak và cộng sự. (2010). Kết quả, chúng tôi đã phát hiện 19 vị trí đa hình, 10 mtDNA haplotype rơi vào 8 trong số 17 nhóm chính haplogroup của mtDNA ngựa, cho thấy sự khác biệt kiểu haplotype ở giống ngựa này, với đa dạng di truyền cao, bao gồm đa dạng haplotype là 0,9221; đa dạng nucleotide bằng 0,02365. Sự đa dạng di truyền cao của ngựa Kushum cho thấy giống này được lai tạo không phải từ một số ít các dòng mẹ ban đầu. Bởi vì giống ngựa Kushum được thành lập bằng cách lai tạo giữa ngựa cái địa phương ở Kazakhstan với ngựa đực giống ngoại, nên các đa hình gen ty thể của ngựa Kushum phải có nguồn gốc từ các quần thể ngựa địa phương vào đầu thế kỷ 20. Do đó, kết quả nghiên cứu liên quan đến đa dạng haplotype gen ty thể này là phù hợp với lý lịch nguồn gốc ngựa Kushum được ghi lại ở Kazakhstan.

Từ khóa:*Ngựa Kazathstan Kushum, Vùng vòng lặp gen ty thể, Đa dạng di truyền, Di truyền dòng mẹ.*

[1] An Giang University, Vietnam National University, Ho Chi Minh City, Vietnam.

* Corresponding Author: Dr. Nguyen Ba Trung, Department of Animal Husbandry and Veterinary Medicine, Faculty of Agriculture and Natural Resources, An Giang University, Vietnam National University, HoChiMinh City (VNU-HCM). 18 Ung Van Khiem street, Long Xuyen city, An Giang Province, Vietnam. Tel: (84)918139960; Email: nbtrung@agu.edu.vn

1. INTRODUCTION

The Kushum horse breed conformed in The West Kazakhstan in the 1950^{s} through crossing between stallions of Russian Don, Thoroughbred, Trotter breeds and the local mare (Dmitriez, Ernst, 1989). The characteristic features of the Kushum are a solid build of a saddleharness horse type; the head is large but not coarse. The stallion's measurements are height at withers 159cm, oblique body length 161cm, chest girth 187cm. The mares measure 154, 157 and 182cm, respectively. They are well adapted to the cold climate, moderate in temperament, and grow rapidly. An adult horse (4-12 years) weighs 340-440kg and the meat production rate is around 57%. A mare can produce 5-6kg milk per day under grazing conditions. Their meat and milk are delicious and rich in nutrients necessary for humans. They play a key role in the health and lives of local people, as well as in the development of the local economy and society (Tiemuerbai, 2014).

The principal goal of the breeding of Kushum horses was to upgrade 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 principally for meat and milk production (Dmitriez and Ernst, 1989). They have important roles in the local community such as herding cattle, sheep, goats, and serve the army. However, molecular genetic characteristics have not been studied.

Understanding of the genetic characteristics of a population is crucial to expropriate conservation strategies that maintain genetic diversity (Hall and Bradley, 1995). Suitable assessment and characterization of populations at the genotypic and phenotypic levels are some of the first and essential steps in the development of conservation strategies (Plante *et al.*, 2007; Gizaw *et al.*, 2008). Molecular data can aid in identifying animals or sets of animals that should be preserved to prevent loss of genetic diversity. Because of organelle maternal inheritance (Hutchinson et al. 1974), mitochondrial DNA (mtDNA) haplotypes should be shared by all individuals within a family.

The equine mtDNA possesses a small of 16.6kb, closed circular double-stranded DNA molecule. The D-loop with an approximately length of 1,100 base pairs (Jansen *et al*., 2002) has two hyper variable regions (HVR1 and HVR2), which are regions with highest mutation rates in the mitochondria. It accumulates mutations about 10-20 times faster than nuclear DNA. These positions are useful for studying about genetic diversity and tracing the phylogenetic. Stability of maternal inheritance within documented horse pedigrees has been demonstrated in both Lippizan (Kavar *et al*., 1999) and Arabian (Bowling *et al*., 2000). Therefore, the aim of the present study is to reveal such genetic characteristics of Kushum horse by analysing mitochondrial DNA to infer the formation process of the Kazakhstan Kushum horses.

2. MATERIALS AND METHODS

The 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.

To determine mtDNA haplotypes, a DNA fragment containing the D-loop region of mtDNA (15,494 to 15,740; total 247bp) was amplified by PCR using primers (Hill *et al.*, 2010), and the amplified fragments were directly sequenced using the dideoxy method. PCR was performed in 10 µl reaction volume containing 10 ng genomic DNA, 0.2µM primers (5'-CTAGCTCCACCATCAACACC-3' and 5'-ATGGCCCTGAAGAAAGAACC-3'), 0.2 µmol/l dNTPs, 2µl 5× PCR buffer, and 1U Go Taq DNA polymerase (Promega, Madison, USA) under the following conditions: initial denaturation at 95°C for 10 min; followed by 35 cycles of denaturation at 94°C for 30sec, annealing at 60°C for 60 sec, and elongation

at 72°C for 30sec; and final extension at 72°C for 10min.

The parameters of the mtDNA sequences, including haplogroup and haplotype number, haplotype diversity, and nucleotide diversity, were calculated using DnaSP 5.10.1 (Librado and Rozas. 2009). The obtained sequence data were aligned using ClustalW in MEGA 7.1 (Kumar *et al.,* 2016), and a dataset of 97 haplotypes of the D-loop region, including 10 Kushum horse haplotypes and 87 haplotypes reported by Cieslak *et al*. (2010), was generated. A median-joining network (Bandelt, et al. 1995) was constructed to detect haplotype differentiation in this population with the NETWORK 4.6 software, based on 247 bp of 22 Kushum D-loop sequences and 87 sequences reported by Cieslak *et al.* (2010).

3. RESULTS AND DISCUSSION

In this study, we analyzed mtDNA based on the 247bp sequence of D-loop region of 22 Kushum horses. We excluded 4 strong mutational hotspots at nucleotide positions 15585, 15597, 15604, and 15650 from the mtDNA haplotype analysis, as described by Cieslak et al. (2010). The statistical results shown that the obtained number of polymorphic sites was 19, genotypic diversity was 0.922±0.034, nucleotide diversity was 0.02365±0.00239, average nucleotide difference was 5.818, ten haplotypes (KZ1-10).

We also constructed a network with 109 horse mtDNA haplotypes, including 22 sequences with 10 Kushum horse haplotypes and 87 haplotypes reported by Cieslak *et al.* (2010), using the Median-joining method (Figure 1) to estimate the phylogenetic relationships of Kushum horse haplotypes with previously reported haplotypes. Among the 10 haplotypes (Table 1), 7 haplotypes, namely KZ1, 2, 3, 4, 7, 8, and 10 were the same as previously reported haplotypes D2, A, B1, K3, K2b, X2, and X3c1, respectively. These haplotypes are known to be ancestral haplotypes survived in modern breeds of horse and widespread among various primitive breeds of the Eurasian continent (Cieslak et al. 2010). The remaining 3 haplotypes, namely KZ5, 6, and 9 were not included in the data set of the 87 previously reported haplotypes (Cieslak *et al.,* 2010), but reported to be present in some primitive breeds or native horse populations in the Eurasian continent (Hristov *et al.,* 2007; Lei *et al.,* 2009).

Following the nucleotide sequences of the D-loop regions of 1,754 modern and 207 ancestral horses, Cieslak *et al.* (2010) classified the 87 observed horse mtDNA haplotypes (sequences) into 17 main haplogroups. Total 22 sequences containing10 haplotypes of Kushum horses dropped into 8 (A, B, D, K, K3, X2, X3 and X7) haplogroups (Figure 1). Particularly, haplogroup A (H18) has 3 sequences (A, F and 1 Kushum); B (H4) has 19 sequences (B B1 B1b B2 A B3 C C1 D D1 E E1 F G2 and 5 Kushum); D (H2) has 12 sequences (D2 D2b D2c D2e D2f and 7 Kushum); K (H19) with 3 sequences (K2b and 2 Kushum); K3 (H3) of 5 sequences (K3 K3a K3b and 2 Kushum); X2 (H1) by 6 sequences (X2 X2b X2c and 3 Kushum); X3 (H28) contented 3 sequences (X3c1 and 2 Kushum) and X7 (H17) included 9 sequences (K X7 X7a X7a1 X7a3 X7a4 X8a and 2 Kushum sequences). Therefore, the distribution of these haplogroups in Kushum horses was essentially similar to that in primitive horse breeds in Central Asia consisting Akhal-Teke, Caspian, and Vyatskaya (Cieslak *et al.,* 2010).

Genetic diversity is the heritable variations between and within populations of organisms. This variation is essential for adapting to environmental changes and has been modulated under long-term and short-term evolutionary impacts of their specific ecosystems elements (Markert et al. 2010). These genetic variations occur due to neutral, deleterious or adaptive variants in chromosomes, individual genes or DNA sequences. Genetic variations are expressed in diverse morphological, physiological between individuals and populations (Frankham *et al.,* 2002). Knowledge about the genetic diversity could help to obtain a measure for the evolutionary history of individuals and populations and estimate the

value of the genetic resources (Tapio *et al.*, 2010; Hasler *et al.*, 2011).

Molecular markers have been developed in the last decades. Different molecular markers were used efficiently to characterize horse populations, and to investigate their origins. The most frequently markers used in horse diversity studies are the single nucleotide polymorphism and mitochondrial DNA (Achilli *et al.*, 2012, Cieslak *et al.*, 2010).

These findings of mtDNA haplotypes indicate high genetic diversity and unique genetic composition of the maternal lineage of Kushum horses. The high genetic diversity of Kushum horses observed in present study suggested that the founder of this breed was not from limited maternal lineages. Since the Kushum breed was established by cross-breeding between mares of Kazakhstan local horse and stallions of exotic breeds, the mtDNA haplotypes of Kushum horses must have originated from the population of local horses in the early 20th century. Therefore, the high diversity of mtDNA haplotypes in Kushum horses likely reflects high genetic diversity of the maternal lineage of the local horse population in Central Asia.

Table 1. Variable nucleotide positions and haplotypes of mtDNA D-loop region observed in Kushum horses

15495	15496	15534	15542	15598	15602	15603	15616	15617	15635	15649	15659	15666	15667	15703	15720	15726	15740	Kazakhstan haplotyp[b]	Numbers of horses	haplotypes in Ceislak et al. (2010)
T	T	A	C	C	T	C	T	A	T	C	A	T	G	A	T	G	G	A	X79547[a]		
.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	KZ-1	5	D2
.	C	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.		KZ-2	2	A
.	C	.	.	.	.	T	.	.	C	.	.	C	.	.	A	.	.		KZ-3	3	B1
.	C	.	.	.	.	T	.	.	.	.	.	.	.	G	C	A	.	.	KZ-4	2	K3
.	C	.	.	.	.	T	.	.	C	.	.	C	.	.	C	A	.	.	KZ-5	1	-
.	C	.	.	.	C	T	.	G	.		.	.		.	C	A	.	.	KZ-6	1	-
.	C	.	.	.	.	T	.	.	.	.	.	.	.	.	C	A	A	G	KZ-7	2	K2b
C	C	G	T	.	.	T	C	.	.	.	G	.	.	.	.	A	.	.	KZ-8	3	X2
.	C	.	.	.	.	T	.	.	.	.	G	.	.	.	C	A	.	G	KZ-9	1	-
.	C	.	.	T	.	T	.	.	.	T	.	.	A	.	C	A	.	.	KZ-10	2	X3c1

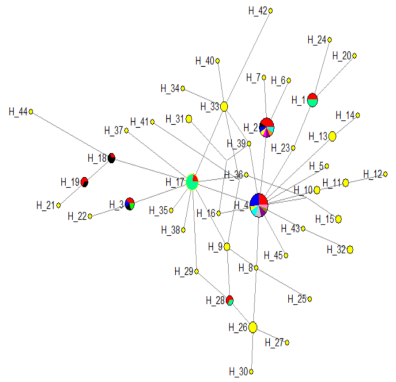

Figure 1. A median joining phylogenetic network constructed for 109 horse mtDNA sequences using 247bp of the control region to show the relationship of haplotypes in Kushum and 87 haps of other horse breeds (Cieslak *et al.*, 2010). Circles represent sequences, the area being proportional to the frequency of the sequence. The positions of the Kushum horse mtDNA sequences are shown as red pie slices

4. CONCLUSION

The breeding of Kushum horses began in the middle of the 20th century, just before World War II, in a major horse producing region of former Soviet Union, Kazakhstan. At that time, producing numerous military horses with high endurance performance, strong adaptability to cold climates might be required to prepare for a possible war in the European continent. Presumably, to match such military demands, Kushum horses were bred through systematic breeding programs using mares of local horse and stallions of exotic breeds for the traits desired as a military horse. The mtDNA diversity observed in this study might reflect these historical situations. The results of the present study indicate that it can infer useful information for better understanding of the origin and breeding history of horse breeds using mtDNA markers.

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INFLUENCE OF APPLYING THE *BACILLUS AMYLOLIQUEFACIENS, PEDIOCOCCUS PENTOSACEUS AND PEDIOCOCCUS ACIDILATICI* BACTERIAL STRAINS IN CONTROLLING AMMONIA AND HYDROGEN SULPHIDE FROM POULTRY, GOAT AND CATTLE MANURES, AND IMPROVING GROWTH PERFORMANCE

Duong Nguyen Khang[1], Dang Hoang Dao[2], Tu Phuong Binh[3], Huynh Vu Duy Khang[3], Nguyen Ba Khanh Tuong[4], Trinh Kim Phuong[4] and Croize Paul-Antoine[4]*

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ABSTRACT

The aim of this study is to evaluate the effectiveness of applying of Manure Pro product containing the *Bacillus amyloliquefaciens, Pediococcus pentosaceus* and *Pediococcus acidilatici* bacterial strains in controlling ammonia and hydrogen sulphide from poultry, goat and cattle manures and improving animal growth performance. The experiment was carried out at farm of Nong Lam University of Ho Chi Minh city, Vietnam. For this aspect, this product was included into four levels of 0, 0.5, 1 and 1.5g of Manure Pro product used to sprayed for 1m² of litter surface. Four hundred Ross 308 broiler chicken at the first day old, eighty Bach Thao x Saanen crossbred goats at 1-2 years old with an average weight of 50kg and eighty Sindhi crossbred cattle at 8-12 months old with an average weight of 180kg were randomly assigned to four experimental groups: Control (MP0), Manure Pro 0.5g (MP0.5), Manure Pro 1g (MP1.0) and Manure Pro 1.5g (MP1.5), respectively. The trial lasted for 35, 60 and 60 days for treatment the chicken, goat and cattle manures, respectively. The concentrations of atmospheric ammonia and hydrogen sulphide were determined and measured using a special instrument called 'single-gas detector' at the end of experiment. The data on feed intake, feed conversion ratio as well as health care parametters were also recorded. In general, concentrations of ammonia and hydrogen sulphide in chicken, goat and cattle manures were highly reduced. The feed conversion ratio was also better, whereas the weight again was increased. Additionally, the incidence of lameness infected cattle in was lower than that in the treatmental groups. Taken together, utilization of Manure Pro applying not only decreased concentrations of ammonia and hydrogen sulphide in the manures but also improved the growth performance and health. Therefore, the effectiveness of Manure Pro might be recommended as a control method to keep or dissipate the odour production from poultry, goat and cattle manures and improving their growth performance.

Keywords: *microorganism, poultry layer house, goat and cattle manures, ammonia, hydrogen sulphide*

TÓM TẮT

Hiệu quả của việc sử dụng chế phẩm Manure Pro có chứa các vi khuẩn *Bacillus amyloliquefaciens, Pediococcus pentosaceus* và *Pediococcus acidilatici* cho việc kiểm soát nồng độ ammonia và hydrogen sulphide sinh ra trong phân gà, dê và bò

Mục đích nghiên cứu này là để đánh giá hiệu quả của việc sử dụng chế phẩm Manure Pro có chứa các vi khuẩn *Bacillus amyloliquefaciens*, *Pediococcus pentosaceus* và *Pediococcus acidilatici* cho việc

[1] Nong Lam University, Ho Chi Minh City
[2] HUTECH University
[3] Department of Animal Husbandry and Veterinary Medicine of Tien Giang province,
[4] Lallemand Vietnam Company Limited, Canada
*Corresponding Author: Prof. Dr. Duong Nguyen Khang. Address: Nong lam University, Ho Chi Minh City; Tel: (84)989390179; Email: duongnguyenkhang@gmail.com

kiểm soát nồng độ ammonia và hydrogen sulphide sinh ra trong phân gà, dê và bò; cũng như cải thiện năng suất tăng trưởng của vật nuôi. Thí nghiệm được thực hiện tại Trại chăn nuôi, Trường Đại học Nông Lâm Thành phố Hồ Chí Minh, Việt Nam. Trong bố trí thí nghiệm, chế phẩm Manure Pro được cho vào bốn mức 0; 0,5; 1 và 1,5g để phun trên 1 m² bề mặt chất độn chuồng. Bốn trăm gà thịt Ross 308 ngày tuổi, tám mươi dê sữa lai Bách Thảo x Saanen lúc 1-2 năm tuổi có khối lượng trung bình 50kg, và tám mươi bò lai Sindhi lúc 8-12 tháng tuổi với khối lượng trung bình 180kg được phân ngẫu nhiên vào bốn lô thí nghiệm: Đối chứng (MP0), Manure Pro 0,5g (MP0,5), Manure Pro 1.0g (MP1,0) và Manure Pro 1,5g (MP1,5), tương ứng. Thí nghiệm kéo dài 35, 60 và 60 ngày để xử lý phân gà, dê và bò. Nồng độ ammonia và sunfua hydro khí thải được xác định và đo bằng 'máy dò đơn khí' lúc cuối thí nghiệm. Các số liệu lượng ăn vào, hệ số chuyển đổi thức ăn cũng như các thông số chăm sóc sức khỏe cũng được ghi lại. Nhìn chung, nồng độ ammonia và sunfua hydro trong phân gà, dê và bò đã giảm rất nhiều. Hệ số chuyển đổi thức ăn tốt hơn, trọng lượng cao lên. Ngoài ra, bò bị chân móng ở các nhóm xử lý có tỷ lệ thấp hơn. Nhìn chung, việc sử dụng Manure Pro không chỉ làm giảm nồng độ ammonia và sunfua hydro trong phân mà còn cải thiện tăng trưởng và sức khỏe. Do đó, chế phẩm Manure Pro có thể được khuyến nghị như là một phương pháp kiểm soát để kiểm soát hoặc làm tiêu tan mùi hôi từ phân gia súc gia cầm đồng thời còn cải thiện năng suất tăng trưởng của chúng.

Từ khóa: *vi sinh vật, chuồng nuôi gia cầm, phân dê và bò, ammonia,* sunfua hydro

1. INTRODUCTION

Poultry and animal farms are a source of odour and attract flies, rodents and other pests that create local nuisances and carry the diseases to other farms. Odour emissions from poultry and animal farms adversely also affect the life of people living. Odour from poultry and animal farms is not caused by a single compound, but is rather the result of a large number of contributing compounds including ammonia, hydrogen sulphide and volatile fatty acid; mostly ammonia (Donham *et al*., 1982). Ammonia gas has a sharp and pungent odour and can act as an irritant when present in elevated concentrations. The emission of odors mostly depends on the frequency of animal house cleaning, the temperature and humidity of the manure, the type of manure storage and on air movements. Hydrogen sulfide also one of the pollutant gases related to chicken and animal manures. It is produced in anaerobic environments from the microbial reduction of sulfate in water and the decomposition of sulfur containing organic matter in manure. Hydrogen sulfide is considered the most dangerous gas when at acute concentration has been responsible for animal as well as human deaths (Donham *et al*., 1982). Many technologies have been developed and investigate in order to reduce odour from poultry and animal farms including the using of effective microorganism (Yongzhen and Waijiong, 1994; Nizaha and Syed, 2008). The use of effective or beneficial microorganisms in poultry and animal manures was claimed to be effective in preventing odour and has no known adverse effects on plants, animals, humans, or environment after over a decade of application (Higa and Wood, 2007). The aim of this study was to evaluate the effectiveness of Manure Pro product as a control method to dissipate the odour production from poultry, goat and cattle manures and improving their growth performance.

2. MATERIALS AND METHODS

2.1. Location

The study was conducted at The Research and Technology Transfer Center (RTTC), Nong Lam University of Ho Chi Minh (NLU), Vietnam from October to December 2021.

2.2. Product profile on ingredients, effectiveness and use

Ingredients: *Bacillus amyloliquefaciens, Pediococcus pentosaceus, Pediococcus acidilactici*. Additives and carriers of beta-glucanase, xylanase, collidal silica and sucrose.

Effectiveness: Manure Pro product contains the high concentrated bacteria for manure treating. It also includes a cellulolytic enzymatic complex working in complete synergy with the bacteria.

Usage: 1kg of Manure Pro used to treat 1,000m² of surface, equivalent to 1 g/m². Dosage and dilution may vary depending on the intended application and sprayer capacity. It should follow technical recommendations before use. Dissolve 1kg in 5l of clean water into a container, transfer in a sprayer and make up to 10-15l. Spray uniformly on the bedding surface or manure according to the below recommendations

Bedding and litter: First time, apply a dose of 1.0 g/m² on the floor after cleaning and/or 24hrs after disinfecting. For rice husk, reapply the application after mulching. Repeat at 0.5, 1.0 or 1.5 g/m² every week.

Manure: Spray of 1 kg Manure Pro product to every 60 to 80 tons of manure.

Slurry: First time, after emptying the pit, apply 5.0 g/m³ to the remaining slurry and stir so that the entire product will be uniformly distributed. Every 15 days, apply Manure Pro product at 5.0 g/m³ under the crust of produced slurry, then stir.

2.3. Materials, animals, experimental design and method

The Manure Pro product in powder form available of Lallemand Company Limited was applied. Four treatments were carried out, the control without (MP0) any application of *Bacillus amyloliquefaciens*, *Pediococcus pentosaceus* and *Pediococcus acidilatici* bacterial strains and three treated groups with microorganism application of 0.5 (MP0.5), 1.0 (MP1.0) and 1.5 (MP1.5)g/m² of the bedding surface. Before starting the trial, the manures were allowed to accumulate for 3 days to meet the appropriate amount in order to run this trial. On the first day of the trial, the microbial mixture was spread on the manures of the treated groups. The next day, additional mixture of microbes was spread in the treated groups. The procedure was repeated every 7 days for poultry manure or 15 days for cattle and goat manures. The trial lasted for 35, 60 and 60 days for poultry, cattle and goat manures, respectively. Atmospheric ammonia and hydrogen sulphide were measured using a special instrument called single gas detector by detection method of infrared and electrochemical sensors (Geotech BIOGAS5000). The levels of ammonia and hydrogen sulphide were measured at the end of experiment.

For poultry manure, time for carry out the study was 35 days. Four hundred Ross 308 broiler chickens one day old, equally divided between males and females in 4 pens with an area of 15 m²/plot, sprinkled with rice husks at 6.5 kg/m². No rice husks addition during the batch. Manure Pro dose for 4 pens at 0, 0.5, 1.0 and 1.5 g/m² of the litter surface. The dose of Manure Pro is maintained weekly by spraying uniformly over the litter surface. The litter quality of moisture, scoring and visual aspect will be measured. The score of litter in order of classification as follows: score 1 was the best with dry and crumbly litter, score 2 was good with crumbly but slightly damp litter, score 3 was medium with crumbly but crusted in some places, score 4 with crusted on the surface and crumbly when digging, score 5 with totally crusted or moist. The pH, moisture, ammonia and hydrogen sulphide concentrations were measured.

The pododermatitis and mortality were recorded at the end of experiment. Mortality rate and score to assess the level of pododermatitis with score 1 was no or mild inflammation, score 2 was moderate inflammation, score 3 was severe level of inflammation. The growth indicators were measured on feed intake, weight gain, feed conversion ratio.

For cattle manure, time for carry out the study was 60 days. There were 80 Sindhi crossbred cattle with 8-12 months old, an average weight of 180 kg/head, equally divided between males and females into 4 pens with

an area of 42 m^2/pen. Manure Pro dose for 4 pens at 0, 0.5, 1.0 and 1.5 g/m^2 of the manure surface. The dose of Manure Pro product is maintained weekly by spraying uniformly over the manure surface. The quality of manure was recorded on the score of manure, pH, humidity, ammonia and hydrogen sulphide concentrations were measured. Feed intake, weight gain and feed conversion ratio were measured and calculated. Health care problem on lameness ratio also recorded at the end of experiment.

Figure 1. Pictures on broiler incubation with bedding materials at the first day old

For goat manure, time for carry out the study was 60 days. There were 80 Bach Thao x Saanen crossbred goats with 1-2 years old, an average weight of 50 kg/head, equally divided between male and female in 4 pens with an area of 25 m^2/pen. The Manure Pro dose for 4 pens at 0, 0.5, 1.0 and 1.5/m^2 of the manure surface. The dose of Manure Pro product is maintained weekly by spraying uniformly over the manure surface. Parameters on the quality of goat manure were measured the score of manure, pH, humidity, ammonia and hydrogen sulphide concentrations. Feed intake was recorded.

2.4. Data analysis

The data are presented as the Mean and SEM. All data were analyzed with one-way ANOVA. Significance was declared at $P<0.05$. Other data were analyzed descriptive statistic such as percentages and frequency distributions in excel program.

3. RESULTS AND DISCUSSION

The composition and quality of the Manure Pro product were given by the manufacturer of the Lallemand Company Limited, Canada (Table 1).

Table 1. Composition and quality of the Manure Pro product

Parameters		From manufacturer	At the beginning	After two months
Appearance	Structure	Fine powder	Fine	Fine
	Color	Beige and White	White	White
Quality	Moisture, max %	7.0	0.04	0.04
	Bacillus spp., min x 10^9 CFU/g	1.0	1.10	0.67
	Pediococcus spp, min x 10^9 CFU/g	4.0	4.20	6.50

Efficient of animal manure treatment on quality of litter surface with score of manure, pH, humidity, temperature, ammonia and hydrogen sulphide concentrations shown in Table 2. The levels of ammonia, hydrogen sulphide was noted to decrease from the first day to the end of experiment in the treated groups.

Table 2. Efficient of Manure Pro product on quality of poultry, cattle and goat manure surfaces

Parameters	Animal	Cont	MP0.5	MP1.0	MP1.5	SEM	P
Score of manures	Poultry	4.0	3.0	2.2	1.7	0.11	0.001
	Cattle	5.0	4.0	2.0	1.75	0.32	0.009
	Goat	3.0	3.0	2.0	2.0	0.19	0.13
pH	Poultry	7.9	8.1	8.4	8.1	0.02	0.001
	Cattle	8.0	8.3	8.4	8.4	0.09	0.42
	Goat	7.5	7.9	8.1	8.1	0.08	0.06
Surface humidity	Poultry	94.2	93.6	93.5	93.5	0.13	0.21
	Cattle	94.3	93.2	92.8	92.3	0.42	0.41
	Goat	92.1	91.2	90.3	90.4	0.23	0.06
Humidity depth 10cm, %	Poultry	17.2	16.4	16.6	16.2	0.13	0.08
	Cattle	23.7	22.9	22.5	22.4	0.52	0.81
	Goat	24.8	23.2	23.1	23.0	0.64	0.72
Surface temperature,%	Poultry	30.12	29.30	29.20	29.52	0.13	0.11
	Cattle	29.45	28.30	28.20	28.62	0.13	0.02
	Goat	30.52	29.54	29.32	29.42	0.27	0.40
Temperature depth 10cm, ^{0}C	Poultry	30.92	30.16	29.80	30.38	0.14	0.07
	Cattle	29.92	29.68	29.25	29.58	0.48	0.97
	Goat	31.62	30.78	29.65	29.78	0.28	0.09
Ammonia, ppm	Poultry	12.0	6.0	3.0	2.8	0.23	0.001
	Cattle	9.0	5.0	2.0	1.5	0.26	0.001
	Goat	10.0	5.0	3.0	2.75	0.62	0.008
Hydrogen sulphide, ppm	Poultry	8.0	4.0	2.0	1.6	0.20	0.001
	Cattle	6.0	4.0	2.0	1.5	0.28	0.001
	Goat	5.0	3.0	2.0	1.75	0.39	0.04

Table 3. Efficient of Manure Pro product on feed intake, weight gain and FCR of poultry, cattle and goat

	Parameters	Cont	MP0.5	MP1.0	MP1.5
FI	Poultry, kg/head	2.99	2.81	2.82	2.68
	Cattle, kg/100kg LW	2.51	2.45	2.40	2.41
	Goat, kg/100kg LW	2.41	2.35	2.30	2.31
WG	Poultry, g/head	1,778	1,825	1,915	1,777
	Cattle, g/head/day	720	755	780	760
FCR	Poultry, kg WG	1.68	1.54	1.47	1.51
	Cattle, kg WG	8.32	8.25	7.95	8.05

Table 4. Poultry mortality and level of pododermatitis

Parameters	Cont	MP0.5	MP1.0	MP1.5
Mortality, %	8	7	4	5
Score for assessment of pododermatitis	3	2	1	1

The accumulation of manure in the litter surface leads to the increase of ammonia and hydrogen sulphide concentrations which contributes to odour problems. However, the application of microbes seemed to have an effect on ammonia and hydrogen sulphide levels starting from the first day until end of the trial. The ammonia and hydrogen sulphide levels were significantly decreasing in the treatment group compared to the control group. The multi-gas detector shown there was few hydrogen sulphide presence in the environment (2ppm) in higher addition of Manure Pro product at 1.0 and 1.5 g/m^2 of the manure surface. The previous study by Yongzhen and Weijiong (1994) shown that the use of probiotic effective microorganism (EM) in drinking water reduced ammonia concentrations within chicken houses by 42.12%. The use of EM fermented feed reduced ammonia concentrations by 54.25% and the combination of the two techniques reduced ammonia concentrations by 69.7%. Our study found some positive influence on the ammonia and hydrogen sulphide levels presence in the environment by adding Manure Pro into the manure.

Table 5. Lameness ratio of cattle

Type of lameness		Cont	MP0.5	MP1.0	MP1.5
Prevalence of lameness symptoms in experimental block	Hard and soft	0	0	0	0
	Worn heel	0	1	1	0
	Feet rot	0	0	0	0
	Skin inflammation	1	0	0	1
	Tissue inflammation	0	0	0	0
	Claw inflammation	2	1	0	0
	Subclinical inflammation	0	0	0	0
	Chronic inflammation	0	0	0	0
	Digital dermatitis	1	1	1	0
	Ulcers	0	0	0	0
	Long claw	0	0	0	1
Total, head		4	3	2	2
Lameness ratio, %		5	3.75	2.5	2.5

4. CONCLUSION

The use of a litter conditioner including Manure Pro product on broiler litter, cattle and goat manure surfaces is an interesting tool to maintaining a better litter quality. The bacterial strains in Manure Pro product significantly break down the sulphur containing compounds in the manure. Manure Pro product have handled livestock and poultry manure very well with a dose of 1g of Manure Pro product per m^2 of barn surface, safe to use, improve weight gain, do not harm animal health. Indeed, the environment in which broiler, cattle and goat growth is essential for animal welfare and helps guarantee broilers, cattle and goat reach their full genetic potential. The litter conditioner including Manure Pro product had a direct action on litter characteristics such as moisture and, consequently, on the improvement of the incidence of pododermatitis and the performances of the animals.

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EFFECT OF DIFFERENT LEVELS OF PREMIX SUPPLEMENTATION ON REPRODUCTIVE YIELD AND EGG QUALITY OF NOI CROSSBRED LAYING HENS

Nguyen Thi Kim Khang[1], Doan Phuong Lam[1], Nguyen Van Truyen[2] and Le Thanh Phuong[3]*

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ABSTRACT

The objective of this study was to evaluate the effect of premix (PM) supplementation on reproduction and egg quality of Noi crossbred hens from 18 to 30 weeks of age. The experiment was arranged in a completely randomized design with 5 treatments and 9 replicates, with 1 hen for each replicate. The treatments were respectively as follows (1) Control using only the basal diet without premix added, (2) PM2.5 including basal diet supplemented with 2.5mg PM/kg feed, (3) PM5 includes basal diet supplemented with 5mg PM/kg feed, (4) PM7.5 includes basal diet supplemented with 7.5mg PM/kgTA, and (5) PM10 includes basal diet supplemented with 10mg PM/kg feed. The experiment was carried out from November 2020 to February 2021 at Thuan Tien B hamlet, Thuan An commune, Binh Minh town, Vinh Long province. The results showed that the first laying age of Noi crossbred hens ranged of 126-129 days, and at 50% of the laying rate in PM5 and PM10 (146-148 days) were earlier than in the control group (151.5 days). Egg laying rate and total egg yield in PM2.5 (36.51% and 66.44 eggs/hen) tended to be higher than control and the other treatments (P>0.05). Feed consumption and feed conversion ratio of hens among treatments were not statistically significant (P>0.05) nor did they affect egg quality of Noi crossbred hens (P>0.05). From the above research results, it can be suggested to add 2.5mg of premix/kg of feed to the diet to improve egg performance.

Keywords: *Vitamin, mineral, egg laying rate, first laying age, hen*

TÓM TẮT

Ảnh hưởng của bổ sung premix lên năng suất sinh sản và chất lượng trứng của gà mái Nòi lai

Thí nghiệm được thực hiện nhằm đánh giá ảnh hưởng các mức bổ sung premix (PM) lên khả năng sinh sản và chất lượng trứng của gà mái Nòi lai giai đoạn từ 18 đến 30 tuần tuổi. Thí nghiệm được bố trí theo thể thức hoàn toàn ngẫu nhiên với 5 nghiệm thức (NT) và 9 lần lặp lại với mỗi lần lặp lại là một gà mái đẻ. Các NT lần lượt như sau (1) đối chứng (ĐC) chỉ sử dụng thức ăn của khẩu phần cơ sở (KPCS) không có bổ sung premix khoáng, (2) PM2,5 gồm KPCS có bổ sung 2,5mg PM/kgTA, (3) PM5 gồm KPCS có bổ sung 5mg PM/kgTA, (4) PM7,5 gồm KPCS có bổ sung 7,5mg PM/kgTA, và (5) PM10 gồm KPCS có bổ sung 10mg PM/kgTA. Thí nghiệm được thực hiện từ tháng 11/2020 đến tháng 02/2021 tại ấp Thuận Tiến B, xã Thuận An, Thị xã Bình Minh, tỉnh Vĩnh Long. Kết quả phân tích cho thấy tuổi đẻ đầu tiên của gà mái Nòi lai ở các NT nằm trong khoảng 126-129 ngày, tuổi đẻ 50% ở PM5 và PM10 (146 -148 ngày) sớm hơn so với ĐC (151,5 ngày). Tỉ lệ đẻ (TLĐ) và năng suất trứng (NST) ở PM2,5 (36,51%; 66,44 trứng/con) có xu hướng cao hơn ĐC và cao hơn các NT bổ sung khác (P>0,05). Tiêu tốn thức ăn (TTTA), hệ số chuyển hóa thức ăn (HSCHTA) của gà giữa các NT khác biệt không có ý nghĩa thống kê (P>0,05). Bổ sung premix khoáng vitamin không ảnh hưởng đến chất lượng trứng của gà mái Nòi lai (P>0,05). Từ kết quả nghiên cứu trên có thể đề nghị bổ sung 2,5mg premix/kg TA vào khẩu phần giúp cải thiện NST ở gà mái Nòi lai.

Từ khóa: *premix, tỉ lệ đẻ, tuổi đẻ quả trứng đầu tiên, gà mái*

[1] CanTho University

[2] Trading Service Manufacturing Friends Co., Ltd.

[3] Emivest Feedmill Vietnam Co. Ltd.

[*] Corresponding Author: Assoc. Prof. Dr. Nguyen Thi Kim Khang, Can Tho University, Campus II, 3/2 street, Ninh Kieu district, Can Tho city, Vietnam. Tel: (84) 939205355, Email ntkkhang@ctu.edu.vn

1. INTRODUCTION

For laying birds, the requirement for minerals, amino acids and vitamins plays an extremely important role. Mineral requirement in laying birds is 4-7% because calcium - phosphorus is needed to create eggshells (Truong Phuoc Thong and Le Van Dang, 2013). Calcium (Ca), which exists in the body mainly in the form of phosphate and calcium carbonate, plays an important role in building and developing the skeleton of poultry and contributes to the formation of egg shells, up to 98% CaCO3 in eggshells (Le Hong Man, 2003). Amino acids are one of the important nutrients in growth and egg production (Baker, 2009). Reproductive requirements in poultry are high in vitamins of the ADE group, in which vitamin A is important to support the metabolic activity of chickens in order to maintain and enhance egg production, vitamin E helps to improve the egg productivity and quality (Bendich, 1988), and finally, vitamin D has a significant effect on supporting proper bone growth and avoiding foot problems.

The results of recent studies have shown that the addition of minerals, vitamins and amino acids to the diets of laying hens has improved the performance and egg quality of Hisex Brown hens at 20-30 weeks of age. Specifically, the research results of Nguyen Ba Nguyen (2014) showed that supplementing with minerals, B vitamins and amino acids at 100 ml/30l of drinking water had higher the rate of laying and egg weight (88.11% and 57.11 g/egg, respectively) than that of the control group (87.43% and 52.11 g/egg). Research results of Tran Thai Chien (2017) showed that supplementing with Calphovit containing minerals and vitamins of the ADE group at 1.5g/kgfeed had higher in feed consumption and laying rate (122 g/day and 87.58%) than that of the control group (117.7 g/day and 83.66%), in addition, the shape index and eggshell thickness were also improved in Hisex Brown chickens at 58-63 weeks of age.

In order to improve the yield and quality of the hens' eggs of Noi chicken breed to introduce into the large scale, it is necessary to add vitamin mineral premix in the diet of this chicken breed. Currently, studies on adding nutrients in the diet of Noi chickens to improve egg production and quality are still very limited. Therefore, the topic "Effect of different levels of premix (PM) supplementation on reproductive yield and egg quality of Noi crossbred laying hens from 18 to 30 weeks of age" was carried out with the aim to investigate the effects of vitamin mineral premix supplementation on yield and egg quality of Noi crossbred hens raised in open barn conditions.

2. MATERIALS AND METHODS

2.1. Animals and management

A total of 45 experimental Noi crossbred hens at 18-30 weeks of age were fully vaccinated against infectious diseases and dewormed before the experiment started.

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 (1990) 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 was 2700 kcal/kg ME and 17% crude protein.

The premix powder used in the experiment is in powder form, milky white and odorless, which includes calcium, lysine, methionine, and ADE vitamins (Table 1). The product is recommended to be used at 5mg/1kg of chicken feed.

Table 1. Ingredients of vitamin mineral premix

Contents	Units	Weight
Vitamin A (min)	IU	350.000
Vitamin D_3 (min)	IU	200.000
Vitamin E (min)	IU	30.000
Lysine (min)	mg	16.000
Methionine (min)	mg	22.000
Calcium (min-max)	g	22-25

2.2. Experimental design and data collection

A total of 45 Noi crossbred hens were randomized design into 5 treatments and 9 replicates, with 1 hen for each replicate. The treatments were respectively as follows (1) Control (Cont) using only the basal diet without premix added, (2) PM2.5 including basal diet supplemented with 2.5mg PM/kg of feed, (3) PM5 includes basal diet supplemented with 5mg PM/kg of feed, (4) PM7.5 includes basal diet supplemented with 7.5mg PM/kg of feed, and (5) PM10 includes basal diet supplemented with 10mg PM/kg of feed.

Feeding procedures were performed uniformly for all chickens in the experiment, differing only in diets with or without the addition of premix powder. Chickens were fed twice a day, fed 33.3% of the diet at 8am and 66.7% of the diet at 14pm. Water was available for free access. Feeders and drinkers were cleaned daily.

The experimental chickens were weighed at the beginning (18 weeks of age) and at the end (30 weeks of age) of the experiment. Leftovers were weighed and recorded the next morning. The age at laying the first egg of the hens as well as the laying age of 50% of the hens were recorded. Chicken eggs were collected at 8:30am every day, the number and weight of eggs were weighed and recorded. At the end of the experiment, the average number as well as the weight of eggs for each treatment was calculated.

The egg quality was analyzed by selecting eggs from the treatments at 23-26 weeks of age. The total number of eggs was 45 eggs (5 treatments x 9 replicates). Chicken eggs were weighed, measured in length and width of egg, and then broken to measure parameters such as width, height of white and egg yolk. The white, egg yolk and egg shell were weighed, eggshell thickness, albumen index, egg yolk index and Haugh unit (HU) were measured (Bui Huu Doan *et al*., 2011). Yolk color was measured by using a Konica Minolta Chroma Meter CR-410 which allows determination of L* (lightness), a* (redness) and b* (yellowness). These parameters were performed two times and the final values were calculated as the averages of the two corresponding values measured.

2.3. Statistical analysis

The collected raw data were recorded and processed by Microsoft Excel software, then statistically processed by Minitab Version 16 software according to GLM-ANOVA model. The mean values were compared using the Tukey method with 95% confidence intervals. The first egg laying age and 50% laying age were treated by χ^2 test (Minitab Version 16). The temperature and humidity data were processed using descriptive statistics.

3. RESULTS AND DISCUSSION

3.1. Body weight and the laying age of Noi crossbred hens

The results in table 2 showed that there was no statistically significant difference among the treatments in terms of body weight of chickens at the beginning, at the end of the experiment and weight gain in Noi crossbred hens (P>0.05). Similarly, age at first laying eggs and 50% laying age of crossbred hens were also not statistically significant among treatments (P>0.05). The body weight of Noi crossbred pullets at 18 weeks of age was in the range of 1.51-1.59 kg/head, the final weight at 30 weeks of age was 1.93-2.09 kg/head, and the weight gain of Noi crossbred chickens. were in the range of 0.39-0.49 kg/head. In general, the addition of premix in the diet did not affect the body weight of the experimental hens. The first laying age of Noi crossbred pullets

in all treatments ranged from 126 to 129 days, 50% of laying age in PM5 and PM10 (146-148 days) was earlier than in control (151.5 days). The results in this experiment on age of the first egg laying and 50% of laying hens were shorter than that reported by Nguyen Thi Kim Khang *et al*. (2020) recorded on Noi crossbred hens at 16-26 weeks of age was 144-154 days and 157-181 days for the first egg-laying age and 50% of laying hens, respectively. This difference may be due to differences in experimental time, additive feeds.

Table 2. Body weight and laying age of Noi crossbred hens

Parameters		Treatments					SEM	P
		Cont	$PM_{2.5}$	PM_5	$PM_{7.5}$	PM_{10}		
Body weight (kg/head)	BW at 18 wks	1.56	1.57	1.54	1.51	1.59	0.05	0.79
	BW at 32 wks	1.98	1.96	1.94	1.93	2.09	0.07	0.57
	Weight gain	0.42	0.39	0.40	0.42	0.49	0.06	0.74
Laying age of hen (day)	First laying age	126	127	127	129	126	0.047	1.00
	50% of laying age	151.5	153.5	148	154	146	0.32	0.99

3.2. Reproductive performance of Noi crossbred hens

The laying egg rate of Noi crossbred hens in Table 3 showed that the laying rate of Noi crossbred hens in PM2.5 gave bester than other treatments through the week of age, especially at 18-22 weeks of age, the laying rate of chickens was high at PM2.5 (25.89%) followed by PM7.5 (23.81%) or at 23-26 weeks of age, PM2.5 was 52.38% of the laying rate compared to PM7.5 (49.49%) and PM5 (47.22%), and in overall at 18-30 weeks of age, PM2.5 was a higher laying rate of 1.87-5.82% compared to the control and other treatments. Similarly, the total egg production of hens at PM2.5 was also higher than that of the control and the other treatments. This proved that the addition of premix with 2.5mg improved the laying rate and egg performance of the experimental hens. However, the laying rate and total egg production of Noi crossbred hens among treatments did not have statistical significance over the weeks of age (P>0.05). Similarly, the egg weight of Noi crossbred hens was not statistically significant among treatments over the weeks of age. These above results indicated that the addition of premix did not affect the laying rate and egg weight of Noi crossbred hens. The results of this experiment differed from the study on Hisex Brown chickens by Nguyen Ba Nguyen (2014) and Tran Thai Chien (2017) in that when adding minerals, vitamins and amino acids in the hen's diet, it improved not only the laying rate in hens but also the hen' egg weight. Compared with the research results of Nguyen Thi Kim Khang *et al.* (2020), the laying rate of 24-26-week-old hens was 34.69-46.94% lower than the results of this experiment.

Table 3. Laying egg rate (%) and egg weight (g/egg) of Noi crossbred chickens

Parameters	Laying age (Week)	Treatments					P
		Cont	$PM_{2.5}$	PM_5	$PM_{7.5}$	PM_{10}	
Laying egg rate, %	18-22	16.96±6.93	25.89±7.75	15.63±6.93	23.81±8.95	14.20±6.93	0.76
	23-26	45.24±8.17	52.38±8.17	47.22±8.17	49.49±9.26	44.44±8.17	0.96
	27-30	48.02±4.83	45.63±4.83	48.02±4.83	45.63±4.83	44.84±4.83	0.98
	18-30	34.23±4.37	36.51±4.37	34.64±4.37	30.69±4.37	32.39±4.37	0.88
Egg weight, (g)	18-22	37.01±2.45	35.45±2.74	34.04±2.45	33.77±3.16	31.16±2.45	0.56
	23-26	42.14±1.51	39.55±1.51	36.27±1.51	39.71±1.72	38.21±1.51	0.11
	27-30	44.92±1.41	42.62±1.41	41.41±1.41	45.11±1.41	41.21±1.41	0.15
	18-30	41.96±1.28	40.36±1.28	38.11±1.28	41.58±1.28	38.33±1.28	0.12

The results in Table 4 showed that feed intake and feed conversion ratio of experimental hens tended to increase gradually in groups of PM5, PM7.5 and PM10 over weeks of age, however, these differences were not statistically significant among treatments (P>0.05). Feed consumption of Noi crossbred hens at the age of 18-30 weeks was in the range of 80.94-86.6 g/head/day. In general, the addition of vitamin mineral premix in the diet did not affect the feed consumption of the experimental hens.

Table 4. Feed intake, FCR and egg mass of Noi crossbred chickens

Parameters	Laying age (Week)	Treatments					P
		Cont	$PM_{2.5}$	PM_5	$PM_{7.5}$	PM_{10}	
Feed intake, g/ hen/day	18-22	73.97±3.72	76.62±3.72	70.70±3.72	65.90±3.72	71.94±3.72	0.34
	23-26	91.9±2.86	91.61±2.86	84.18±2.86	88.45±2.86	87.77±2.86	0.32
	27-30	88.17±2.55	91.56±2.55	87.97±2.55	88.49±2.55	93.16±2.55	0.51
	18-30	84.68±2.03	86.6±2.03	80.95±2.03	80.94±2.03	84.29±2.03	0.22
FCR, gfeed/g egg	18-22	0.91±0.27	1.41±0.31	1.27±0.27	1.01±0.35	1.14±0.27	0.76
	23-26	1.70±0.21	1.94±0.21	1.99±0.21	1.76±0.24	1.91±0.21	0.85
	27-30	1.98±1.15	2.05±1.15	2.03±1.15	1.63±1.15	2.08±1.15	0.20
	18-30	1.39±0.14	1.54±0.14	1.58±0.14	1.11±0.14	1.54±0.14	0.15
Egg mass, g egg/hen	18-22	6.55±2.79	9.84±3.13	4.91±2.79	8.68±3.61	4.73±2.79	0.70
	23-26	20.70±3.55	20.80±3.55	18.28±3.55	15.66±3.55	16.75±3.55	0.79
	27-30	21.82±2.22	19.66±2.22	20.02±2.22	20.36±2.22	18.68±2.22	0.90
	18-30	15.39±1.83	14.94±1.83	13.68±1.83	12.97±1.83	12.68±1.83	0.79

There was no statistically significant difference in egg mass of Noi crossbred hens among treatments (P>0.05). Egg mass from 18 to 30 weeks of age in supplemented diets were lower than that of the control group, 0.45-2.71g eggs/head.

3.3. Egg quality traits of Noi crossbred chickens

The results in Table 5 showed that the addition of premix in the crossbred hen's diet on the egg quality parameters was not statistically significant among treatments (P>0.05). However, the Haugh, egg yolk index and egg shell percentages of most treatments with the addition of premix tended to be higher than that of the control group.

Table 5. Egg quality traits of Noi crossbred hens

Parameters	Treatments					SEM	P
	Cont	$PM_{2.5}$	PM_5	$PM_{7.5}$	PM_{10}		
Egg weight, g	40.49	38.85	37.83	39.5	35.85	1.56	0.29
Albumen, %	54.01	54.24	51.92	54.39	52.78	1.56	0.75
Egg yolk, %	33.04	32.66	34.53	32.40	33.97	1.42	0.80
Egg shell, %	12.95	13.10	13.55	13.21	13.25	0.51	0.94
Egg yolk/albumein	1.69	1.70	1.55	1.72	1.57	0.12	0.79
Eggshell thickness, mm	0.31	0.31	0.32	0.30	0.31	0.01	0.80
Egg shape	1.34	1.34	1.32	1.4	1.29	0.03	0.13
Egg yolk index	4.14	4.48	5.13	4.26	3.90	0.43	0.34
Albumen index	0.05	0.05	0.06	0.06	0.06	0.01	0.93
HU	69.59	70.25	70.08	69.19	70.88	2.3	0.99
L*	49.19	51.39	50.24	50.46	50.85	1.05	0.66
a*	11.84	8.54	11.77	9.86	9.01	1.08	0.11
b*	47.26	46.89	51.45	45.97	44.08	1.88	0.10
Egg yolk color	11.11	9.89	10.11	10.22	10.11	0.52	0.52

4. CONCLUSION

Supplementation of Premix in the diets of crossbred hens did not affect the reproductive performance and egg quality of the hens, however, PM2.5 improved the laying rate and egg production of Noi crossbred hens. From the above study results, it can be suggested to add 2.5mg Premix/kg feed to the diets of laying hens to help improve egg productivity and laying rate in hens.

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EFFECTS OF PROTEASE ENZYME SUPPLEMENTATION AT DIFFERENT LEVELS ON CARCASS PERFORMANCE, MEAT QUALITY AND LAYING PERFORMANCE OF NOI FEMALE CHICKENS

Nguyen Thi Kim Khang[1], Le Hoa Hiep[1], Nguyen Van Truyen[2] and Le Thanh Phuong[3]*

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ABSTRACT

The experiment was conducted to evaluate the effect of protease enzyme supplementation on carcass performance, meat quality and laying performance of Ben Tre Noi female chickens at 14-22 weeks of age. The experiment was arranged in a completely randomized design with 4 treatments and 11 replicates and each replicate was 2 females at 14 weeks of age. The experimental groups were as follows (1) the control (control) using only the feed of the basal diet without the addition of protease enzyme; (2) Pro100 includes basal diet supplemented with 100mg protease/kg of feed; (3) Pro125 including basal diet supplemented with 125mg protease/kg of feed; and (4) Pro150 including basal diet supplemented with 150mg protease/kg of feed. The experimental results showed that the body weight of chickens at the beginning and the end of the experiment was not statistically significant (P>0.05). There was a statistically significant difference in the absolute weight gain of chickens (P<0.05), the highest was in Pro100 (17.48 g/head) and the lowest was in the control group (11.79 g/head). Among treatments, there were no differences in carcass yields nor were there any difference in pH, driploss, a*, and b* of breast and thigh meat (P>0.05). However, L* 30p on breast meat color at 30 minutes after slaughter was highest in Pro125 (43.16) and lowest in control (36.68). Similarly, L*d48h of thigh meat at 48 hours after slaughter was highest in Pro100 (41.04) and lowest in Pro125 (36.68) and Pro150 (37.57). Egg production, laying egg rate, egg weight, age at first laying, egg mass, feed consumption and feed conversion ratio were not significantly different among treatments (P>0.05), however, Pro100 tended to improve the laying egg rate and egg mass compared to control and other supplementation treatments.

Keywords: *Protease enzyme, weight gain, meat color, hen.*

TÓM TẮT

Ảnh hưởng của các mức bổ sung enzyme premix lên năng suất thân thịt, chất lượng thịt và năng suất trứng của gà mái Nòi Bến Tre

Thí nghiệm được thực hiện nhằm đánh giá ảnh hưởng của bổ sung enzyme protease lên năng suất thân thịt và chất lượng thịt của gà mái Nòi Bến Tre giai đoạn 14-22 tuần tuổi. Thí nghiệm được bố trí theo thể thức hoàn toàn ngẫu nhiên với 4 nghiệm thức (NT), 11 lần lặp lại, mỗi lần lặp lại là 2 gà mái 14 tuần tuổi. Các NT lần lượt là: (1) đối chứng (ĐC) chỉ sử dụng thức ăn của khẩu phần cơ sở (KPCS) không có bổ sung enzyme protease; (2) Pro100 gồm KPCS có bổ sung 100mg protease/kgTA; (3) Pro125 gồm KPCS có bổ sung 125mg protease/1kgTA; và (4) Pro150 gồm KPCS có bổ sung 150 mg protease/kgTA. Kết quả thí nghiệm cho thấy khối lượng gà đầu kì và cuối kì giữa các NT khác biệt không có ý nghĩa thống kê (P>0,05). Tăng trọng tuyệt đối có sự khác biệt có ý nghĩa thống kê (P<0,05), cao nhất là ở Pro100 là 17,48 g/con và thấp nhất ở ĐC là 11,79 g/con. Các chỉ tiêu về năng suất thân thịt như KL sống, KL sau nhổ lông, KL lòng, KL tim,...khác biệt không có ý nghĩa thống kê giữa các NT (P>0,05). Tương tự, pH, độ rỉ dịch (ĐRD), a*, và b* của thịt ức và thịt đùi khác

[1] Can Tho University

[2] Trading Service Manufacturing Friends Co., Ltd.

[3] Emivest Feedmill Vietnam Co. Ltd.

[*] Corresponding Author: Assoc. Prof. Dr. Nguyen Thi Kim Khang, Can Tho University, Campus II, 3/2 street, Ninh Kieu district, Can Tho city, Viet Nam. Tel: (84) 939205355; Email ntkkhang@ctu.edu.vn

biệt không có ý nghĩa thống kê giữa các NT (P>0,05). Tuy nhiên, L*30p ở thịt ức 30 phút sau khi giết mổ cao nhất ở Pro125 (43,16) và thấp nhất ở đối chứng (36,56). Tương tự, L*đ48h của thịt đùi ở 48 giờ sau khi giết mổ cao nhất ở Pro100 (41,04) và thấp nhất ở Pro125 (36,68) và Pro150 (37,57). Năng suất trứng, tỉ lệ đẻ, khối lượng trứng, tuổi đẻ quả trứng đầu tiên, sản lượng trứng (SLT), tiêu tốn thức ăn (TTTA) và hệ số chuyển hóa thức ăn (HSCHTA) khác biệt không có ý nghĩa thống kê giữa các NT (P>0,05), tuy nhiên Pro100 có khuynh hướng cho tỉ lệ đẻ và sản lượng trứng cao hơn đối chứng và các NT còn lại.

Từ khóa: *Protease,tăng trọng tuyệt đối, màu sắc thịt, gà mái.*

1. INTRODUCTION

Nowadays, many enzyme preparations added to animal feed have been widely used, including protease enzymes and protein digestibility varies between feeds due to differences in feed composition and amino acid structure (De Coca-Sinova *et al*., 2008). The use of exogenous proteases can help livestock improve protein digestibility in feed (Lemme *et al*., 2004; Cowieson and Ravindran, 2008). Protease (also known as peptidase or proteinase) is an enzyme that catalyzes the hydrolysis of peptide bonds in proteins and converts them into amino acids, which are then uptake and use by cells (Lopez-Otín *et al*., 2015). In most living organisms, protease enzymes are essential for protein digestion and absorption. Research results of Freitas *et al*. (2011) showed that the addition of protease to the diet improved the protein and lipid digestibility in chickens.

According to Nir *et al*. (1993), poultry has the ability to hydrolyze protein, but a significant amount of protein in the diet is not fully digested and excreted (Wang and Parsons, 1998; Lemme *et al*., 2004). Therefore, the addition of enzymes to the diet is considered an effective measure to overcome the above problem. Indeed, incorporation of protease enzymes in poultry diets has been reported to increase nutrient utilization (Romero *et al*., 2013; Cowieson and Roos, 2016; Stefanello *et al*., 2016). In addition, protease was used to incorporate into poultry diets to improve feed conversion ratio, weight gain, and meat performance of Cobb 500 chickens raised in hot and humid tropical environments (Law *et al*., 2018). As protease increases crude protein digestibility (CP) and metabolic energy (ME) in low CP diets (Angel *et al*., 2011; Fru-Nji *et al*., 2011), therefore, protease enzymes can be considered as part of the solution for the poultry industry when antibiotics are not used as growth promoters.

Saleh *et al.* (2019) reported that supplementation of 200-300mg protease/kg of feed increased growth performance in Egyptian chickens reared in open cages at 0-9 weeks of age, while Fang *et al.* (2019) concluded that the addition of 500mg protease/kg of feed had the effect of improving feed efficiency of Cobb chickens reared in close house. Ho Le Huynh Chau *et al.* (2021) also reported that supplementation with 500mg protease/kg of feed improved feed conversion ratio and protease digestibility accumulation of Ri chickens reared at 1-12 weeks of age. The unpublished results of Noi crossbred chickens under open house conditions showed that supplementation of 250mg protease/kg of feed improved body weight, feed conversion ratio and ADG at 28-84 days of age (unpublished data, 2021). Currently, studies on supplementing these additive nutrients in the diet of Ben Tre Noi chickens to improve reproductive performance and meat quality are still very limited.

Therefore, this study was conducted with the aim of investigating the effect of protease enzyme supplementation on the performance of 14-22-week-old Ben Tre female chickens raised in open barn conditions.

2. MATERIALS AND METHODS

2.1. Animals and management

The experiment was conducted from December 7, 2021 to January 31, 2022 at Phong Dien hatchery, Truong Long commune, Phong Dien district, Can Tho city.

The experiment was carried out on a total of 88 females of Ben Tre breed from 14 weeks old to 22 weeks old. All experimental chickens were carefully selected for their appearance, good health, fully vaccinated and dewormed according to the regulations of the farm.

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 (1990) 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 was 2,850 kcal/kg ME and 18% CP, 3.5% calcium, 1.0% phosphorus.

The protease enzyme used in the experiment is in the form of a powder, brown in color, with a slight aroma, with the trade name JEFO PROTEASE® purchased from JEFO Canada Company, branch on the 1st floor, No. 23B, 3rd Street, Quarter 2, Binh An Ward, District 2, HCMC.

2.2. Experimental design and data collection

The experiment was arranged in a completely randomized design with 4 treatments (NT) corresponding to 4 diets supplemented with protease enzyme (Pro) as follows:

Control: Basal feed;

Pro100: Basal feed+100mg protease/kg feed

Pro125: Basal feed+125mg protease/kg feed

Pro150: Basal feed+150mg protease/kg feed

The experiment was repeated 11 times with a total of 44 experimental units, each of which was 2 hens. The total number of experimental chickens was 88 at the age of 14-22 weeks.

Experimental chickens were raised under the same conditions, differing only in diets with or without protease enzyme supplementation. Chickens were fed twice a day, given 40% of the diet at 6:30am and 60% of the diet at 2pm. Drinking water was provided freely by automatic drinking knob. Regularly check and clean feeders and drinkers to minimize the risk of pathogens attacking chickens and avoid possible risks during the experimental period.

Feeds and leftovers were weighed and recorded the next morning. Chicken body weight (g/head) was weighed and recorded at 14, 18 and 22 weeks of age. At 20 weeks of age, the experimental chickens selected for slaughter had an average weight of chickens in the rearing experiment. The slaughter process in chickens was carried out according to the method of Bui Huu Doan *et al.* (2011).

Chickens used for slaughter were weighed and given water only without feeding 24h before slaughter, the chickens was weighed before slaughter, after blood removal, feathers, head, legs and internal organs were removed viscera, and finally the carcass. After slaughter, chicken breast meat and thigh meat after removing the skin and bones were stored in the refrigerator at 4^{o}C for 24 and 48h to evaluate the criteria for color and meat quality. The pH values of breast and thigh meat were measured at 30min, 24 and 48h post-mortem using a pH meter (Hanna Mettler HI991001, USA) that inserted the probe directly into the breast or thigh meat untill the screen shows a constant number. The color of thigh and breast meat was measured with a Chromatometer CR-400 (Japan) with 3 values of L*, a*, b*. L* determines the reflectance of light on the sample at 30min, 24 and 48h post-mortem. Driploss of thigh and breast meat were also recorded.

Eggs were collected at 8am every day, collected eggs are cleaned, weighed and stored in a cool place. The age at laying the first egg of the hens as well as the laying age of 50% of the hens were recorded.

2.3. Statistical analysis

The collected raw data were recorded and processed by Microsoft Excel software, then statistically processed by Minitab Version 16 software according to GLM-ANOVA model. The mean values were compared using the Tukey method with 95% confidence intervals. The first egg laying age and 50% laying age were treated by Chi-Square test (Minitab Version 16).

3. RESULTS AND DISCUSSION

3.1. Growth performance of Ben Tre Noi chickens

It was shown that there was no statistically significant difference among treatments in terms of body weight (BW) at the beginning, the middle and the end of the experiment, as well as the ADG14-18 and ADG14-22 (P>0.05), however the treatments with protease addition tended to be higher than the control. The results in Table 1 showed that ADG18-22 had a statistically significant difference among the treatments, the highest in Pro100 and Pro150 was 17.48 and 17.08 g/head and the lowest in control was 11.79 g/head (P<0.05). This difference may be due to the fact that the addition of exogenous protease increased protein hydrolysis in the diet, increasing the presence of nutrients in the gastrointestinal tract, thereby stimulating intestinal villi growth and Crypt. This is consistent with the conclusion of Angel *et al.* (2011) and Fru-Nji *et al.* (2011) protease enzyme combined with feed in the diet helps to increase the digestibility, making it easier for chickens to absorb, thereby improving their BW. In addition, the supplementation of beneficial exogenous yeast preparations such as protease in poultry diets helps to reduce feed costs and improve intestinal physiology by increasing CP digestibility and metabolic energy in diets with low CP (Angel *et al.*, 2011; Fru-Nji *et al.*, 2011) as well as incomplete amino acid digestibility in all animals (Wang and Parsons, 1998; Lemme *et al.*, 2004).

Table 1. Body weight and laying age of Noi crossbred hens

Parameters	Treatments				SEM	P
	Control	Pro100	Pro125	Pro150		
BW14wks (g/bird)	1,151.2	1,117.0	1,154.1	1,132.0	27.15	0.78
BW18wks (g/bird)	1,336.2	1,331.2	1,317.9	1,299.1	52.25	0.97
BW22wks (g/bird)	1,285.0	1,482.0	1,395.0	1,430.8	146.3	0.68
ADG_{14-18} (g/bird)	6.61	8.97	5.85	7.51	0.84	0.07
ADG_{18-22} (g/bird)	11.79^{b}	17.48^{a}	12.32^{ab}	17.08^{a}	1.54	0.03
ADG_{14-22} (g/bird)	6.37	7.15	5.79	8.40	0.83	0.14

3.2. Carcass yield and meat quality of Ben Tre Noi chickens

The slaughter results of Ben Tre Noi hens in Table 2 showed that there were differences but not statistically significant among treatments (P>0.05) in terms of carcass parameters such as live weight (LW), liveweight loss, carcass weight and dressing percentage with the additional treatments tended to be higher than in control, whereas the internal organs such as heart, liver, gizzard and the proportion of these internal organs tended to be higher in the control group. The results of this analysis showed that enzyme supplementation had no clear beneficial effect on carcass performance of the experimental chickens. The carcass results in this experiment are consistent with Fang *et al.* (2019) that the addition of 300mg protease/kg feed to the broiler diet did not affect the carcass performance of the chickens.

Table 2. Carss yield parameters of Ben Tre Noi chickens

Parameters	Treatments				SEM	P
	Control	Pro100	Pro125	Pro150		
LW (g/bird)	1,177.5	1,190.0	1,155.0	1,252.5	69.10	0.77
LW loss (%)	8.11	7.00	5.71	6.68	0.81	0.26
Carcass weight (g/bird)	782.50	807.50	775.00	850.00	48.61	0.70
Dressing (%)	66.37	67.89	67.06	67.85	0.70	0.41
Breast weight (g/bird)	167.89	176.93	169.79	201.45	14.95	0.39
Breast meat (g/bird)	135.19	140.16	140.32	170.97	13.36	0.26
Breast meat (%)	21.23	21.94	21.93	23.76	1.14	0.47
Thigh weight (g/bird)	241.15	227.03	230.63	262.24	17.48	0.50
Thigh meat (g/bird)	194.69	182.62	190.98	231.95	15.24	0.54
Thigh meat (%)	15.37	14.01	14.94	15.53	0.38	0.06
Gizzard weight (g/bird)	26.36	23.64	21.76	23.21	2.21	0.54
Gizzard (%)	3.35	2.92	2.79	2.74	0.17	0.11
Heart weight (g/bird)	6.26	5.11	5.58	6.30	0.71	0.60
Heart (%)	0.80	0.63	0.71	0.72	0.06	0.30
Liver weight (g/bird)	31.57	25.90	27.24	26.96	2.69	0.48
Liver (%)	4.07	3.19	3.56	3.13	0.32	0.21
Intestine length (cm)	129.75	133.50	127.00	114.75	6.00	0.19
Cecum length (cm)	18.37	16.37	17.50	17.37	1.13	0.67

The results on drip loss (DR), pH, a* and b* of breast and thigh meat at 30min, 24 and 48h among treatments did not have statistical significance (P>0.05). However, L*u30p and L*d48h were statistically significant differences among treatments (P<0.05), where L*u30p had the highest value in Pro125 and lowest in control, while L*d48h had the highest value in Pro100 and lowest in Pro125 and Pro150. The present experimental results showed that the exudate of breast meat and thigh meat tended to be higher in the additional treatments than in the control, which could not be explained by the short trial period. In all poultry species, a pH of breast meat between 5.8-6.0 and thigh meat between 6.2-6.6 is considered normal. Thus, the pH of chicken meat in this experiment after 30min post-mortem was at normal level. After 24 and 48h, the meat pH decreased slightly due to the anaerobic breakdown of muscle glycogen to produce lactic acid. Regarding the color of the breast and thigh meat, Ben Tre Noi chicken meat colors were lighter, less red. According to the color classification of Quiao *et al.* (2001), Ben Tre Noi chickens in this experiment had light colors.

Table 3. Quality of breast and thigh meat

Parameter	Treatments				SEM	P
	Contr	Pro100	Pro125	Pro150		
$DLu_{24h(\%)}$	1.80	2.51	2.16	5.51	1.29	0.21
$DLu_{48h(\%)}$	2.24	3.17	2.93	8.54	1.92	0.13
L^*u_{30p}	36.56^{b}	39.66^{ab}	43.16^{a}	40.84^{ab}	1.00	0.004
a^*u_{30p}	1.63	1.95	0.87	0.68	0.42	0.16
b^*u_{30p}	3.66	4.68	5.37	2.93	0.76	0.16
L^*u_{24h}	41.41	41.92	45.56	42.51	1.26	0.14
a^*u_{24h}	1.01	1.47	0.86	0.98	0.38	0.70
b^*u_{24h}	4.67	6.09	5.45	4.69	0.59	0.31
L^*u_{48h}	42.12	42.89	43.75	40.77	1.46	0.54
a^*u_{48h}	0.68	0.67	0.85	0.87	0.35	0.96
b^*u_{48h}	4.93	5.73	6.04	5.15	0.53	0.45
pHu_{30p}	5.88	5.67	5.71	5.84	0.16	0.76
pHu_{24h}	5.79	5.80	5.68	5.64	0.09	0.57
pHu_{48h}	5.91	5.83	6.08	6.04	0.19	0.78
$DLđ_{24h(\%)}$	1.40	2.49	2.30	4.30	0.89	0.19
$DLđ_{48h(\%)}$	1.36	2.53	2.33	3.89	1.18	0.53
L^*d_{30p}	40.41	34.55	43.01	36.81	2.13	0.07
a^*d_{30p}	5.42	10.1	5.65	7.10	1.28	0.08
b^*d_{30p}	3.49	5.19	4.56	5.30	1.17	0.69
L^*d_{24h}	40.16	41.14	39.07	41.90	1.69	0.67
a^*d_{24h}	7.60	7.36	7.69	7.72	1.21	0.99
b^*d_{24h}	5.69	6.41	5.76	5.95	0.98	0.95
L^*d_{48h}	38.67^{ab}	41.04^{a}	36.68^{b}	37.57^{b}	0.66	0.003
a^*d_{48h}	8.21	7.79	9.21	8.34	0.53	0.33
b^*d_{48h}	6.01	5.81	6.50	6.33	0.72	0.91
pHd_{30p}	5.89	5.88	6.02	5.95	0.12	0.81
pHd_{24h}	6.12	5.81	5.92	6.02	0.11	0.25
pHd_{48h}	5.83	5.62	5.58	5.66	0.15	0.68

3.3. Reproductive performance of Ben Tre Noi chickens

Experimental results recorded that at the beginning of the experiment on 14-week-old chickens and ending at 22 weeks of age, the laying rate of chickens among treatments over weeks of age did not have a statistically significant difference (P>0.05) (Table 4). Average laying rate of Ben Tre Noi hens was in the range of 47.5-75%, gradually increasing through weeks of age, except Pro150. There was no statistically significant difference in egg weight among treatments (P>0.05), egg weight at 19-22 weeks old was from 35.06-36.06 g/egg. The age of first laying eggs of Ben Tre chickens is in the range of 131-139.8 days (P>0.05). The experimental results in Table 4 showed that the age of first laying egg was earlier and the laying rate was higher than that reported by Khang *et al.* (2020) that the age of first laying eggs was 144-154 days and egg laying rate was 12.33-26.19% on Noi chickens at the age of 20-24 weeks old. The difference between the research results may be due to the difference between the chicken breed origin, rearing conditions, supplementary feed sources.

Table 4. Laying rate, egg weight and the age of first laying egg

Parameters	Perious (month)	Treatments (Mean±SD)				P
		Control	Pro100	Pro125	Pro150	
Laying rate (%)	19-20	49.5±10.6	52.0±21.9	71.0	49.5±10.6	0.74
	20-21	46.0±24.4	67.5±37.5	85.0	42.6±24.8	0.50
	21-22	70.7±20.2	71.0±22.2	71.0	56.7±11.8	0.67
	Average laying rate (%)	54.0±18.4	61.3±20.2	75.0	47.5±11.3	0.48
Egg weight (g)	19-20	38.0±1.41	35.6±1.15	34.0	34.0±4.24	0.43
	20-21	33.5±7.14	35.7±4.64	36.0	33.6±6.65	0.94
	21-22	36.3±4.62	36.7±2.05	35.6	37.5±7.27	0.97
	Age of first laying egg (day)	138.7±5.74	137.6 ±12.6	131.0	139.7± 7.97	0.88

The results of Table 5 showed that the effect of protease supplementation on egg mass over the weeks of age was different but not statistically significant among treatments (P>0.05), egg mass of Ben Tre Noi hens at 19-22 weeks of age was in the range of 17.5-26g eggs/head/day. Similarly, feed consumption and feed conversion ratio of chickens among treatments were not statistically significant (P>0.05). The experimental results showed that the addition of protease in the diet did not affect egg production, nor did it affect feed consumption and FCR of Ben Tre Noi chickens.

Table 5. Egg mass, feed consumption and feed conversion ratio

Parameters	Treatments (Mean±SD)				P
	Control	Pro100	Pro125	Pro150	
Egg mass (g egg/hen): 19-20	18.5±3.53	18.3±7.63	24.0	17.0±5.65	0.83
20-21	15.2±9.54	24.0±13.3	30.0	15.3±10.6	0.52
21-22	25.2±7.84	26.1±8.51	25.0	21.0±5.59	0.76
19-22	19.0±7.07	21.8±7.25	26.0	17.5±6.24	0.63
Average feed intake (g/hen/day)	78.7±1.89	79.6±0.51	80.0	78.7±0.50	0.42
FCR (g feed/g egg)	1.75±0.50	2.00±0.00	2.00	1.75±0.50	0.64

4. CONCLUSION

Treatments with protease supplementation not only improved chicken weight gain but also improved meat quality at L*u30p and L*d48h. Pro100 tended to improve the laying egg rate and egg mass compared to control and other supplementation treatments.

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EFFECTS OF SUPPLEMENTARY LEVELS OF *POLYSCIAS FILICIFOLIA* ON THE GROWTH AND QUALITY OF NOI CHICKEN'S MEAT

*Nguyen Huynh Khang[2] and Nguyen Ba Trung[1]**

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ABSTRACT

Noi chicken is widely raised in the Mekong Delta. This experiment was carried out to investigate the effect of different levels of powdered of stem and leaf of *Polyscias filicifolia* (BĐL) on growth and meat quality of Noi chickens, at 5-12 weeks of age. A total of 240 chickens were arranged in a completely randomized design, 5 treatments (NT), 3 replications, the experimental unit was one chicken cage, 16 chickens (8♂:8♀). Five treatments corresponding to 5 levels of (BĐL) supplement: 1) Control (BĐL0) using the basal diet without BĐL, 2) BĐL0.25: basal diet with 0.25% BĐL/kg feed, 3) BĐL0.5: basal diet with 0.5% BĐL/kg feed, 4) BĐL0.75: basal diet with 0.75% BĐL/kg feed, and 5) BĐL1.0: basal diet with 1.0% BĐL/kg feed. As a result, chickens consuming basal diet increased statistically significantly (P<0.05) when increasing BĐL. Chicken weight gain, digestibility of DM, OM and CP was highest in NT3, lowest in NT1 (P<0.05). Feed conversion coefficient ranged from 3.22 to 3.49, the difference was statistically significant, the lowest in NT3 (3.22). BĐL supplementation did not significantly affect dry matter, organic matter, crude protein, fat and total minerals in chicken meat. Thus, adding BĐL at 0.5% to the diet of Noi chickens for the best utilization.

KeyWords: *Polyscias Filicifolia, Noi chicken, gain weight.*

TÓM TẮT

Ảnh hưởng các mức bổ sung hỗn hợp bột thân lá đinh lăng đến sinh trưởng và chất lượng thịt gà Nòi

Gà Nòi được nuôi phổ biến ở Đồng bằng sông Cửu Long. Thí nghiệm này được thực hiện nhằm tìm hiểu ảnh hưởng các mức bổ sung hỗn hợp bột thân lá đinh lăng lá to (*Polyscias Filicifolia*) lên sinh trưởng và chất lượng thịt gà Nòi, giai đoạn 5-12 tuần tuổi. Tổng số 240 con gà được bố trí theo thể thức hoàn toàn ngẫu nhiên, 5 nghiệm thức (NT), 3 lần lặp lại, đơn vị thí nghiệm là một ô chuồng, nuôi 16 con gà (8♂:8♀). Năm NT ứng với 5 mức bổ sung bột đinh lăng (BĐL): 1) Đối chứng (BĐL0.00) sử dụng khẩu phần cơ sở (KPCS) không có BĐL, 2) BĐL0.25: KPCS có bổ sung 0.25% BĐL/kg TA, 3) BĐL0.50: KPCS có bổ sung 0.5% BĐL/kg TA, 4) BĐL0.75: KPCS có bổ sung 0.75% BĐL/kg TA, và 5) BĐL1.00: KPCS có bổ sung 1.00% BĐL/kg TA. Kết quả, gà tiêu thụ KPCS tăng lên có ý nghĩa thống kê (P<0.05) khi tăng BĐL; gà tăng khối lượng (g/con/ngày), tiêu hóa DM, OM và CP (g/con/ngày) cao nhất ở NT3, thấp nhất ở NT1 (P<0,05). Hệ số chuyển hóa thức ăn dao động 3,22-3,49, khác biệt có ý nghĩa thống kê, thấp nhất ở NT3 (3,22). Bổ sung BĐL không ảnh hưởng khác biệt đến vật chất khô, vật chất hữu cơ, đạm thô, béo thô và khoáng tổng số trong thịt gà. Như vậy, bổ sung BĐL ở mức 0.5% vào khẩu phần nuôi gà Nòi cho hiệu quả tốt nhất.

Từ khóa: *Cây đinh lăng (Polyscias Filicifolia), gà Nòi, tăng khối lượng.*

1. INTRODUCTION

Noi chicken is widely raised in the Mekong Delta. In households and some farms, chickens often get sick and the mortality rate is quite high. Therefore, antibiotics are often used to prevent and treat chicken diseases. However, the use of antibiotics is no longer recommended, and should not be mixed into feed as a growth promoter. Therefore, besides using some additives such as organic acids, probiotics, etc., herbs are being studied to replace antibiotics and supplement in poultry diets (Nguyen Thi Kim Khang *et al.*, 2020; Ho Le Quynh Chau *et al.*, 2021; Ho Thi Bich Ngoc *et al.*, 2021; Van Ngoc Phong *et al.*, 2021).

[1] An Giang University

[2] Master student, An Giang University (Sub-Depart. of Fisheries, Depart. of Agr. and Rur. Dev. An Giang).

* Corresponding Author: Dr. Nguyen Ba Trung, Depart of Anim. Hus. Vet. Med., Faculty of Agriculture and Natural Resources, An Giang University, Vietnam National University HoChiMinh City (VNU-HCM). Tel: (84) 0918 139 960; Email: nbtrung@agu.edu.vn.

Therefore, using herbs in poultry production is a positive trend that needs to be studied to replace antibiotic position.

Herbs are often used in powder form, fresh form, or plant extracts (Eltazi, 2014; Le Thi Men and Nguyen Hieu Nghia, 2016; Nguyen Thi Thuy *et al.*, 2020; Nguyen Thao Nguyen *et al.*, 2021; Nguyen Cong Oanh *et al.*, 2021). Recent studies using herbs as additives in chicken feed have helped increase weight gain, improve feed efficiency, and reduce mortality (Cu Thi Thien Thu *et al.*, 2018).

An Giang has rich medicinal resources, in which, *Polyscias Filicifolia* is a familiar plant to many families and is widely grown; roots, stems, leaves are used as medicine with many uses such as increasing physical strength, anti-stress, stimulating the brain, antioxidant, protecting the liver, stimulating immunity. The area of planting *Polyscias Filicifolia* is about 18.8ha, focused in That Son, Tinh Bien district. According to research by Nguyen Thi Thu Huong *et al.* (2001), at the Center for Ginseng and Medicinal Plants in Ho Chi Minh City, *Polyscias Filicifolia* has the same effect as ginseng, but it is cheaper and easier to grow than ginseng. *Polyscias Filicifolia* can be a potential antibiotic alternative, so it needs to be researched and tested as a local natural feed additive that can replace commercial products in poultry production medium and small. Therefore, a study on "*Effect of levels of Polyscias Filicifolia supplementation on growth performance and meat quality of Noi chickens*" is needed.

2. MATERIALS AND METHODS

2.1. Materials

Polyscias Filicifolia over 3 years old was collected at the medicinal garden, at the top of Cam Mountain, Tinh Bien district, An Giang province, the plants were washed and drained. Stems and leaves were thinly sliced and dried at 60°C. After drying, the parts of the plant were ground to a fine powder and sieved through a 3mm sieve.

Powder mixture (stems and leaves) of *Polyscias Filicifolia* (BĐL) added to the diet was calculated based on the daily feed intake of each experimental unit (on dry matter). BĐL is added to the Commercial concentrate food by mixing BĐL into the basal diet, spraying lightly with water, then continuing to mix and continue to spray water until the BĐL adheres evenly to the food, the amount of BĐL mixed is slightly excess to compensate for the drop during chicken feeding, and to ensure that the chickens ate the required amount of BĐL for each experimental unit.

Experimental diets were designed based on the basic diet of complete chicken feed, trademark Koudijs, code 6242, product by De Heus Co., Ltd. (DM: 85%, CP: 19% and ME: 3,000 kcal/kg). Chickens were fed 4-5 times/day with feed and water freely provided. The barn is built with good ventilation. Chickens are raised in cages, 0.8m from the ground, the size of each cage for an experimental unit is 1.5x1.7x1.5m. The barn floor is spread with biological padding (rice husk mixed with product Balasa N01). The cages and breeding equipment are cleaned, disinfected and sun-dried for 7 days before the chickens are brought in. Chickens in all treatments were vaccinated against Newcastle disease, Gumboro, pox and avian influenza at 3, 7, 10 and 15 days of age.

2.2. Experimental design

The experiment was arranged on chickens from 5 to 12 weeks old, from Apr 2022 to Aug 2022 at a household, Cho Moi district, An Giang province; Samples were analyzed at Can Tho University and Center for Quality Management of Agriculture, Forestry and Fisheries Region VI.

A total of 240 chickens were arranged in a completely randomized design, 5 treaments, 3 replicates, corresponding to 15 experimental units. Each experimental unit was a cage with 16 chickens (8♂:8♀) at 5 weeks of age, with an average live weight of 242±1.57g (Mean±SD). Dietary supplements (based on dry matter) included:

BĐL0.00: basal diet + 0.00% BĐL (Control)

BĐL0.25: basal diet + 0.25% BĐL

BĐL0.50: basal diet + 0.50% BĐL

BĐL0.75: basal diet + 0.75% BĐL

BĐL1.00: basal diet + 1% BĐL

Management and measurements: The growth and meat quality parameters of chickens were analyzed according to methods of Bui Huu Doan *et al.* (2011). Survival rate (%) = (number of chickens alive at the end of the period×100)/ number of chickens at the beginning of the period. In which, the number of chickens alive at the end of the period = the number of chickens at the beginning of the period minus the number of chickens that die.

Growth: Chickens were individually weighed on a fixed day each week, early in the morning, before feeding. Feed intake and leftovers were recorded each day. This information will be used to calculate the growth parameters, including: Live weight (LW, g/head), Weight gain (WG, g/head/day), Feed intake (FI, g/head/day), and FCR (g feed/g gain).

Carcass quality: After 12 weeks, 4 chickens (2♂ and 2♀) in each cage with a live weight equivalent to the mean weight of the treatment were selected for carcass survey. About 12 hours before the survey, chickens were kept in normal conditions, exposed to water and not provided with food. Then conduct survey to evaluate carcass yield according to the method of Bui Huu Doan *et al.* (2011). The chemical composition of breast meat weight was analyzed based on the AOAC method (2005), CP was determined by the Kjeldahl method.

2.3. Data analysis

Collected data were sorted by Excel software, and analyzed by the General Linear Model of Minitab 16.2.1 software (Minitab, 2010). The difference between the experimental means was analyzed by Tukey in this program (P<0.05).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of diets

Analysis of the nutrient composition of the Commercial concentrate food and the BĐL, results are shown in Table 1.

Table 1. Chemical composition of diets (%DM)

Feed	DM	OM	CP	Ash
Basal diet	87.0	93.8	19.0	6.25
BĐL	89.2	93.4	6.92	6.63

DM: Dry Matter, OM: Organic matter, CP: Crude Protein, Ash: Total Minerals

Table 1 shows that basal diet has DM and CP of 87 and 19.0% (DM, respectively). The CP content in basal diet is suitable for raising Noi chickens, 28 days old to slaughter. According to a study by Nguyen Thi Thuong (2014), basal diet for Noi chickens from 5 weeks to slaughter had CP of 19.3% and DM of 90.36%. Following to Pham Tan Nha and Nguyen Thi Kim Dong (2020), basal diet raising Tau Vang chickens 7-14 weeks old has a CP of 18%. In this study, the BĐL has a chemical composition of 89.2% DM and 6.92% CP. *Polyscias Filicifolia* is a medicinal plant, helping to strengthen the immune system and increase digestion, so it can be added to the chicken diet within certain limits.

3.2. Effect of BĐL on feed consumption and nutrient intake of chickens

Add different levels of BĐL to the diet of Noi chickens. The results, feed consumption and nutrient intake of chickens in this experiment are presented in Table 2 showing the consumption of basal diet of chickens increased with the increase of BĐL supplementation. BĐL supplementation increased feed intake of chickens, the most obvious difference was in diets supplemented with 0.5-1% DM of BĐL compared with diets not supplemented with BĐL (P<0.05). The increase in basal diet of chickens can be explained by the fact that in BĐL there are antioxidants, increase energy, stimulate brain activity and increase the body's adaptability (Do Huy Bich *et al.*, 2006). In addition, BĐL also has the ability to stimulate eating and to treat poor digestion (Nguyen Tran Anh Thu, 2021). Since then, adding BĐL to the diet has stimulated the eating ability of chickens.

Table 2. Feed intake and nutrient intake of chickens

Variable	Variable	Treatments					SE	P
		BĐL0	BĐL0.25	BĐL0.5	BĐL0.75	BĐL1.0		
Feed intake, gDM/ head/day	Basal diet	57.6^{b}	60.7^{ab}	62.3^{a}	62.1^{a}	62.3^{a}	0.836	0.011
	BĐL	0.00^{e}	0.15^{d}	0.321^{c}	0.481^{b}	0.645^{a}	0.005	0.001
Nutrient intake, g/head/day	DM	57.6^{b}	60.9^{ab}	62.7^{a}	62.5^{a}	62.9^{a}	0.839	0.005
	OM	54.0^{b}	57.1^{ab}	58.7^{a}	58.6^{a}	60.0^{a}	0.787	0.005
	CP	10.9^{b}	11.5^{ab}	11.9^{a}	11.8^{a}	11.9^{a}	0.159	0.008
	Ash	3.60^{b}	3.81^{ab}	3.92^{a}	3.91^{a}	3.93^{a}	0.052	0.005

BĐL0, BĐL0.25, BĐL0.5, BĐL0.75 and BĐL1.0: are additional levels of BĐL by 0.0, 0.25, 0.5, 0.75 and 1% (DM) to basal diet; Mean values with different letters on the same row are statistically significant at P<0.05.

Consumption of DM, OM and CP of chickens was lowest in treatment BĐL0 (P<0.05). The consumption of DM and CP of chickens in the experiment was equivalent to the research results of Pham Tan Nha and Nguyen Thi Kim Dong (2020) when using garlic powder supplemented with 7-14 week old Tau Vang chickens, the values of this is respectively of 61.1-63.6 and 11.1-11.5 g/ head/day. In summary, BĐL affects increased nutrient consumption, helps chickens digest and absorb feed better, the level of 0.5% gives good utilization.

3.3. Effect of BĐL on weight gain FCR

Add different levels of BĐL to the Noi chicken diet. The results, WG, FCR of chickens through the treatments are presented in Table 3 shows the LW of chickens at 5-week-old was not statistically significant (P>0.05), and this value ranged from 241-243 g/head. There was a statistically significant difference in the LW of chickens at the end of the experiment (P<0.05). The highest LW of chickens at the end of the experiment in treatment BĐL0.5 was 1,873g, followed by treatments BĐL0.25, DL0.75 and DL1.0 at 1,767; 1,786 and 1,771g, respectively. BĐL0 treatment gave the lowest result of 1,628g.

Chicken WG was statistically significant between treatments (P<0.05): the highest was 19.4 g/head/day in treatment BĐL0.5, and treatments BĐL0.25, BĐL0.75 and BĐL1.0 were 18.1, 18.4 and 18.2 g/head/day, respectively. Chickens fed diets not supplemented with BĐL had the lowest WG, 16.5 g/head/day. Chickens gain weight when supplemented BĐL, which can be explained as follows: when increasing BĐL helps to increase FI (Table 2), BĐL contain antioxidants, a variety of amino acids, and digestive stimulants, enhance metabolism, as well as increase vitality (Do Huy Bich, 2006; Tran Nguyen Anh Thu, 2021), thereby helping to improve the WG of chickens.

Table 3. Weight gain and feed conversion ratio

Variable	Treatments					SE	P
	BĐL0	BĐL0.25	BĐL0.5	BĐL0.75	BĐL1.0		
LW 5-week-old, g	242	243	241	243	243	0.894	0.415
LW 12-week-old, g	1,628^{c}	1,767^{b}	1,873^{a}	1,786^{b}	1,771^{b}	7.780	0.110
WG, g/head/day	16.5^{c}	18.1^{b}	19.4^{a}	18.4^{b}	18.2^{b}	0.100	0.001
FCR	3.49^{a}	3.35^{ab}	3.22^{b}	3.40^{ab}	3.46^{a}	0.049	0.026
CP/WG, g/kg	663^{a}	636^{ab}	611^{b}	643^{ab}	653^{ab}	9.32	0.025

The FCR of chickens was 3.22-3.49 and there was a statistically significant difference between treatments (P<0.05). When compared with other chicken breeds, the FCR of chickens in this experiment was lower. According to Pham Tan Nha *et al.* (2021) in Tau Vang chickens, adding Black Turmeric powder from 0.0-0.4% DM to the basal diet of commercial concentrate food with 18% CP resulted in an FCR of 3.44-3.57. Thus, adding 0.5% BĐL to the diet reduced FI/kg of BW gain, while still giving growth and higher accumulation compared to no supplementation, as well as supplementing levels 0.25, 0.75 and 1.0% BĐL.

3.4. Effect of BĐL on chicken meat quality

Adding different levels of BĐL to the Noi chicken diet, the results, chemical composition of chicken meat is shown in Table 4. The pH index of meat ranged from 5.767 to 5,857 and the difference was not statistically significant between treatments (P>0.05). The chemical composition of meat was not significantly different between treatments (P>0.05). The DM of chicken meat ranged from 26.82 to 27.57% and the CP value through the treatments ranged from 23.5 to 23.74%. Thus, increasing the level of BĐL in the diet did not affect the DM, OM, CP, EE, and Ash of Noi chicken meat.

Table 4. pH value, chemical composition in chicken meat after slaughter

Variable	Treatments					SE	P
	BĐL0	BĐL0.25	BĐL0.5	BĐL0.75	BĐL1.0		
pH	5.800	5.817	5.767	5.857	5.783	0.053	0.788
DM	27.57	26.82	26.85	27.09	27.51	0.457	0.668
OM	98.38	98.35	98.45	98.85	98.83	0.180	0.205
CP	23.66	23.45	23.74	23.50	23.50	0.331	0.961
EE	1.998	2.050	2.087	2.058	2.161	0.070	0.588
Ash	1.616	1.651	1.552	1.149	1.171	0.180	0.205

4. CONCLUSION

Treatments using the supplemented BĐL in the diets of Noi chickens gave better results in terms of FI, nutrient intake, improved FCR and WG compared to the treatments without the supplement. Adding BĐL at 0.5% to the diet of Noi chicken gave the best utilization. BĐL did not significantly affect the DM, OM, CP, EE, and Ash of Noi chicken meat.

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EFFECTS OF VITAMIN C SUPPLEMENTION IN THE DRINKING WATER ON HEMATOLOGICAL INDICATORS OF GROWING AND REPRODUCTIVE DOE RABBITS

Truong Thanh Trung[1], Tran Long Hai[1] and Pham Thi Cam Nhung[1]*

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ABSTRACT

This study aimed to evaluate the effects of vitamin C supplementation in the drinking water on the ability to ameliorate heat stress in rabbits, through the evaluation of blood physiological and biochemical, and growth and reproductive performance of rabbits. The experiment was conducted in 2 rabbit groups: growing rabbits and reproductive rabbits. The first experiment: fifty local crossbred rabbits at 40-55 days of age (691.2±12.5g) were arranged in a completely randomized design with 5 treatments, 5 replications. Two rabbits including 1 male and 1 female were in one experimental unit (a cage). Five treatments were levels of vitamin C supplementation: 0, 250, 500, 750 and 1,000 mg/l in drinking water, respectively. The second experiment: 20 reproductive crossbred doe rabbits at the second litter of 8-8.5 months of age were also arranged in a completely randomized design with 5 treatments (corresponding to the levels of vitamin C supplementation as the first experiment), 4 replicates, 1 doe rabbit per experimental unit. The study results showed that the WBC, RBC and hemoglobin counts were improved in the first group, however, this difference was not significant (P>0.05). Contributing to enhancing the resistance and ability to absorb nutrients hence it could improve daily weight gain (DWG). Supplementation at 500 mg/liter/day and 750 mg/liter/day gave the best effect (P<0.05), the average DWG was 20.68 g/per/day and 21.18 g/per/day, respectively. In the reproductive rabbits, vitamin C did not significantly affect the change in physiological and biochemical parameters. Although, supplementation at 500 mg/liter/day resulted in weaning weight was highest (2,946 g/litter) and lowest at 750 mg/liter/day (1,713 g/litter) (P<0.05). In conclusion, vitamin C contributed to increased nutrient absorption and improved the red and white blood cells count and supplementation at 500 mg/liter of water gave the best result in both growth and reproductive performance rabbits.

Key words: *Blood biochemical and physiology, heat stress, rabbits, vitamin C.*

TÓM TẮT

Ảnh hưởng của việc bổ sung vitamin C trong nước uống lên các chỉ tiêu sinh lý và sinh hóa máu của thỏ tăng trưởng và thỏ sinh sản

Nghiên cứu này nhằm đánh giá ảnh hưởng của việc bổ sung vitamin C trong nước uống lên khả năng cải thiện tình trạng stress nhiệt, thông qua việc đánh giá sinh lý và sinh hóa máu, năng suất sinh trưởng và sinh sản. Thí nghiệm được tiến hành ở 2 nhóm: thỏ tăng trưởng và thỏ sinh sản. Thí nghiệm đầu tiên: năm mươi con thỏ lai địa phương ở 40-55 ngày tuổi (691,2±12,5g) được bố trí theo thể thức hoàn toàn ngẫu nhiên với 5 nghiệm thức và 5 lần lặp lại. Hai con thỏ (1 cái và 1 đực) là một đơn vị thí nghiệm. Năm nghiệm thức là 5 mức độ bổ sung vitamin C: 0, 250, 500, 750, 1000 mg/l nước. Thí nghiệm thứ hai là 20 con thỏ cái lai ở lứa thứ hai từ 8-8,5 tháng tuổi, bố trí theo thể thức hoàn toàn ngẫu nhiên với 5 nghiệm thức (mức độ bổ sung vitamin C như thí nghiệm đầu tiên), 4 lần lặp lại, một đơn vị thí nghiệm là một con thỏ cái. Kết quả cho thấy số lượng bạch cầu, hồng cầu và hemoglobin được cải thiện ở nhóm thỏ đầu tiên (P>0,05). Bổ sung 500 mg/l/ngày và 750 mg/l/ngày cho kết quả tốt nhất (P<0,05), tăng trọng trung bình hằng ngày tương ứng lần lượt là 20,68 g/con/ngày và 21,18 g/con/ngày. Nhóm thứ 2 không có sự thay đổi về sinh lý và sinh hóa máu. Ở mức 500 mg/lít/ngày cho kết quả tốt nhất về khối lượng cai sữa (2946 g/ổ) và thấp nhất ở

[1] Can Tho University

* Correspondence Author: Dr. Truong Thanh Trung, Department of Animal Sciences, Faculty of Agriculture, Can Tho University, Vietnam. Campus II, 3/2 street, Ninh Kieu ward, Can Tho city. Tel: (84)988911650; Email: tttrung@ctu.edu.vn

750 mg/l/ngày (1.713 g/ổ) (P<0,05). Tóm lại, vitamin C góp phần làm tăng sự hấp thu dinh dưỡng và cải thiện số lượng hồng cầu và bạch cầu và bổ sung 500 mg/lít nước cho kết quả tốt nhất cả năng suất sinh trưởng và sinh sản của thỏ.

Từ khóa: *Sinh lý và sinh hóa máu, stress nhiệt, thỏ, vitamin C.*

1. INTRODUCTION

Rabbit breeding is a fairly developed and potential profession in the Mekong Delta due to the great advantage of vegetable resources as a good natural food source for the development of rabbit breeding. However, rabbits are very sensitive to temperature, high temperature leading to stress adversely affecting the ability to increase weight, poor intake, poor food efficiency, reduced meat quality and high mortality (Marai *et al*., 2008; Hassan *et al*., 2016). Heat stress also reduces the amount of milk for breastfeeding mothers. According to Sayed-Ahmed *et al*. (2018) hemoglobin, hematocrit and red blood cell count levels have decreased significantly, while cholesterol, creatinine, white blood cells and lymphocytes increased in the summer compared to winter. Vitamin C plays an important role in the prevention of stress, having an improved effect on immunity and growth (Ibiyo *et al*., 2006). Vitamin C is resistant to the effects of heat stress through the activity of free radicals (Lee, 2002). According to Al-Shanty (2003) showed that vitamin C (1.0 g/l) significantly improved weight gain per day of the developing Flander rabbit compared to the monitoring group. Supplementing with 400mg of vitamin C per 1kg of feed in the diet of breeding rabbits improved feed intake, infant population, infant/drive volume and milk intake of the mother rabbit (Abdel-Khalek *et al*., 2008). Abd-El-Hamid (1994) indicates that the addition of ascorbic acid significantly improves the number of red blood cells (RBC) and white blood cells (WBC) of rabbits. Vitamin C supplementation at 40 mg/kg of body weight significantly increases hemoglobin, red blood cell count and red blood cell volume of male New Zealand White rabbits (Yousef *et al*., 2003). The study of vitamin C supplementation in drinking water is based on natural vegetable rations in the Mekong Delta to assess the ability of vitamin C to improve heat stress in rabbits. This study is conducted to determine the optimal vitamin C levels and the ability to improve the thermal stress of vitamin C in rabbits through the evaluation of improvements in weight gain, reproduction and changes in physiological and biochemical indicators of blood.

2. MATERIALS AND METHODS

2.1. Location, time and animals

The experiment consisted of 50 post-weaning rabbits (including 25 males and 25 females) for growth experiment and 20 breeding female rabbits in the first and second litter for the reproductive experiment. Rabbits are fully vaccinated against parasitic diseases, hemolytic failure and respiratory diseases. The experiment was carried out at the experimental farm which is located in Thoi Hoa ward, O Mon district, Can Tho city, Vietnam. The chemical analysis of feed, feces, urine was done at the laboratory E205 of the Department of Animal Sciences, College of Agriculture and Applied Biology, Can Tho University. The implementation of this study was from July to October 2021.

2.2. Growth experiment design

The growth experiment was arranged in a completely randomized design with 5 treatments and 5 replications. Five treatments were 5 vitamin C levels adding to drinking water at 0, 250, 500, 750, 1,000mg per one litter corresponding to C0, C250, C500, C750 and C1000 treatments, respectively. Two rabbits including one male and one female were in an experimental unit. Drinking water was supplied for rabbits by automatic drinking water bottle. The experiment lasted 10 weeks.

Table 1. The diet composition for growth rabbit

Feed (g/per/day)	**Treatments**				
	C0	**C250**	**C500**	**C750**	**C1000**
Ipomoea aquatica	90	90	90	90	90
Soybean extraction meal	9	9	9	9	9
Soya waste	90	90	90	90	90
Pennisetum purpureum	36	36	36	36	36
Nutritional content in food/per/day					
%CP	23.0	23.0	23.0	23.0	23.0
%NDF	38.0	38.0	38.0	38.0	38.0
DE (MJ/kg of dry matter)	10.8	10.8	10.8	10.8	10.8

The amount of food presented in the feeding state, CP: Crude protein, ME: Metabolizable energy, C0, C250, C500, C750 and C1000: 0, 250, 500, 750 and 1.000 mg/liter of water.

2.3. Feeds, Feeding And Management

Ipomoea aquatica, soybean extraction meal, soya waste and ascorbic acid and *Pennisetum purpureum* were used in the experiment. *Ipomoea aquatica* and *Pennisetum purpureum* were collected daily in the areas surrounding farm. Soybean extraction meal was bought at a local stock feed store in during the experiment while soya waste was taken every day from tofu store. Vitamin C (Ascorbic acid, assay ≥99%) was imported from Ningxia Yuan Pharmaceutical Company. These feeds were given in fresh and were offered three times a day at 7:00am, 12:00pm and 17:00pm. Ascorbic acid was added to drinking water and freely available. The refusals were weighed daily in the morning to calculate the feed intake. The rabbits were vaccinated to prevent hemorrhagic and parasite diseases. The experimental diets containing 23.0% crude protein, 38% neutral detergent fiber and 10.8 MJ/kg DM. The feedstuffs were increased weekly at 10-15% to cover the nutrient requirements of growing rabbits. Ambient temperature (0C) was measured by Thermos recorder TR-73U at 6am, 8am, 10am, 12pm, 14pm, 16pm and 18pm during the experiment.

2.4. Reproductive experiment design

The reproductive experiment was arranged in a completely randomized design with 5 treatments and 4 replications. Five treatments were 5 vitamin C levels adding to drinking water at 0, 250, 500, 750 and 1000mg per one litter corresponding to C0, C250, C500, C750 and C1000 treatments, respectively. Each unit of the experiment is a 4.0-4.5 month-old crossbred does rabbit in two litters.

Table 2. The diet composition of reproductive rabbit

Feed (g/per/day)	**Treatments**				
	C0	**C250**	**C500**	**C750**	**C1000**
Pennisetum purpureum	200	200	200	200	200
Soya waste	200	200	200	200	200
Soybean extraction meal	30	30	30	30	30
Commercial feed pellet	30	30	30	30	30
Nutritional content in food/per/day					
CP (%)	22.0	22.0	22.0	22.0	22.0
ME, MJ (kcal)	9.96	9.96	9.96	9.96	9.96

Soybean extraction meal, soya waste and ascorbic acid, commercial feed pellet and *Pennisetum purpureum* were used in the experiment. *Pennisetum purpureum* were collected daily in the areas surrounding farm. Soybean extraction meal was bought at a local feed store in one occasion during the experiment while soya waste was taken every day from tofu store. These feeds were given in fresh and were offered three times a day at 7:00am, 12:00pm and 17:00pm. The experimental diets containing 22.0% crude protein and 9.96 MJ/kg DM. Ambient temperature (^{0}C) was measured by Thermos recorder TR-73U at 6am, 8am, 10am, 12pm, 14pm, 16pm and 18pm during the experiment. The refusals were weighed daily in the morning to calculate the feed intake. All feeds were analyzed chemical composition and calculated ME for the treatments.

The feeds and refusals were taken for analysis of DM, OM, CP, EE, NDF, ADF, and ash following procedures of AOAC (1990), Van Soest *et al.* (1991) and Robertson and Van Soest (1981). The metabolizable energy (ME) values of feeds were calculated according to the formula proposed by Cheeke (1987) and Maertens *et al.* (2002).

Rabbits were weighed individually every week. Daily feed intakes, growth rate, and

feed conversion ratios were measured and calculated. After finishing, the experimental rabbits were slaughtered for evaluating carcass quality. The economic analysis was also done among the treatments.

Apparent of nutrient digestibility and nitrogen retention were determined by collecting and analyzing offered and refused feeds, feces, and urine daily. The animals had one week for getting samples according to McDonald *et al.* (2002). Feeds and refusals were daily measured. Urine was also collected for nitrogen analysis to calculate the nitrogen retention. The DM, CP, EE, NDF, ADF digestibility were employed according McDonald *et al.* (2002).

2.5. Blood draw method and test quota

Blood draw method: The rabbit blood was taken 2 times, the first time when the rabbit finished 10 weeks of the growth experiment, the second time when the rabbit finished the reproductive experiment completed the 2nd litter. Rabbit blood is taken through the ear veins, put into 2 different test tubes. Test tubes contain EDTA and test tubes contain heparin. After that the blood was analyzed at The Center Lab Vietnam medical laboratory. The hematological indicators are analyzed by machine: Cell-DynR 1700 (manufacturing: Abort). Biochemical indicators are analyzed by machine: Humalyer 2000 (production: Humen).

Test norms: WBC (White blood cell): The number of white blood cells present in a unit of blood; % LYM: Three-bridge forestry rate; RBC: The number of red blood cells present in a unit of blood; HGB: Hemoglobin levels in the blood; HCT: Red blood cell volume ratio; PLT: Platelet count. Biochemical indicators: Protein, Glucose, Triglycerrid, Cholesterol TP, HDL-C, LDL-C.

2.6. Statistical analysis

The data is preliminarily processed on an Excel spreadsheet, processed and analyzed according to the General Linear Model of the Minitab 16 program (Minitab, 2014). Data were analyzed using the model $yijk = \mu + T_i + A_j + P_k + e_{ijk}$; *where yijk: the dependent variable,* μ*: the overall mean,* T_i *= the effect of treatment (i = 1 to 4), Aj: the effect of animal (j = 1 to 4),* P_k*= the effect of period (j = 1 to 4), and* e_{ijk} *= the random error*. Compare the differences between the tests by the Tukey method of the Minitab 16 program (2014).

3. RESULTS AND DISCUSSION

3.1. Growth experiments

Table 3 showed that the number of WBCs and RBCs in the vitamin C-supplemented treatments tends to be higher than in the control treatment, although this difference has not been significant (P>0.05). The percentage of lymphocytes in the vitamin C-supplemented treatments tended to be lower than in the control, which indicates that the increased WBC was due to the effect of vitamin C and not due to the effect of disease in rabbits. According to Ahmadu *et al.* (2015) when pathogens invade, vitamin C enhances white blood cells and protects phagocytic cells from oxidative damage. Hence, vitamin C increased the ability of phagocytosis, increasing the body's resistance. Earlier studies indicated that vitamin C supplementation increases red and white blood cell counts (Abd-El-Hamid, 1994; Sahota *et al.*, 1994). Khaled *et al.* (2019) when supplementing with vitamin C 40 mg/kg body weight, the red blood cell count was $5.8\pm0.23x10^{12}$/l higher than the control treatment. According to Abd El-Hamid and El-Adawy (1999) the WBC and RBC values of NZW rabbits increased significantly with increasing levels of vitamin C supplementation. However, the above results showed that, in the experimental treatment The C1000 had a lower erythrocyte count compared with C0, although other treatments tended to be higher. This result was similar when compared with the hemoglobin (HGB) and hematocrit (HCT) content of rabbits in the experiment. According to Kird and Bistner (2000), there is a positive correlation between hemoglobin content and red blood cell count. The higher

the hemoglobin content, the higher the red blood cell count and vice versa. Thereby, it was shown that vitamin supplementation at C1000 level did not contribute to improving the red blood cell count in rabbits. Many research results show that vitamin C increases the absorption of iron in the diet (Hallberg *et al.*, 1986; Cook and Reddy, 2001) thereby increasing the synthesis of HGB, the oxygen transporter, and the metabolism.

Table 3. Growth rabbit blood physiological results

Item	Treatments					SEM	P
	C0	C250	C500	C750	C1000		
WBC,10^3/µl	8.02	10.33	9.04	8.66	8.33	0.56	0.11
LYM, %	51.2	44.1	40.9	43.9	43.7	2.82	0.20
RBC, 10^{12}/l	5.43	5.88	6.06	5.91	5.34	0.20	0.10
HGB, g/dl	13.23	14.48	14.60	14.18	13.10	0.35	0.07
HCT, %	44.30	47.80	49.60	47.70	43.60	1.79	0.17
PLT, 10^9/l	504^{ab}	557^{ab}	503^{ab}	648^{a}	471^{b}	32.1	0.02

Note: WBC: White Blood Cell, LYM: Lymphocyte, RBC: Red Blood Cell, HGB: Hemoglobin, HCT: Hematocrit, PLT: Platelet Count. The value bearing different letters on the same row is statistically significant difference (P<0.05).

The number of platelets (PLT) in C750 was statistically significant compared with C1000 (P<0.05). The main function of platelets is to protect vascular endothelial cells, participate in protein and lipid synthesis, and contain proteins that resist the effects of heparin. Release of thrombokinase involved in blood clotting.

Table 4 showed that there was no significant difference in the initial live weight (P>0.05). Final live weight and daily weight gain were highest at C500 and C750 (P<0.05). Comparing the results of weight gain with the results of blood physiology in Table 3, the results gave to C500 and C750 had a higher number of red blood cells than the other treatments. The above results showed that vitamin C increased the ability to better absorb nutrients from the diet through improving the number of red blood cells and enhances metabolism, which would help improve daily weight gain in rabbit. According to Al-Shanty (2003) and Yassein *et al.* (2008) showed that adding vitamin C to water at the concentration of 1.0 g/l significantly improved the final live weight and weight gain when compared with the control group.

Table 4. Daily weight gain of the growth rabbit

Item	Treatments					P	SEM
	C0	C250	C500	C750	C1000		
Initial live weight, g	688.7	687.3	678.7	698.2	703.7	0.70	13.14
Final live weight, g	1957.9^{b}	2054.3^{ab}	2126.5^{a}	2180.6^{a}	2110.6^{ab}	0.01	35.63
Daily weight gain, g/day	18.13^{b}	19.53^{ab}	20.68^{a}	21.18^{a}	20.1^{ab}	0.01	0.50

Tables 3 and 4 showed that vitamin C supplementation in rabbits contributes to enhancing resistance and absorption capacity, thereby improving weight gain in rabbits. Supplementing at C500 and C750 gave the best effect. However, C1000 supplementation did not have a positive effect on reducing platelet count and was not significant in the results of weight gain in rabbits.

3.2. Reproductive experiment

Table 5 showed that when supplementing with different levels of vitamin C in reproductive rabbits, there were fluctuations in blood physiological parameters such as red blood cells, white blood cells, platelets, hemoglobin content, blood platelet count and lymphocyte count. However, this difference had not been statistically significant (P>0.05), these parameters were within the normal physiological value of rabbits at the reproductive stage. The RBC count, HGB and HCT indiced in the vitamin C supplementation treatments tended to be lower than in the control treatment, the results were similar to the study results (Hassnaa *et al.*, 2008), when supplementing with vitamin C in reproductive rabbits to improve the

effects of heat stress on breeding rabbits. This could explain that the increase in red blood cell count in the control treatment could be due to the effects of heat stress-induced dehydration. Oral vitamin C supplementation in summer rabbits may protect animals from the harmful effects of heat stress. The beneficial effect of vitamin C in alleviating heat stress was reported by Alam (2000) and Marai *et al.* (2002). Alam (2000) states that vitamin C is involved in the synthesis of some stress hormones such as epinephrine. These hormones control respiratory rate, blood flow, and pressure and maintain a roughly constant body temperature during heat stress.

Table 5. Reproductive rabbit blood physiological results

Item	Treatments					P	SEM
	C0	C250	C500	C750	C1000		
WBC, 10^9/l	5.73	5.41	7.35	4.96	6.43	0.43	0.91
LYM, %	19.4	15.9	27.7	26.8	24.0	0.08	3.00
RBC, 10^{12}/l	5.25	4.64	5.06	4.83	4.82	0.30	0.20
HGB, g/dl	11.1	10.1	10.9	10.0	10.5	0.43	0.48
HCT, %	32.8	30.6	32.8	30.2	32.1	0.59	1.41
PLT, 10^9/l	275.3	339.3	345.3	487.3	421.3	0.18	59.13

Table 6 showed that rabbits in the vitamin C supplementation treatments had cholesterol (0.4-0.9 mmol/l), HDL-C (0.43-0.76 mmol/l) and LDL-C levels (0.32-0.5 mmol/l) tended to be lower in rabbits in the non-vitamin C-supplemented treatment. The higher the LDL-C and triglycerides, the higher the risk of fat metabolism diseases. However, this difference was not statistically significant (P>0.05). These values are all within the normal physiological values.

The protein of rabbits in the vitamin C-supplemented treatment tended to be higher than that of the control. It showed that the vitamin C-supplemented rabbit group absorbed better nutrition than the non-supplemented rabbits, the difference in total protein value between the two groups of rabbits was not statistically significant (P>0.05). Protein content in normal physiological state ranges from 49-71 g/l (Hewitt *et al.*, 1989). According to Nguyen Thi Kim Dong and Ho Thanh Tham (2017), protid accounts for 6-8% of the total plasma. Most total protein is synthesized in the liver, so testing for total protein is helpful in the diagnosis of liver and kidney disease. Low protein may suggest liver or kidney dysfunction or protein digestion or absorption.

Through the above results, when supplementing with vitamin C with a high content of 1000 mg/liter/day for 10 consecutive weeks, it did not cause liver and kidney toxicity. Besides, it contributes to improving the ability to absorb protein well and metabolize bad fat.

Table 6. Reproductive rabbit blood biochemical results

Item	Treatments (mmol/l)					P	SEM
	C0	C250	C500	C750	C1000		
Glucose	6.67	5.96	6.6	6.94	6.16	0.19	0.29
Triglycerrid	1.13	1.33	0.72	0.27	0.89	0.54	0.45
Cholesterol TP	1.2	0.4	0.9	0.67	0.63	0.46	0.31
HDL-C	0.79	0.43	0.72	0.76	0.50	0.41	0.16
LDL-C	0.76	0.34	0.5	0.32	0.38	0.54	0.20
Protein, g/l	59.7	65.7	63.7	61.7	59.7	0.76	3.79

HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol,

Table 7 shows that vitamin C supplementation in the drinking water does not affect the reproductive performance on litter size at birth, litter weight at birth and mortality embryos at birth. In addition it does not affect the milk yield of the doe rabbit, weight of 21-day-old rabbits and litter size at weaning (P>0.05). In C500 treatment, litter weight at weaning is the highest and differs significantly (P<0.05). From the above results show that vitamin C contributes to good improvement of litter weight at weaning because vitamin C supplementation in drinking water improved nutrient absorption better than adding to food. Litter size at birth and weaning is higher than in the control treatment. According to the results of Trung (2020) when using the same experimental

ration and adding vitamin C to drinking water at C500 levels, litter size at birth and litter size at weaning improved significantly. Litter size at birth is 5.0-7.0 kits and litter size at weaning is 4.75-6.5 kits. Vitamin C contributes to improved reproductive performace, increasing the survival rate of kits from birth to weaning.

Table 7. Reproducive performance of does rabbit

Item	Treatments					P	SEM
	C0	C250	C500	C750	C1000		
Litter size at birth	5.40	6.40	8.00	6.20	6.60	0.18	0.71
Litter weight at birth (g)	322.1	347.9	422.1	309.7	355.5	0.24	35.4
Litter size at weaning	5.20	5.80	7.40	5.60	5.60	0.06	0.52
Litter weight at weaning	1950^{ab}	2177^{ab}	2946^{a}	1713^{b}	2059^{ab}	0.02	230
Milk yield of the doe rabbit (g)	97.12	92.38	94.01	87.90	87.12	0.76	6.17
Weight of 21-day-old rabbits (g)	234.8	228.3	223.5	204.1	217.3	0.72	16.2

Through the results presented in Table 5, 6 and 7, vitamin C supplementation in rabbits at the reproductive stage did not significantly affect the change in blood physiological and biochemical indicators. However, vitamin C supplementation at 500 mg/l/day provided the best end-experiment weaning weight results. Besides, the addition of vitamin C also brought many positive improvements in reducing heat stress, good absorption of nutrients and improved bad fat.

4. CONCLUSION

From the results of this study, vitamin C supplementation contributes to increased nutrient absorption and improved the number of red blood cells and white blood cells on rabbits. Vitamin C supplementation at 500 mg/liter of water gains the best results in daily weight gain of growing rabbits and reproductive performace of doe rabbits.

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EFFECTS OF SPICE HERBS ON THE GROWTH PERFORMANCE OF NOI CHICKENS

Nguyen Tuyet Giang[1,2], Do Vo Anh Khoa[3], Le Thi Thuy Hang[1,2] and Nguyen Thi Hanh Chi[1,2]*

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ABSTRACT

The study was conducted to investigate the effects of dietary spice herbs (a mixture of turmeric, lemongrass and garlic) on the growth performance of Noi chickens from 28 to 98 days of age. A total of 240 Noi chickens were randomly allocated into five dietary treatments: T0, T25, T50, T75 and T100, corresponded to 5 levels of herb supplementation (0, 0.25, 0.5, 0.75 and 1.0%) to the basal diet. Each treatment was replicated three times with 16 birds (8 males and 8 females) per each. The results showed that body weight, average daily gain and feed conversion ratio (FCR) were significantly better (P<0.05) in groups of chickens fed with a mixture of spice herbs, particularly at 56 days of age, while average daily feed intake was not affected (P>0.05) during whole trial time. In overall, the FCR and mortality rate were lowest (3.18 and 0%, respectively) with the inclusion of 1.0% in the diet. From these findings, it can be concluded that the herbal mixture of garlic, lemongrass and turmeric can be added up to 1.0% (10 g/kg feed) in the diet to improve the growth performance of chickens.

Keywords: *Growth performance, Noi chicken, mixture, spice herb.*

TÓM TẮT

Ảnh hưởng của gia vị thảo dược đến năng suất tăng trưởng của gà Nòi

Nghiên cứu được thực hiện nhằm khảo sát ảnh hưởng của các mức bổ sung hỗn hợp gia vị thảo dược (từ bột tỏi, bột sả và bột nghệ) đến năng suất tăng trưởng của gà Nòi từ 28 đến 98 ngày tuổi. Tổng số 240 con gà Nòi được bố trí ngẫu nhiên vào 05 nghiệm thức: T0, T25, T50, T75 và T100, tương ứng với 05 mức bổ sung hỗn hợp thảo dược (0, 0,25, 0,5, 0,75 và 1,0%) vào khẩu phần cơ bản. Mỗi nghiệm thức được lặp lại ba lần với 16 con gà Nòi (8 con trống và 8 con mái) cho mỗi lần lặp lại. Kết quả cho thấy khối lượng cơ thể, tăng khối lượng bình quân/ngày và hệ số chuyển hóa thức ăn (FCR) tốt hơn đáng kể ở nhóm gà được cho ăn hỗn hợp gia vị thảo dược, đặc biệt ở 56 ngày tuổi, trong khi lượng thức ăn bình quân/ngày không bị ảnh hưởng (P>0,05). Nhìn chung, FCR và tỷ lệ tử vong thấp nhất (tương ứng 3,18 và 0%) ở nghiệm thức bổ sung 1,0% thảo dược. Từ các kết quả trên, có thể kết luận rằng hỗn hợp thảo dược từ tỏi, sả và nghệ có thể được bổ sung đến mức 1,0% (10 g/kg thức ăn) để cải thiện năng suất tăng trưởng của gà.

Từ khóa: *năng suất tăng trưởng, gà Nòi, hỗn hợp, gia vị thảo dược.*

1. INTRODUCTION

Poultry is the most commonly farmed species in Vietnam and the second largest meat contributor in the country. Their population has grown significantly in the previous two decades, around 5.14% annually. In 2020, a population of 513 million birds in the country produced about 1.5 million tonnes of meat and 15 billion eggs (Birhanu *et al.*, 2021). However, with the increase in number, poultry production is undergoing a continuous challenge to optimize chickens' efficiency while ensuring food safety. Traditionally, pharmaceutical products, such as antimicrobials, have been employed in poultry production as certain drugs and feed additives for rapid growth and health (Mund *et al.*, 2017). Nevertheless, inappropriate use of these products causes an accumulation of

[1]An Giang University
[2]Vietnam National University Ho Chi Minh City
[4]Animal Husbandy Association of Can Tho, Vietnam
* Corresponding Author: Dr. Nguyen Tuyet Giang, Department of Animal Science and Veterinary medicine, An Giang University, An Giang province, Vietnam. Tel: (84) 902 719 021, Email: ntgiang@agu.edu.vn

harmful residues in meat and eggs, which in turn threats public health by a spread of microbial resistance. This results in treatment failures, economic losses and could act as a gene pool for zoonotic diseases (Agyare *et al.*, 2018; Muaz *et al.*, 2018). Therefore, emergence concerns must be taken to provide safe animal origin food to consumers.

There are a broad spectrum of alternatives to antibiotics being proposed such as vaccines and other immunity modulating agents, organic acids, probiotics, prebiotics, and synbiotics, phytobiotics, enzymes (Cheng *et al.*, 2014). Phytobiotics are also known as herbs or phytogenic additives, have shown their efficacy on improving palatability, feed conversion, daily weight gain, dressing percentage and carcass traits, which in turn lead to enhanced poultry production. They are classified based on medicinal properties, essential oil extracts and bioactive compounds (Abdelli *et al.*, 2021). Some of the herbs are spices which are easy found in Vietnam, such as garlic (*Allium sativium*), lemongrass (*Cymbopogon citratus*) and turmeric (*Curcuma longa*). These plants with their bioactive compounds (such as allicin, allyldisulfide, curcumin, turmerones, citral, myrcene,...) have been reported to possess antioxidative antimicrobial, anti-inflammatory and immuno-modulatory properties (Odoemelam *et al.*, 2013; Manvitha and Bidya, 2014; Krauze, 2021). The synergistic combination of these components may give a complementary effects on the growth performance of chickens. Therefore, this study was designed to evaluate the effect of mixed herb product from garlic, lemongrass and turmeric on the growth performance of Noi chickens, a famous indigenous chicken breed in Vietnam.

2. MATERIALS AND METHODS

The experiment was conducted from February to April 2022, at a experimental farm in Long Xuyen city, An Giang province. A total of 240 Noi chickens at 28 days of age were evenly distributed by gender for use in the present study. There were five treatments: T0, T25, T50, T75 and T100, corresponded to 5 levels of herb supplementation (0, 0.25, 0.5, 0.75 and 1.0%) to the basal diet. Each treatment was replicated three times with 16 birds (8 males and 8 females) per each. The spice herb powder was mixed from three ingredients: garlic, lemongrass and turmeric with an equivalent ratio of 1:1:1 by weight. The basal diets were formulated to meet the nutritional requirements of broiler chickens as indicated by NRC (1994), as shown in Table 1. Water and mash feed were offered *ad libitum*. All birds were vaccinated against Newcastle disease, Gumboro disease, fowl pox and Avian influenza according to the recommended veterinarian program.

Table 1. Ingredients, chemical composition of basal diet

Ingredients (%)	**28-56 days old**	**≥56 days old**
Broken rice	23.0	23.0
Rice bran	39.0	40.0
Maize	17.0	19.0
Fish meal	10.0	8.0
Soybean meal	10.0	9.0
Mineral-vitamin premix*	0.5	0.5
Dicalcium phosphate	0.5	0.5
TOTAL	*100.0*	*100.0*
Metabolizable energy and chemical composition **		
ME (MJ/kg)	13.3	13.4
Ash (%)	6.61	6.18
DM (%)	89.5	89.8
CP (%)	18.4	17.1

**1kg premix contains 2,500,000IU vitamin A; 350,000IU vitamin D_3; 1,000mg vitamin E; 1,500,000mg B_1; 2,500,000mg vitamin B_2; 8,000mg vitamin B_5; 650mg vitamin B_6; 9,000mg vitamin PP; 127-130mg Fe; 380mg Zn; 127-130mg Mn; 40mg Co; 35,000-42,500 NaCl; 3,365-4,115mg KCl; 17,000mg D, L-methionine. **ME was estimated according to database of McDonald et al. (2011); The chemical composition was analysed followed standard methods of AOAC (2005).*

The initial weight of the birds was taken at the beginning of the study and body weight (BW) measurement was fortnightly recorded to calculate average daily gain (ADG). The average

daily feed intake (ADFI) was determined on a fresh basis as the difference between the quantity of feed offered and leftover. The feed conversion ratio (FCR) was calculated as the ratio of the ADFI to the ADG. All cages were checked for viability throughoutly.

Data collected were statistically analyzed using GLM procedure of Minitab 16.0. Differences among the treatments were compared by the Tukey test at the 5% significance level.

3. RESULTS AND DISCUSSION

The growth performance, as impacted by herbals mixture powder, is shown in Table 2. The BW of chickens at the beginning of the experiment (28 days old) was similar (P>0.05). After 70-day trial, the BW improved in the treatments supplemented with spice herbs. Among the treatments, BW and ADG of birds fed diets enriched with herbs were influenced (P<0.05) only at 56 days of age, although during the study time, chickens fed diets enriched with herbs, particularly T100, showed higher BW compared to the control. At 56 days of age and during the whole period, chickens received herbal mixture at level 1.0% had the lowest FCR values, compared to the control and other groups. The best FCR recorded in chickens fed herbs at level 10 g/kg feed (3.18), while the worst FCR was recorded in group fed herbs at level 2.5 g/kg feed (3.59). Data in Table 2 also present insignificant impact (P>0.05) of added herbs on bird ADFI among the treatments during the whole period.

This noticeable improvement in FCR for the whole experimental period (28-98 days of age) might be due to improved digestion and absorption of diet nutrients by adding some active compounds in the phytogenic additives. Therefore, that may enhance the feed efficiency and the growth rate, consequently. Ashour *et al.* (2020) cleared that the growth rate of Hubbard broilers was not adversely influenced by adding a herbal mixture (including *Capsicum annuum, Thymus vulgaris, Salvia rosmarinus, Pimpinella anisum, Mentha spicata, Nigella sativa* and *Allium sativum*) in the diet. The results also showed no statistical difference in ADFI due to the dietary supplementation of herbs. In agreement, Sadeghi *et al.* (2012) claimed that feed intake of Ross 308 broiler chickens was not affected by the adding infusion (5g per liter) of cinnamon, thyme and turmeric in equal ratio in replacement of drinking water.

Table 2. Growth performance of Noi chickens

Age (day)	**T0**	**T25**	**T50**	**T75**	**T100**	**SEM**	**P**
Boby weight (g/bird)							
28	249	254	252	255	252	7.53	0.98
42	466	463	456	461	488	11.5	0.35
56	632^{b}	661^{ab}	652^{b}	680^{ab}	712^{a}	12.5	0.01
70	1008	1005	1026	1024	1057	28.6	0.72
84	1289	1340	1387	1421	1430	43.3	0.15
98	1574	1570	1629	1666	1711	54.0	0.32
Weight gain (g/bird/day)							
42	15.5	15.0	14.6	14.7	16.9	0.62	0.09
56	11.9^{b}	14.1^{ab}	14.0^{ab}	15.6^{a}	16.0^{a}	0.70	0.01
70	26.8	24.6	26.7	24.6	24.6	1.73	0.75
84	20.1	23.9	25.8	28.3	26.6	2.36	0.17
98	20.4	16.4	17.3	17.5	20.1	1.62	0.34
42-98	18.9	18.8	19.7	20.1	20.8	0.73	0.28
Feed intake (g/bird/day)							
42	40.0	39.4	40.3	42.2	40.3	1.32	0.66
56	55.0	56.6	58.2	55.6	55.4	2.24	0.85
70	68.8	71.2	68.2	71.9	69.4	2.29	0.75
84	83.9	84.1	82.7	84.8	81.3	3.48	0.96
98	87.4	85.7	85.9	86.3	84.3	3.61	0.98
42-98	67.0	67.4	67.1	68.2	66.1	2.20	0.98
FCR							
42	2.59	2.63	2.80	2.86	2.42	0.11	0.07
56	4.67^{a}	4.03^{ab}	4.25^{ab}	3.62^{b}	3.51^{b}	0.24	0.01
70	2.58	3.03	2.59	3.05	2.83	0.21	0.37
84	4.54	3.84	3.23	3.10	3.10	0.43	0.11
98	4.33	5.62	5.11	5.04	4.33	0.42	0.18
42-98	3.55^{ab}	3.59^{a}	3.42^{ab}	3.39^{ab}	3.18^{b}	0.09	0.03
Mortality (%)	4.2	2.1	2.1	2.1	0.0	-	-

T0: diet supplemented with 0% herb; T25: diet supplemented with 0.25% herb; T50: diet supplemented with 0.50% herb; T75: diet supplemented with 0.75% herb; T100: diet supplemented with 1.0% herb. Means in the same row with different superscripts are significantly different (P<0.05).

Data in Table 2 showed that lowest mortality rate was observed at T100 (0%), followed by T25, T50, T75 (sharing 2.1%), highest mortality rate was at T0 (4.2%). This was similar with the finding of Alagbe and Oluwafemi (2019) who noted that 12% mortality was found in Ross-308 broiler strain given control diet, but 0% mortality was recorded in treatments with lemongrass-garlic extract mixture added at levels 3.0, 6.0, 9.0 and 12.0 ml/l.

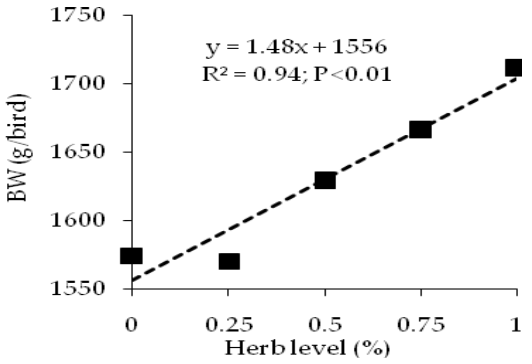

Figure 1. The relationship between herb level and final BW of Noi chickens

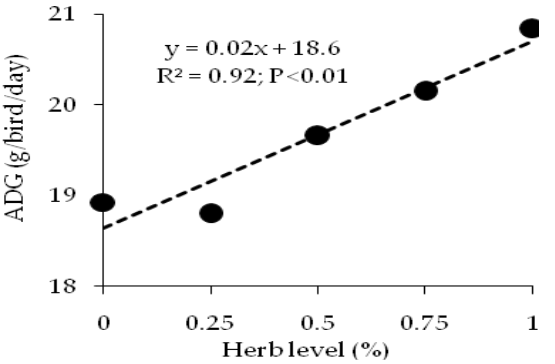

Figure 2. The relationship between herb level and ADG of Noi chickens

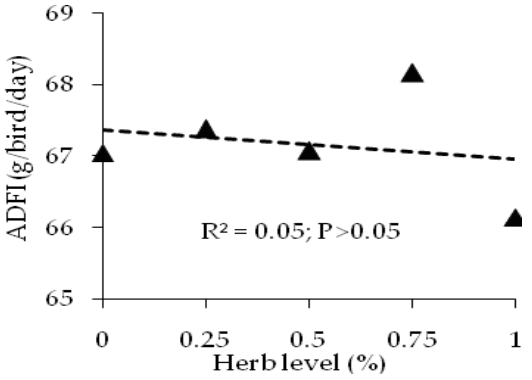

Figure 3. The relationship between herb level and ADFI of Noi chickens

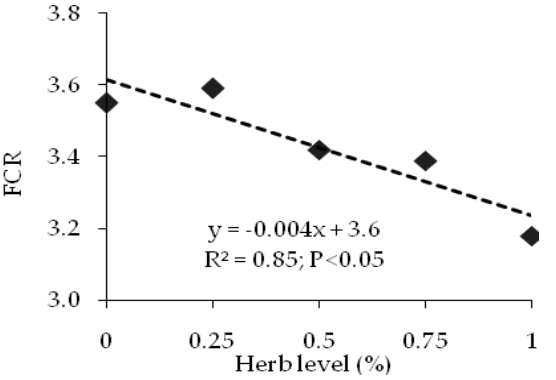

Figure 4. The relationship between herb level and FCR of Noi chickens

The effect of herbal mixture (garlic, lemongrass and turmeric) on the growth performance of Noi chickens was most pronounced on the final BW of Noi chickens at 98 days of age (Figure 1), ADG and FCR of the whole period (Figure 3 and 5). The level of herbal supplementation positively affected the final BW (y=1.48x+1556, R^2=0.94, P<0.01) and the ADG (y=0.02x+18.6, R^2=0.92, P<0.01) but was inversely proportional to the FCR (y=-0.004x+3.6, R^2=0.85, P<0.05). However, the levels of herb supplemented to the diet did not affect the ADFI of the chickens (R^2=0.05, P>0.05). The herbal mixture of turmeric, lemongrass and garlic contains many active ingredients that improve the growth performance of chickens. The combination of active ingredients such as allicin in garlic, citral in lemongrass and curcumin in turmeric may support body digestion and metabolism, stimulate the growth, develop useful microbiome and limit the harmful actions of pathogens (Manvitha and Bidya, 2014; Ansary *et al.*, 2020; Sharifi-Rad *et al.*, 2020). Other studies also shown that herbs could stimulate the growth of intestinal cells, increasing the contact area of the intestinal mucosa, particularly the duodenum, which in turn regulate the secretion of digestive enzymes and balance the intestinal microflora, thereby supporting the growth performance of chickens (Bongiorno *et al.*, 2008; Alizada and Hemmaty, 2020). In addition, as figured out by Flees *et al.* (2021), phytobiotic additives derived from herbs and spices, was also used to promote animal performance due to their beneficial effects on reducing the hepatic lipogenesis, enhancing the lipolysis and

stimulating the muscle protein synthesis, which in turn improve BW and carcass traits of the treated birds.

4. CONCLUSION

The results showed that BW, ADG and FCR were significantly better in groups of chickens fed with a mixture of spice herbs, particularly at 56 days of age, while ADFI was not affected during whole trial time. In overall, the FCR and mortality rate were lowest with the inclusion of 1.0% in the diet. These findings suggests that the herbal mixture of garlic, lemongrass and turmeric can be added up to 1.0% (10 g/kg feed) in the diet to improve the growth performance of chickens.

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CHARACTERISATION OF LARGE RUMINANT SYSTEMS AND THEIR MULTIPLE CONTRIBUTION IN A HIGHLAND COMMUNE, DIEN BIEN PROVINCE

Melanie Blanchanrd[1], Han Anh Tuan[1], Duc Do Van[2], Le Tien Dung[2] and Le Thi Thanh Huyen[2]*

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ABSTRACT

Upland regions cope with many difficulties compared to the delta region. Cattle are considered as the most important livestock species. This study uses an ontology to define relevant indicators to evaluate multiple functions of these grassland livestock systems for characterisation of main livestock farm types and their contribution to the sustainable development of farms, and community. The study was implemented in Quai Nua commune, Tuan Giao District, Dien Bien Province North-West Vietnam. 48 smallholders of seven villages were selected for data collection in an in-depth survey. In addition, key person interviews with other stakeholders were also implemented. Four main types of farms are identified including (i) the extensive livestock farms; (ii) the semi-intensive livestock farms; (iii) the crop-oriented farms; and (iv) the farms without ruminants. Livestock contributes multifunction to all kinds of household farms with high contribution from value of ruminants that implicates the role of grazing land in the study region.

Keywords: *Livestock system, highlands, livestock contribution, Dien Bien, Vietnam.*

TÓM TẮT

Đặc điểm các hệ thống chăn nuôi gia súc nhai lại và đóng góp của chúng ở xã vùng cao, huyện Điện Biên

Vùng cao gặp nhiều khó khăn so với vùng đồng bằng. Đại gia súc được coi là vật nuôi quan trọng nhất. Nghiên cứu này sử dụng bản thể luận để xác định các chỉ số liên quan nhằm đánh giá đa chức năng của các hệ thống chăn nuôi sử dụng bãi chăn và xác định đặc điểm của các loại hình chăn nuôi chính và đóng góp của chúng vào sự phát triển bền vững của nông hộ và cộng đồng. Nghiên cứu được thực hiện tại xã Quài Nưa, huyện Tuần Giáo, tỉnh Điện Biên, Tây Bắc Việt Nam. Tổng số 48 hộ dân được lựa chọn từ bảy bản tham gia vào cuộc điều tra sâu. Ngoài ra các cuộc phỏng vấn với các tác nhân khác cũng được thực hiện để thu thập số liệu. Bốn loại hình chăn nuôi nông hộ chính được xác định bao gồm (i) chăn nuôi quảng canh; (ii) chăn nuôi bán thâm canh; (iii) nông hộ chủ yếu dựa vào trồng trọt; và (iv) nông hộ không có chăn nuôi động vật nhai lại. Chăn nuôi đóng góp đa chức năng cho tất cả các loại hình chăn nuôi hộ gia đình với đóng góp cao từ giá trị đàn gia súc nhai lại, kết quả này đồng thời thể hiện vai trò của đất sử dụng làm bãi chăn trong vùng nghiên cứu.

Keywords: *Hệ thống chăn nuôi, vùng cao, đóng góp của chăn nuôi, Điện Biên, Việt Nam.*

1. INTRODUCTION

Upland regions cope with many difficulties in terms of steep slope, uneven terrain, accessing difficulties, low soil quality, poor infrastructure, and high poverty rate (Minot *et al*., 2003; Tran Duc Vien, 2003). In Northwest Viet Nam, rain-fed crop monoculture is dominated on sloping lands. Cattle are considered as the most important livestock species. Farmers are largely dependent on grazing lands as main feed source for their animals (Huyen *et al*., 2006; Phung, 2009; Trung, 2011).

On the one hand, the intensification of large ruminant systems (cattle and buffaloes) relies on the establishment of forage crops

[1] CIRAD, UMR Selmet

[2] National Institute of Animal Science

[*] Corresponding Author: Asoc. Prof. Dr. Le Thi Thanh Huyen, Senior Researcher in Farming System Livestock System and Environment Research Depart, National Institute of Animal Science. Tel: (84) 904854499; Email: lehuyen1973@yahoo.com

production promoted by research and development projects and agricultural extension services, however, the areas currently available for forage production remain very limited. On the other hand, pastoral or grazing systems are considered archaic, not intensive enough to meet the production challenges and not remunerative enough, therefore, they are little taken into account, studied and supported by extension services and development. It is taken into account that an assessment of the contribution of these grassland livestock systems for the territory development is necessary in order to initiate a dialogue with the local authorities on the basis of scientific data.

This study uses an ontology to define relevant indicators to evaluate multiple functions of grassland livestock systems for characterisation of main livestock farm types and their contribution to the sustainable development of farms, and community. This essential information can help local authorities in their strategic planning of investment and orientation of agricultural and livestock production, resource management and spaces and territories in general. All can help to consider the trade-offs between issues of production, protection of space and resources, economic development.

2. MATERIALS AND METHODS

To assess the place and role of grazing livestock in the mountainous areas of North-West Vietnam in terms of the sustainability, an ontology of grazing livestock systems developed by Muller *et al*. (2020) was mobilized to identify the multi-functionality provided by these systems. The indicators used in the ontology included a series of 21 criteria and 129 indicators those cover the four dimensions: Production, Local development, Ecosystem and Social, and the different levels and entities herd, farm, community and landscape, services and value chain. From this list, we have selected 28 indicators that make sense in the local context, in relation to the challenges of livestock systems, recognized or not by the diversity of the actors involved, and responding to the expectations of the various actors involved in territory and the value chain linked to grazing livestock system.

The study was implemented in Quài Nưa commune, Tuần Giáo District, Điện Biên Province North-West Vietnam. Seven villages were selected for data collection in an in-depth survey after a rapid survey on farm typology that was carried out based mainly on scales of livestock production, land areas, land use, and types of grazing systems in 10 high and intermediate highland villages out of total 21 villages of Quai Nua commune (at least 15 households per village were quick accessed).

A series of surveys was carried out afterwards with the diversity of the actors involved: diversity of mixed farms, local authorities, collectors, retailers, butchers and producers of dried meat. An in-depth survey was conducted in 48 farms selected to represent the diversity of farm types: i. Farm without ruminant; ii. Farm with a small herd (<5 cattle) and cultivates large areas (>1.5ha); iii. Farm with a small herd and cultivates small areas (<1.5ha); iv. Farm with an average herd (5-8 cattle) and cultivates large areas (>1.5ha); v. Farm with an average herd (5 to 8 cattle) and cultivating small areas (<1.5ha); v. and Farm with a large herd (>9 cattle). The farms surveyed also represent the diversity of grazing management systems from animals to free grazing with more or less close monitoring to control grazing animals, at certain times of the day, or even completely stabled animals. The farm survey focused on farm structure; the agricultural system with the available and cultivated surfaces on the farm, the production costs, the harvests and their futures, and the income from different crops; the livestock system with the composition of the herd, the costs of production, the herd exploitation (reproduction, donations, purchases, sales), the livestock income, the practices of animals feeding on pasture and barn; family alimentation and other farm income sources.

To build a global image of the farming systems of the town, we relied on a typology of livestock farms. The diversity of livestock farming systems was analysed through a Principal Component Analysis (PCA) on the variables representative of this diversity: family size (number of people), size of the cropping area in low land, highland and forage crop (in m²), herd size (in tropical livestock unit (TLU), feed distributed to animals (in kg DM /TLU / year), part of feeding taken during grazing (%) and off-farm time in the rainy season and in the dry season (%). A Hierarchical Ascending Classification (CAH) was carried out to identify homogeneous farm groups, according to these criteria. Each type of livestock identified by this analysis represented a defined percentage of the commune's farms, which made it possible to go from individual evaluation of certain indicators to an assessment of the value of indicators at the commune level.

3. RESULTS AND DISCUSSION

3.1. Diversity of livestock farming systems in Quài Nưa commune

The three farm types with ruminants are represented according to the main CPA plan in figure 1. The non-ruminant farm is an added type of farm (type 0) is no represented in the figure.

On the first axis of the PCA, the variables illustrate the size of the herd, part of feeding taken during grazing and the off-farm time to the right and the size of the cropping area in the uplands on the left. The second axis differentiates farms with larger forage areas and those with feed distributed to animals upwards. The size of the family is not discriminating for farm types.

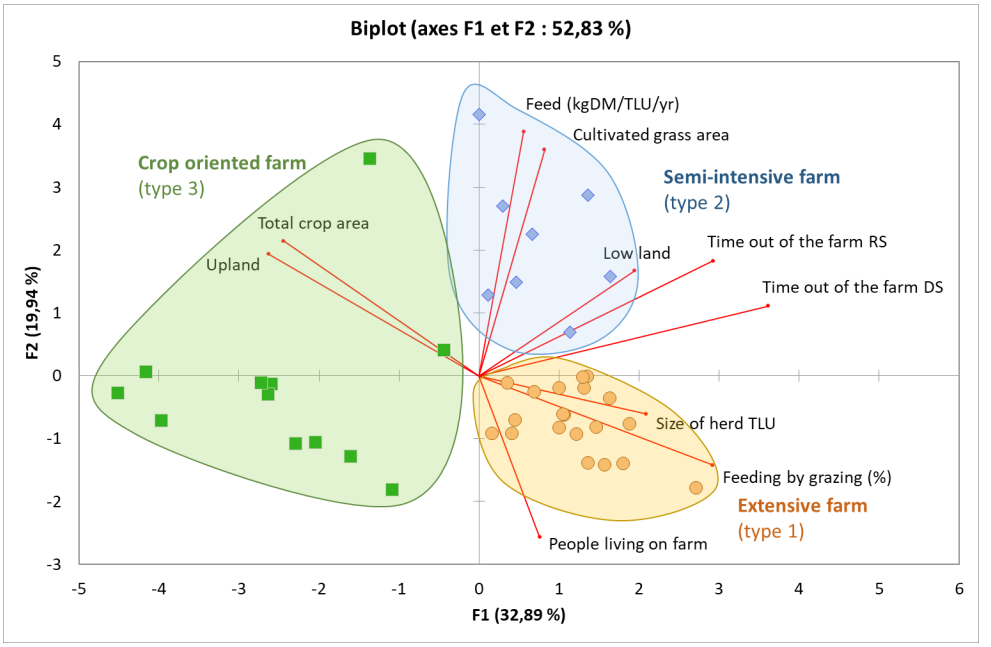

Figure 1. First plan of the main component analysis with projection of 3 types of farm

The analysis makes it possible to distinguish 4 types of exploitation.

The extensive livestock farms (type 1): They have average cultivated area of 6,235m², composed mainly of upland field, and with a medium area of the lowland field. They have no or little forage crop. The herd of ruminant is large (>6 TLU) whose feeding is largely ensured by grazing (60%) with a little supplementation to the trough. The animals

spend a lot of time in whole year around grazing outside the farm.

The semi-intensive livestock farms (type 2): They cultivate in intermediate surfaces (11,870m²), with the highest area of lowland surfaces (2,070m²) compared to other types of farm, intermediate highland areas, and an average of 825m² that is the largest area for forage among all surveyed farm types. They also own a large herd (>6 TLU) that are grazed in all seasons but remain largely fed to the trough (2,330kg DM/TLU/year).

The crop-oriented farms (type 3): They have the largest cultivated areas (20,160m²) compared to other farm types. The land area mainly contains of high land field, but without forage crop area. They have a smaller herd (>3 TLU) that are kept under a barn. The contribution from pastures outside to this farm type was smallest compared to the others. The animals are mainly draft animals.

In addition, the farms without ruminants (type 0): This type of farm shares about 30% of the total farms in the commune according to the local authority's data. These farms have small cultivated areas (2,240m²) distributed between the lowlands and the highlands. They rear pig and poultry and practice agriculture with rice and some maize production. The detailed characteristics of these four types of farms are presented in Table 1.

Table 1. Characteristics of the diversity of livestock farm types in Quài Nưa Commune

	Type 1	Type 2	Type 3	Type 0	Significance
Number of surveyed	20	8	12	8	
%	41.7	16.7	25.0	16.7	
People living on farm (person)	5.7^{a}	3.8^{a}	5.3^{a}	3.9^{a}	ns
Total crop area (m²)	6 235^{bc}	11 870^{b}	20 163^{a}	2 238^{c}	***
Low land crop area (m²)	1 605^{ab}	2 070^{a}	1 079^{b}	900^{b}	*
Upland crop area (m²)	4 630^{bc}	9 800^{b}	19 083^{a}	1 338^{c}	***
Cultivated grass area (m²)	10^{b}	825^{a}	0^{b}	0^{b}	***
Size of herd (TLU)	6.1^{a}	6.1^{a}	3.6^{a}	0.0^{b}	***
Feed (kg DM/ TLU/year)	432^{b}	2 330^{a}	303^{b}	0^{b}	***
Feeding by grazing (%)	59.4$_{a}$	29.0^{b}	16.8^{b}	0.0^{c}	***
Time of cattle grazing in the rainy season (%)	61.8^{a}	66.1^{a}	28.0^{b}	0.0^{c}	***
Time of cattle grazing in the dry season (%)	65.0^{a}	62.0^{a}	12.2^{b}	9.6^{b}	***

*ns : non significance ; *: P-value<0,05 ; **: P-value<0,01 ; ***: P-value<0,001*

Huyen *et al*. (2011) also describe similar main types of household farms in Son La province, however, without quantified value of different production practices. In addition, the specialized beef farm type is not existed in the current study area.

3.2. Contribution of grazing livestock systems to the production, local development, ecosystem and social aspects

The total contribution from crops and livestock production to the whole commune is estimated about 152.712 billion VND/year, in which, 40.6% from crops, and the rest of 59.4% from livestock production. Ruminants contribute 41.097 billion VND out of 90.766 billion VND of total animal production value in the commune (45.3%), in which pastoral based ruminant shares of 16.3%. Nevertheless, roles of different production segments are not the same on different farm types. The proportion of contribution value from pastoral ruminants in the total income of extensive livestock farms (type 1) is higher than on other farm types. While the share of non-pastoral ruminant in the income of semi-intensive farms (type 2) is larger than of other farm types; and not much different between

extensive and crop- oriented farms (type 3). Even though, on ruminant keeping farms (at farm level), the contribution from ruminants is quite high, especially on type 1 and 2, however, the proportion of these farms is lower than cropping farm and mono-gastric animal keeping farms in the commune. This results in lower contribution value of ruminants to the whole commune compared to crops and mono-gastric livestock (at communal level).

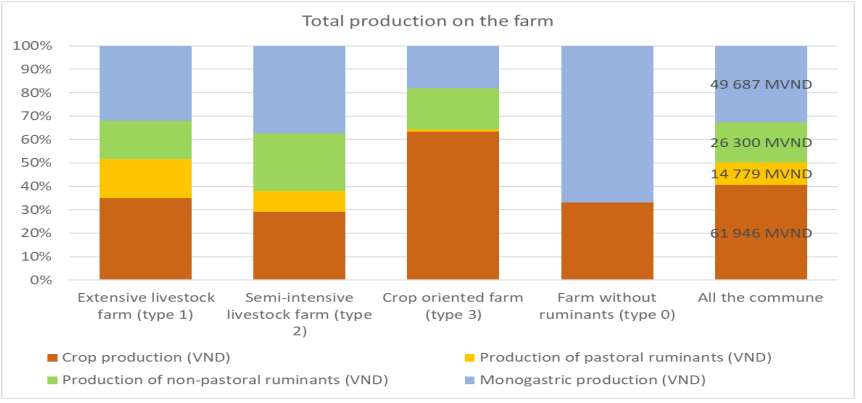

Figure 2. Total values of crop and animal production at farm and communal levels

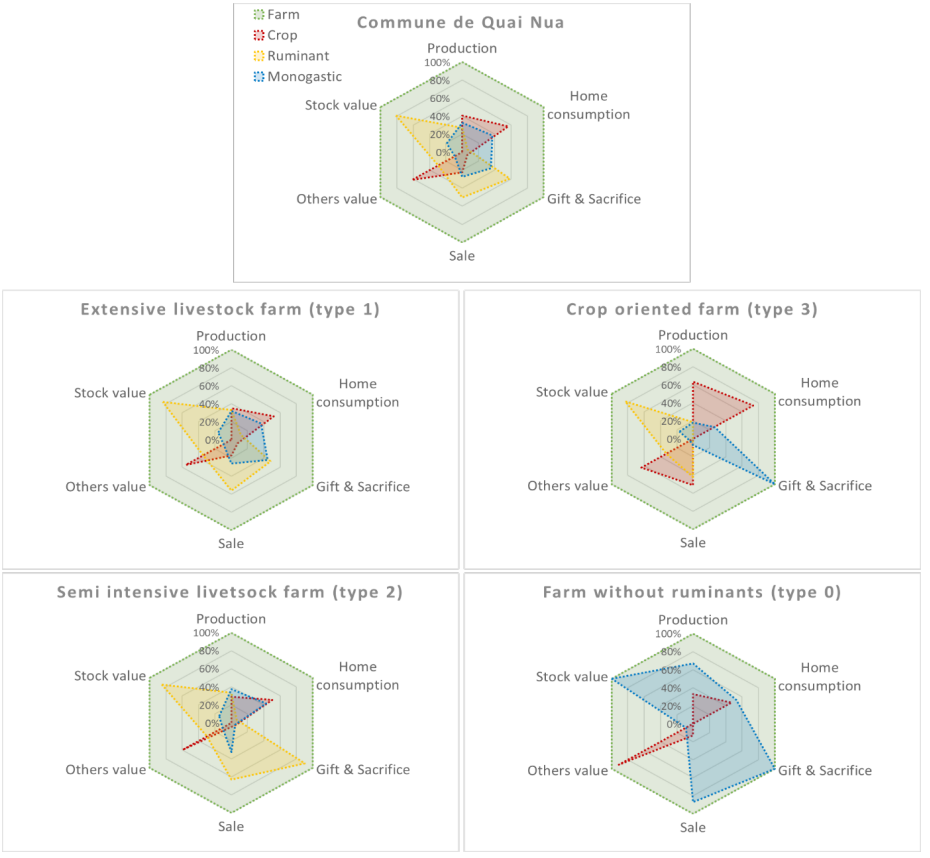

Figure 3. Multifunction roles of livestock in the commune and different farm types

Figure 3 also illustrates multifunction of livestock in the whole commune as well as at different farm types. Livestock production systems are not just noble animal products for sale. Particularly in extensive and semi-intensive livestock farm those are strongly dependent on pastures, the ruminants play multiple roles on the farms and community, such as meat for home self-consumption (protein source of the farm), sacrifices and donations (cultural and social roles), traction force (important for slope land), and the production of manures (necessary for maintenance of organic soil fertility and increase crop productivity). Stock value of ruminants plays an important role as bank saving for all farmers.

Results of the current study found that livestock contributed multifunction to all kinds of household farms, inclusive of non-ruminant keeping farms, crop-orientation farm, extensive ruminant farms, and semi-intensive ruminant farms. In which, cattle and buffaloes brought high contribution to the two latter farm types from different values of meat for home consumption, sacrifices and donations, traction force, production of manures for cropping and stock value of ruminants as bank saving for families. High contribution of non-cash value from cattle and buffaloes is also identified in Son La province by Huyen *et al*. (2010). A number of authors (eg.: Choocharoen *et al*., 2014; Huyen *et al*., 2018) state that pastoral systems are characterized by multi-functions of livestock with strong linkages of livestock, grassland, forest and cropping. The grazing areas were identified as in the review of Huyen *et al*. (2018) those are composed of uncovered grasslands, but also of forest lands, fallows and interstitial areas (border of fields, roads, etc.).

4. CONCLUSIONS AND RECOMMENDATIONS

In a highland commune, livestock production systems (cattle and buffaloes) are diversified and tend to be sustainable adapted to the local conditions. In each system, livestock plays multiple contribution to the farm and the development of the community. The contribution of large ruminant implicates the role of grazing land that is need to be improved and more concerned by the local authority and farmers.

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THE EFFECTS OF THE OESTRUS INDUCTION METHODS ON THE ESTROUS SYNCHRONIZATION IN GOATS

Nguyen Khanh Van[1], Vu Thi Thu Huong[1], Quan Xuan Huu[1], Phan Trung Hieu[1] and Pham Doan Lan[2]*

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ABSTRACT

A total of 12 crossbred goats Boer were used to evaluate the effect of the estrous method induction on the estrous synchronization efficiency in goats. Experiments were performed three times. According to two methods, experimental goats were divided into two groups to induce estrous: (1) using CIDR in combination with PGF2α; (2) do not use CIDR. The estrous expression of goats was observed at the time of CIDR withdrawal and PGF2α injection for group 1 and PGF2α injection for group 2. Progesterone serum was quantified at day 0 before the estrous synchronization in goats; since the goats begin to show estrous and goats show signs of acceptability of male. After the PGF2α injection 24h and 48h, the rate of estrous in group 1 was higher than that of group 2: 61.11, 94.44 and 94.44% vs. 38.89, 55.56 and 55.56% (P<0.05). The time of onset of estrous after PGF2α injection of group 1 was shorter than that of group 2 (20.02% vs. 30.13%, respectively (P<0.05). The estrous time of group 1 was longer than that of group 2: 36.23% vs. 32.81% (P<0.05). There was no difference in serum progesterone concentrations for estrous goats in groups 1 and 2 (P>0.05). This study showed that using CIDR in combination with PGF2α resulted in higher estrous efficiency than using single PGF2α.

Keywords: *Goats, synchronization, CIDR, progesterone serum.*

TÓM TẮT

Ảnh hưởng của phương pháp gây động dục đến hiệu quả gây động dục hàng loạt cho dê

Tổng số 12 dê Boer lai được sử dụng để đánh giá ảnh hưởng của phương pháp gây động dục đến hiệu quả gây động dục hàng loạt cho dê. Tất cả các dê thí nghiệm đều được gây động dục lặp lại 3 lần. Các dê thí nghiệm được chia thành hai nhóm gây động dục theo hai phương pháp: (1) sử dụng CIDR kết hợp với PGF2α; (2) không sử dụng CIDR. Theo dõi biểu hiện động dục của dê tại thời điểm rút CIDR và tiêm PGF2α đối với nhóm 1 và tiêm PGF2α đối với nhóm 2. Định lượng Progesterone huyết thanh tại thời điểm ngày 0 trước khi gây động dục đồng loạt cho dê, dê bắt đầu có biểu hiện động dục và khi dê có biểu hiện chịu đực. Tỷ lệ động dục sau 24 giờ và 48 giờ tiêm PGF2α: tỷ lệ động dục chung của nhóm 1 cao hơn nhóm 2, tương ứng là 61,11; 94,44; 94,44% so với 38,89; 55,56; 55,56% (P<0,05). Thời gian bắt đầu xuất hiện động dục sau tiêm PGF2α của nhóm 1 ngắn hơn nhóm 2, tương ứng là 20,02% so với 30,13% (P<0,05). Thời gian động dục của nhóm 1 là 36,23% dài hơn so với nhóm 2 (32,81%) (P<0,05). Không có sự khác nhau về nồng độ Progesterone huyết thanh đối với các dê có biểu hiện động dục ở nhóm 1 và nhóm 2 (P>0,05). Kết quả nghiên cứu cho thấy gây động dục sử dụng CIDR kết hợp với PGF2α mang lại hiệu quả động dục đồng loạt cao hơn so với việc chỉ sử dụng PGF2α.

Từ khóa: *Dê, động dục, CIDR, progesterone serum.*

1. INTRODUCTION

The management of goats' reproductive cycle is an assisted reproductive technique to enhance goat production efficiency. Goats are a type of ruminant; reproductive management of goats is a problematic issue due to the seasonal fertility of the oestrus cycle. It is essential to control the estrous cycle of goats during the breeding and non-breeding seasons. Estrous synchronization effectively improved and maintained meat and milk production and reduced labor costs in goat farms (Llane

[1] Key Laboratory of Animal Cell Biotechnology
[2] National Institute of Animal Sciences
[*]Corresponding Author: Dr. Pham Doan Lan, National Institute of Animal Sciences, 9 Tan Phong, Thuy Phuong, Bac Tu Liem, Hanoi, Viet Nam. Tel: (84) 914366975; Email: pdlanvn@yahoo.com

et al., 2019). Estrous synchronization helps control the reproductive process of goats, thereby improving the efficiency of goat husbandry. Besides, Estrous synchronization is the basis for oestrus synchronization during goat embryo transfer.

The principle of synchronized oestrus is to control the luteal phase of the estrous cycle. There are two basic mechanisms of synergism used: (1) the use of prostaglandins or analogs to shorten the duration of the corpus luteum, or to induce corpus luteum breakdown, thereby generating cystic waves of the next oestrus; (2) the use of exogenous progesterone to prolong the lifespan of the corpus luteum, so it is similar to the natural progesterone produced by the corpus luteum.

Various methods are used to induce estrous synchronization in goats, the most common of which is the use of a progestagen-containing vaginal device (Hasani *et al*., 2018). Simultaneous oestrus induction using a progestagen-containing vaginal device is usually applied in two different ways: (1) short-term (5-7 days) progesterone treatment or (2) administration with long-term progesterone (12-14 days) (Gore *et al*., 2020). In addition, intramuscular administration of prostaglandin F2α (PgF2α), PMSG, and GnRH were also used to induce oestrus synchronization through the control of follicular activity and luteal activity (Hameed *et al*., 2020). Each method of oestrus synchronization has its advantages and disadvantages; the success of the technique of oestrus synchronization in goats depends on breeds, breeding seasons, methods of oestrus, etc.

In recent years, the demand for goat farming products has increased rapidly in Vietnam. The research and application of techniques in reproductive technology such as inducing ovulation, embryo transfer … in goats. Procedures to induce ovulation in goats have been performed in developed countries. However, in Vietnam, no reports evaluate the effect of the estrous method on estrus efficiency in goats. Stemming from that actual need, this study was conducted to assess the impact of the estrous method on the efficiency of oestrus synchronization in goats in Vietnam.

2. MATERIALS AND METHODS

2.1. Materials

An total of 12 crossbred goats Boer at the age of 8-9 months, bodyweight 25-35kg were divided into two experimental groups: group 1: Oestrus synchronization using a combination of CIDR, PGF2α; Oestradiol and GnRH, and group 2: Oestrus synchronization using a combination of PGF2α and GnRH.

The CIDR of Interag (Newzealand), PGF2α, Oestradiol Benzoat, GnRH.

2.2. Methods

2.2.1. Oestrus induction using CIDR

The method of oestrus using CIDR was performed as follows:

- Day 0: Set CIDR

- Day 5: Inject PGF2α (3ml Lutalyse/goat) and withdraw CIDR

- Day 6: Inject Oestradiol Benzoat (0.5ml Oestradiol/goat)

- Day 7: Inject GnRH (1ml Goat GnRH)

2.2.2. Oestrus induction without using CIDR

The method of estrous using CIDR is performed as follows:

- Day 0: Inject GnRH (1ml Goat GnRH)

- Day 6: Inject PGF2α (3ml Lutalyse/goat)

2.2.3. Monitoring the estrous expression of goats

The monitor estrous expression of goats after estrus induction was based on symptoms such as wagging tail, frequent urination.

2.2.4. Determination of P4 (Progesterone) in the blood

Blood samples were taken at day 0 before the oestrus synchronization since the goats started showing estrous and when the goats showed the signs of standing oestrus.

The process of taking goat blood samples to quantify P4 (Progesterone) content was carried out as follows:

- Fix the goat, then disinfect the ear at the blood collection site.

- Use an 18G needle to suck about 2ml of blood/goat, then immediately put it into a dedicated tube, shake well to prevent blood from clotting.

- The blood sample tubes are stored at 4-8°C and brought to the laboratory within 3 hours.

A quantitative process of P4 (progesterol) in blood was conducted by Medlatec hospital.

2.3. Data analysing

The data were processed by Microsoft Excel 2010 software; the ANOVA function checked the significant difference, the difference was significant with P<0.05.

3. RESULTS AND DISCUSSION

3.1. The effect of oestrus methods on oestrus efficiency of goats

The oestrus of the crossbred goats Boer after oestrus were in Table 1 showed the rate of oestrus in group 1 (using a combination of CIDR, PGF2α; Oestradiol and GnRH) at 24 and 48hrs after injection of PGF2α were both higher than group 2 (using PGF2α and GnRH) (61.11 vs 38.89% and 94.44 vs 55.56%, respectively (P<0.05).

In this study, CIDR in combination with PGF2α and GnRH for estrous induction yielded higher estrous efficiency than when CIDR was not used. This result was similar to Majid and Mahazer (2017) report. According to Majid and Mahazer (2017), 94% of goats were estrous when using CIDR and 30% showed oestrus without using CIDR.

CIDR was made of Silicone impregnated with progesterone. When experimenting, CIDR was placed into the vagina of laboratory animals. Progesterone in CIDR was released slowly, permeating through vaginal blood vessels and entering the blood of laboratory animals. CIDR was commonly used in progesterone delivery in sheep and goats (Jackson *et al*., 2014).

The use of Progesterone or Progesterone derivatives in combination with PGF2α is highly effective on the oestrus induction in goats (Mohamad *et al*., 2021). Progesterone or Progesterone derivatives inhibit oestrus and ovulation for a long time enough to cause spontaneous degeneration of the corpus luteum. After the removal of Progesterone, oestrus and ovulation will coincide. However, to get the oestrus synchronization, the total time the goats are under treatment using progesterone must be long enough, equivalent to the luteal phase of the natural cycle.

Table 1. The effect of oestrus methods on estrous efficiency in goats

Tracking criteria	Method 1	Method 2
The number of goats under treatment (n)	18	18
The rate of oestrus after 24h of PGF2α injection	61,11^{a}±5,55	38,89^{b}±5,56
The rate of oestrus after 48h of PGF2α injection	94,44^{a}±5,56	55,56^{b}±5,55
The avarage rate of oestrus	94,44^{a}±5,56	55,56^{b}±5,55
The time of onset of oestrus after PGF2α injection	20,02^{a}±2,63	30,13^{b}±2,67
The total time of oestrus	36,23^{a}±2,62	32,81^{b}±2,12

Values with different superscripts in the same row are significantly different (P<0.05).

The percentage of goats in oestrus after induction using a combination of CIDR and PGF2α, Oestradiol, and GnRH was different between studies. The proportion of goats showing oestrus after induction using a combination of CIDR and PGF2α, Oestradiol, and GnRH were lower than those of Silva *et al*. (2011); Muayad *et al*. (2019) but higher than Vilarino *et al*. (2011). According to Silva *et al*. (2011), 100% of goats showed oestrus when induced in heat using a combination of CIDR and PGF2α. Muayad *et al*. (2019) reported that only 90-100% of goats showed oestrus when using CIDR for oestrus. Meanwhile, according to the research results of Vilarino *et al*. (2011), only 68% of female goats showed oestrus when inducing estrus with CIDR and PGF2α. Differences between studies may be due to

the goat breeds used in experiments, seasons, nutritional factors.

GnRH helps the follicles develop evenly, PGF2α is a Prostaglandin hormone that destroys the corpus luteum and sends the ovary into the next follicular wave. The effect of PGF2α in estrous induction is demonstrated through its ability to control corpus luteum function. During the normal estrous cycle in goats, PGF2α is secreted from the non-pregnant uterus from day 16 after oestrus. Administration of PGF2α after removal of the CIDR ring mimics uterine PGF2α secretion, induces ovarian corpus luteum degeneration, and initiates a new follicular phase (Fatet *et al*., 2011). PGF2α is only effective at inducing corpus luteum degradation between day 3 and day 14 of the estrous cycle in goats (Abecia *et al*., 2011).

The time of onset of oestrus and duration of estrus is crucial in oestrus synchronization; based on this time, scientists can well manage insemination to improve the conception rate or choose the optimal time for embryo transfer. In this study, we evaluated the effect of oestrus methods on the time of oestrus appearance and duration of oestrus in goats. The results of Table 1 showed a difference between the time of onset of estrus, time of oestrus in method 1 and method 2 (20.02 vs 30.13hrs and 36.23 vs 32.81hrs, respectively; $P<0.05$).

In this study, the CIDR usage with PGF2α resulted in a faster onset and a longer duration of estrus than without CIDR. Our results are similar to those reported by Nur *et al*. (2020). According to Nur *et al*. (2020), when using CIDR to induce oestrus, the time to oestrus onset was faster, and the time to oestrus was longer than that of not using CIDR (22.15 vs. 57.5hrs and 24.4 vs. 56hrs, respectively).

The total time of oestrus in both methods was consistent with that in the report of Greyling (2000) but different from Motlomelo *et al*. (2002), Lehloenya *et al*. (2005). According to Greyling (2000), the total time of estrous in Boer goats was expected at 36hrs, while the report of Motlomelo *et al*. (2002) found that the total time of estrous in Boer goats was 35.2hrs and that of Lehloenya *et al*. (2005) is 37.0hrs. However, according to Greyling (2000), the total time of estrous in Boer goats could vary from 22-60hrs; therefore, the total time of estrous in Boer goats in this study was entirely reasonable.

The time of oestrus onset in this study was earlier than that of Motlomelo *et al*. (2002), Romano (2004), Hashemi and Safdarian (2018). In the report of Motlomelo *et al*. (2002), Romano (2004), Hashemi and Safdarian (2018), the time of onset of oestrus respectively reached 30.1, 36 and 40.2hrs. Differences in the onset of oestrus may be due to the methods of oestrus induction, goat breeds, nutritional conditions, and nutritional care, etc.

The use of CIDR in combination with PGF2α during the oestrus synchronization shortened the time since CIDR removal to the onset of oestrus (Whitley and Jackson, 2004). Induction of estrus using CIDR or PGF2α alone delays oestrus initiation. Even the onset of estrus began only 52hrs after Progesterone treatment.

In this study, we used the short-term Progesterone treatment by placing CIDR in the vaginas of goats with an interval of 5 days. One of the benefits of short-term Progesterone treatment is the high potential for oestrus. Female goats often show oestrus within a short period after finishing treatment. It is beneficial for the producer to plan artificial insemination or use it for an embryo transfer program (Jackson *et al*., 2014). Jackson *et al*. (2014) also found that when inducing oestrus simultaneously for female goats during the non-breeding season with vaginal CIDR for 5 days, the pregnancy rate after oestrus and artificial insemination reached 55%. Vilarino *et al*. (2011) also showed no difference between breeding and non-breeding seasons when inducing oestrus synchronization for goats with CIDR within 5 days. According to the same authors, the combined use of CIDR

and PGF2α for simultaneous estrus increased conception rates by up to 75%.

3.2. The effect of the estrous methods on the Progesterone serum concentration of goats

One of the criteria to evaluate the effectiveness of the method of oestrus synchronization is to determine the Progesterone serum concentration before and after the simultaneous induction of oestrus. In this study, to accurately evaluate the ovulation of goats after oestrus, we determined the serum Progesterone concentration on the day 0 before CIDR was applied to induce oestrus synchronization to goats, at the point at which the goat began to show oestrus and at the time when the goat showed the sign of oestrus standing. The results were shown in Table 2.

Table 2. The effect of the estrous methods on the Progesterone serum concentration of goats

Oestrus methods	Day 0	Time of oestrus starting	Time of oestrus standing
Method 1	3.71±0.9	0.42±0.12	0.32±0.11
Method 2	3.66±1.01	0.44±0.31	0.35±0.21

The results in Table 3 showed that Progesterone serum concentrations of goats at the time of initiation of oestrus, the time of onset of oestrus, and the time of oestrus standing in our experiment did not differ between the two experimental groups (3.71 vs 3.66; 0.42 vs 0.44 and 0.32 vs 0.35, respectively (P>0.05).

Our experiment's serum Progesterone concentrations at the time of the oestrus initiation, the time of oestrus onset, and the time of oestrus standing were also consistent with . According to these authors, the Progesterone serum was deficient on oestrus day (0.35 ng/ml) and peaked (5 ng/ml) at days 13-14 of the cycle. The results of our study showed that although the rate of oestrus induction of method 2 was lower than that of method 1 (Table 1), the Progesterone serum levels between the two methods were similar. Goats after the oestrus induction by both method 1 and method 2 were eligible to use for insemination and fertilization.

The effectiveness of estrous induction depends on many factors: oestrus techniques, methods of oestrus, goat breeds, breeding seasons. According to Meza-Herrera *et al*. (2008), goats with a high body index got more corpus luteum than goats with a low body index when producing oestrus synchronization. Serin *et al*. (2010) demonstrated that the goat group with a body index lower than 1.5 will have a low conception rate. Mellado *et al*. (2004) also reported that goats with body index ≤ 3 have about 20% lower conception rate than goats with body index >3.

4. CONCLUSION

Induction of oestrus using CIDR in combination with PGF2α resulted in higher estrous efficiency than using a single PGF2α.

Repeated oestrus induction did not affect the estrous efficiency of crossbred goats Boer.

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CLINICAL FEATURES AND LESIONS IN BUFFALOES INFECTED WITH *TRYPANOSOMA EVANSI*

Vu Thi Anh Huyen[1], Do Thi Van Giang[1], Nguyen Van Binh[1] and Nguyen Thi Bich Nga[1]*

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ABSTRACT

Microscopic lesions have been found in the internal organs of the buffaloes experimentally infected with *Trypanosoma evansi.* The result of infecting five buffaloes with *T. evansi* in the experiment group compared to three buffaloes in the control group found that: After four to seven days all five buffaloes showed *T. evansi* in the peripheral blood. Infected buffaloes had a marked change in some hematological indicators compared to control buffaloes. Dissection of infected buffalo demonstrated the percentage of organs showing lesions ranged from twenty to a hundred percent. On the other hand, three control buffaloes had no clinical symptoms and there were no lesions at autopsy. The internal organs of all the infected buffaloes had obvious microscopic lesions.

Keywords: *Buffalo, Trypanosomiasis, T. evansi.*

TÓM TẮT

Đặc điểm bệnh lý và lâm sàng bệnh do *Trypanosoma evansi* gây ra ở trâu

Gây nhiễm *Trypanosome evansi* cho 5 trâu thuộc lô thí nghiệm và 3 trâu thuộc lô đối chứng cho thấy: sớm nhất sau 4 ngày và muộn nhất sau 7 ngày cả 5 trâu gây nhiễm đều xuất hiện *T. evansi* trong máu ngoại vi. Trâu nhiễm *T. evansi* có sự thay đổi rõ rệt về một số chỉ số huyết học so với trâu đối chứng. Mổ khám trâu gây nhiễm thấy tỷ lệ các cơ quan có biểu hiện bệnh tích 20,00-100%. Ba trâu đối chứng không có triệu chứng lâm sàng và mổ khám không có bệnh tích.Các nội quan của trâu gây nhiễm đều có bệnh tích vi thể rõ rệt.

Từ khóa: *Trâu, bệnh tiên mao trùng, T. evansi.*

1. INTRODUCTION

Trypanosomiasisor Trypanosomosis is a common disease in cattle and buffaloes causing great loss for cattle husbandry in Vietnam and many other countries in the world. The tsetse fly, Glossina palpalis is a vector of the trypanosome that causes sleeping sickness in humans and nagana in cattle along with associated human health problems and massive economic losses (Illiassou *et al.*, 2013). Alessandra *et al.* (2019) indicate that *Trypanosoma evansi* (Kinetoplastea Trypanosomatidae) is the Trypanosoma species that infects the greatest variety of mammals worldwide.

According to Do Thi Van Giang (2014), trypanosomiasis has appeared in many areas throughout the country with high infection rates (13-30% of buffaloes and 7-14% of cattle), the mortality rate of affected animal was 6.3-20%. Suheir *et al*. (2020) indicate that *Trypanosoma evansi* is the causative agent of surra, a disease that occurs in many animal species. The disease is responsible for substantial losses in global production and can be fatal if not diagnosed early. Payne *et al.* (1991); Wuyts *et al.* (1994); Da Silva *et al.* (2010) indicate that clinical signs in trypanosome-infected buffaloes and cattle include falling and rising fever, emaciation, anemia, edema, corneal inflammation, swelling of the testes and testitis, swollen lympho nodes, limb paralysis, and abortion. According to Damayanti *et al.* (1994); Mekata *et al.* (2013) gross lesions in *T. evansi* infected buffaloes include hemorrhage in pericardium membrance, pneumonia,

[1]College of Economics and Techniques-Thai Nguyen university
* Corresponding Author: Dr. Do Thi Van Giang, College of Economics and Techniques - Thai Nguyen university. Address: Group 15, Thinh Dan Ward, Thai Nguyen City, Thai Nguyen Province. Tel: (84) 904227272; Email: vangiang208@gmail.com

hepatitis, swelling and edema of the spleen, swollen lympho nodes, enlarged bone marrow.

The aims of the study were to assess the clinical features and pathological characteristics of the disease of buffalo caused by *T. evansi* inorder to make a contribution to the control of trypanosomiasis in buffaloes and cattle.

2. OBJECTS AND METHODS OF STUDY

2.1. Object, places and time period of study

- Trypanosomiasis caused by *T. evansi* in catle.

- Buffaloes experimentally infected with *T. evansi.*

- Samples from animals experimentally infected with *Trypanosomes*.

- Auto hematology analyser Cellta - Mek - 6420K - Nihon Kohden (Japanese).

- Light microscope, chemicals and other experimental instruments.

2.2. Methods of study

- Measuring body temperature of buffaloes by thermometer 43^0C.

- Dissection and examination of internal organs of animals experimentally infected which were dead or were still alive. Observing internal organs by naked eyes or by using magnifier, taking pictures of sections in the body that manifested typical gross lesions.

- Testing blood indices by using auto hematology analyzer-Cellta - Mek - 6420K - Nihon Kohden (Japan).

- Data collected is treated by methods of biostatistics (According to document of Nguyen Van Thien *et al*., 2008), on Excel software.

3. RESULTS AND DISSCUSTION

3.1. Appearance time of T. evansi in blood of buffaloes after infecting

After infecting buffaloes of the experimental infected group, monitoring time of *T. evansi* appearance in blood of buffaloes in all groups. The results were illustrated in table 1.

Table 1. Appearance time of *T. evansi* in blood of buffaloes after infecting

Group	Experimental infected buffaloes	Infecting dose (*T. evansi*/ buffalo)	Path of disease	Time of *T. evansi* appearance after being experimentally intected
Experimental infected group 1	1	2×10^8	Peritoneal sinus	6
	2		Peritoneal sinus	7
Experimental infected group 2	3	3×10^8	Peritoneal sinus	4
	4		Peritoneal sinus	4
	5		Peritoneal sinus	5
Control group	3 busfaloes	0	*T. evansi* does not appear in the blood	

Table 1 indicates that, in experimental infected group 1 (at dose $2x10^8$ *T. evansi* per buffalo), the time *T. evansi* appeared in the blood of buffalo No. 1 was 6 days and buffalo No. 2 was 7 days after infection (average 6.5 days). In experimental infected group 2 (at dose $3x10^8$ *T. evansi* per buffalo) 4 and 5 days after infection, *T. evansi* was found in buffalo blood (mean 6.5 days). The control group: *T. evansi* does not appear in the blood.

3.2. Body temperature after being experimentally infected

Monitoring the body temperature of buffaloes in the experimental group and the control group found in the control group; the body temperature curve in all of 3 buffaloes varied from 37.8^0C to 38.5^0C. Fever was not found during the experiments.

In the experimental group, fever was found in 5 buffaloes experimentally infected with *T. evansi* (over 38.5^0C). There were 3-3 times of fever in the 5 buffaloes corresponding to 3-4 waves fevers of trypanosomes.

According to Damayanti *et al.* (1994),

relapsing fever was related to the waves of parasitaemia and fluctuations of pulse and respiration rates. Our scientific research results are similar to this scientific research results.

3.3. Main clinical signs of buffaloes after being experimentally infected

Monitor manifested clinical signs in the group was experimentally infected with *T. evansi* and buffaloes in the control group. The results were illustrated in table 2.

Table 2 showed 5 buffaloes being experimentally infected with 2 different doses of trypanosomes manifested the same clinical signs: in some early days their appetite was normal, not manifesting any clinical signs of trypanosomiasis, later their symptoms appeared (rate of animals manifesting clinical signs varied 20-100%).

All buffaloes in the control group had no clinical symptoms of trypanosomiasis.

Infected buffaloes in the group was experimentally infected with *T. evansi* had symptoms similar with those of Sarah *et al.* (2006).

Table 2. Main clinical signs of buffaloes experimentally infected with trypanosomes

Groups	Main clinical signs	Number of buffaloes monitored	Number of buffaloes showing clinical signs	%
The group was experimentally infected with *T. evansi*	Undulant fever	5	5	100
	Swollen eyes with mucus running out of eyes		5	100
	Dry rough hair coat		5	100
	Edema in jaws		4	80.00
	Edema in chest and abdomen		3	60.00
	Trembling		5	100
	Paralysis of the hindlegs		1	20.00
Control group	Clinical signs mentioned above	3	0	0.00

3.4. Changes of some blood cell indices, number of leucocytes and proportion of various types of leucocytes in buffaloes experimentally infected with trypanosomes compared with that in control buffaloes

Changes of some blood cell indices buffaloes experimentally infected were illustrated in table 3 indicates that the mean erythrocyte count of buffaloes infected with *T. evansi* was lower than that of the control buffaloes. This difference is quite obvious (P<0.01). The mean leukocyte and thrombocytes count of 5 buffaloes infected with *T. evansi* were significantly higher than that of the control buffaloes (P<0.01).

Table 3. Changes of some blood cell indices of buffaloes after being infected

Blood cell indices	Control bufaloes (Mean±SD)	Buffaloes experimentally infected with trypanosomes (Mean±SD)	P
Number of buffaloes (buffalo)	3	5	
Number of erythrocytes (million/ml of blood)	8470±142.11	6936±943.00	P<0.001
Number of leucocytes (thousand/ml of blood)	13600±2250.53	17260±1344.70	P<0.001
Number of thrombocytes (thousand/ml of blood)	213.67±89.88	342.80±33.75	P<0.001
Hemaglobin content (g/l)	125.33±0.33	114.00±1.46	P>0.05
Average volume of erythrocytes (fl)	47.20±3.16	51.58±0.17	P>0.05
Mean corpuscular hemoglobin concentration (g/l)	302.33±2.40	300.4±1.25	P>0.05

The average hemoglobin concentration and mean corpuscular hemoglobin concentration of infected buffaloes were lower than that of the control buffaloes (125.33 and 302.33 g/l compared to 114.00 and 300.4 g/l). However, this difference was not significant (P>0.05).

The average volume of erythrocytes of the infected buffaloes increased slightly compared to the control buffaloes: in the control buffaloes it was 47.20 femtoliter (fl), in the infected buffaloes it was 51.58 fl (P>0.05).

Results of testing 5 *T. evansi* infected buffaloes showed that *T. evansi* infection led to increased number of leucocytes and thrombocytes and decreased number of erythrocytes compared with the control buffaloes (table 3). According to Damayanti *et al.* (1994), packed cell volume, total platelet and red cell counts, and haemoglobin values were below those of two uninfected control buffaloes, as well as below the normal range. Our scientific research results are similar to this scientific research results. Amount and proportion of various types of leucocytes were illustrated in table 4 shows that amount and rates of granulocytes in the buffaloes experimentally infected with trypanosomes were higher than that in the control ones. In contrast, amount and rates of lymphocytes and monocytes in the buffaloes experimentally infected with trypanosomes were much lower than that in the control ones (P<0.001).

Table 4. Amount and proportion of various types of leucocytes in the buffaloes experimentally infected compared with the control buffaloes

Group	Control buffaloes		Experimentally infected buffaloes		P
Type of leucocytes	Amount (Mean±SD)	Percentage (%)	Amount (Mean±SD)	Percentage (%)	
Number of buffaloes	3		5		
Lymphocytes (thousand/ml)	10,333±1,782.48	75.98	7,040±182.35	40.79	P<0.001
Monocytes (thousand/ml)	2,333±47.14	17.16	1,180±74.16	6.84	P<0.001
Granulocytes (thousand/ml)*	934±232.14	6.86	9,040±1,295.96	52.37	P<0.001

Notes: ** Granulocytes include eosinophils, basophils and neutrophils*

Ohaeri and Eluwa (2011) has researched and announced that: The effect of trypanosome infection in ruminant animals showed that infected animals had significantly lower (P<0.05) packed cell volume, erythrocyte count and higher (P<0.01) mean cell volumes than uninfected animals. Leucocytosis, reticulocytosis and thrombocytopenia were also observed. The infection also produced a decrease in albumin (P<0.001), significant increase in total protein and bilirubin levels. These changes were not seen in the animals that were not infected. Our scientific research results are similar to this scientific research results.

3.5. Main gross lesions in buffaloes experimentally with Trypanosomes

Thirty days after infecting buffalo with *T. evansi,* at necropsy of buffaloes in the group was experimentally infected with *T. evansi* and buffaloes in the control group to find lesions. The results were illustrated in table 5 shows that buffaloes experimentally infected with *T. evansi* all had lesions in internal organs, making up 20-100%, in detail 100% of buffaloes infected with *T. evansi* had enlarged swollen and hemorrhagic spleen; 80% of buffaloes infected with *T. evansi* had enlarged flabby heart muscle, swollen and hemorrhagic liver, swollen and hemorrhagic lungs; 40% of buffaloes infected with *T. evansi* had enlarged swollen kidneys; 20% had enlargedsubcutaneous tissues had yellow viscous gel like.

Table 5. Gross and microscopic lesions in the buffaloes experimentally infected with trypanosomes

Group	Main gross lesion	Number of buffaloes at necropsy	Number of buffaloes showing lesions	Percentage (%)
The group was experimentally infected with *T. evansi*	Flabby heart muscle	5	4	80.00
	Swollen kidneys		2	40.00
	Swollen and hemorrhagic liver		4	80.00
	Swollen and hemorrhagic lungs		4	80.00
	Swollen and hemorrhagic spleen		5	100
	Subcutaneous tissues had yellow viscous gel like		1	20.00
Control group	Lesions mentioned above	3	0	0.00

Damayanti *et al.* (1994), Dyah and Rini (2021) indicate that: Mice infected with *T. evansi* cause various clinical manifestations and histopathological changes. the histopathological lesions of mice infected with *T. evansi* isolates (high virulence) showed very acute animal death and showed mild to moderate histopathological lesions, namely non-suppurative encephalitis, non-suppurative pneumonia, hepatitis non-suppurative with intravascular trypanosomiasis, tubular degeneration and necrosis. Our scientific research results are similar to this scientific research results.

3.6. Microscopic lesions in some internal organs of affected buffaloes due to being experimentally infected with trypanosomes

Microscopic lesions in the internal organs of buffaloes experimentally infected with trypanosome consisted of dilated myocardium, enlargement of cardiac muscle fibers; dilated hepatic sinusoid, hepatic vein like spokes being dilated, degeneration of liver cells; congested lungs, accumulated with edema fluid; hemorrhage of splenic tissues filtrated inflammatory cells and macrophages; hemorrhagic kidney tissues, dilated and hemorrhagic renal ducts. Damayanti *et al.* (1994) indicate that hydropericardium, petechial to larger haemorrhages in the pericardium, pneumonia, congested liver and spleen, oedematous enlargement of the superficial lymph nodes and hyperplastic bone marrow were the major gross pathological changes. Histologically, the most consistent lesions were interstitial pneumonia, interstitial myocarditis, splenic multifocal necrosis, interstitial myositis and hyperplastic bone marrow. Infected buffaloes in the experimental group infected with *T. evansi* had gross and microscopic lesions similar to those described by the same author.

4. CONCLUSIONS

At high infective dose of *T. evansi*, time to appear *T. evansi* in peripheral circulation is early and oppositely at low dose.

Buffaloes infected with *T. evansi* manifest waves fever, averagely, 3-8 days.

Five buffaloes infected with *T. evansi* all appear clinical signs about 20-100%.

In trypanosome infected buffaloes there are apparently decreased amount of erythrocytes and increased amount and rates of granulocytes compared with the controls.

Lesions in viscera of buffaloes experimentally infected with *T. evansi* have been found in heart, lungs, spleen, liver, kidneys (20-100%). Microscopic lesions have been found in the internal organs of the buffaloes experimentally infected with *T. evansi.*

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OVERVIEW THE SITUATION AND ORIENTATION FOR DEVELOPMENT OF LIVESTOCK PRODUCTION IN VIET NAM IN PERIOD 2015-2021

1. LIVESTOCK PRODUCTION IN VIET NAM IN PERIOD 2015-2021

1.1. Population of livestock production

Item	Unit	2015	2016	2017	2018	2019	2020	2021	Growth rate, %
1. Buffalo	*1,000 head*	2,523.7	2,519.4	2,491.7	2,425.1	2,349.9	2,410.0	2,282.3	-2.0
2a. Beef casttle	*1,000 head*	5,367.1	5,496.6	5,654.9	5,802.9	5,640.7	5,875.3	6,236.0	3.0
2b. Dairy cattle	*1,000 head*	275.3	283.0	290.0	294.4	321.2	346.5	375.0	6.4
3. Pig	*1,000 head*	27,751.0	29,075.3	27,406.7	28,151.9	20,209.5	22,028.0	28,100.0	0.3
4. Poultry	*mill head*	341.9	361.7	385.5	409.0	481.0	512.7	523.2	8.9

1.2. Growth of livestock products

Products	ĐVT	2015	2016	2017	2018	2019	2020	2021	Growth rate (%)
1. Meat	*1.000 tons*	4,806.6	5,043.5	5,199.5	5,368.2	6,354.1	6,502.1	6,679.2	6.8
Pig meat	*1.000 tons*	3,491.6	3,664.6	3,733.3	3,816.4	4,085.0	4,020.1	4,180.0	3.7
Poultry meat	*1.000 tons*	908.1	961.6	1,031.9	1,097.5	1 681.5	1,881.3	1,992.0	17.0
Buffalo meat	*1.000 tons*	85.8	86.6	88.0	92.1	125.3	120.5	96.0	2.3
Beef	*1.000 tons*	299.3	308.6	321.7	334.7	430.7	441.5	372.5	4.5
Goat meat, lamb	*1.000 tons*	21.8	22.1	24.6	27.5	31.6	38.7	38.7	12.1
2. Eggs	*Mill eggs*	8,874.6	9,446.2	10,637.1	11,645.6	15,470.9	16,681.9	17,500.0	14.5
3. Milk	*1.000 tons*	723.2	795.1	881.3	936.7	986.1	1,049.3	1,200.0	10.7

In 2021, pork production: 4,18 mil tons, occupied 62,4% total meat production in Vietnam.

1.3. Distribution of livestock herds of Vietnam

The 10 provinces with the largest beef cattle: Nghe An (485,900 heads), Gia Lai (395,984 heads), Son La (357,952 heads), Binh Dinh (296,657 heads), Quang Ngai (279,305 heads), Thanh Hoa (260,356 heads), Dak Lak (245,279 heads), Tra Vinh (225,068 heads), Ben Tre (223,432 heads) and Quang Nam (172,328 heads). The number of beef cattle in these 10 provinces accounts for 46.51% of the total beef cattle herd of the country.

The 10 provinces with the largest number of pigs: Dong Nai (2,398,403 heads); Binh Phuoc (1,945,038 heads); Hanoi (1,465,075 heads); Thanh Hoa (1,185,000 heads); Binh Dinh (960,588 head); Bac Giang (942,400 heads); Nghe An (916,522 heads); Dak Lak (901,200 heads); Binh Duong (712,547 heads); Phu Tho (684,548 heads). The total pig herd in these 10 provinces accounts for 42% of the country's total herd.

The 10 provinces with the largest poultry flocks: Hanoi (33.1 million heads), Dong Nai (27.4 million heads), Nghe An (29.4 million heads), Thanh Hoa (22.6 million heads); Bac Giang (19.2 million heads), Tien Giang (19.2 million heads), Phu Tho (15.8 million heads), Thai Nguyen (14.5 million heads), Thai Binh (14.1 million heads), Hai Duong (13.9 million heads). The total poultry herd in these 10 provinces accounts for 40% of the whole country.

The distribution of beef cattle, pig and poultry herds in different regions in Vietnam are:

Regions	Beef cattle (%)	Pig (%)	Poultry (%)
Mekong river delta	15	9	17
Southeast	7	20	13
Western highlands	13	8	5
North central & central coast	38	20	21
Red river delta	8	20	24
Northern Midlands and Mountains	19	23	20

1.4. Meat production in Vietnam

Comparing with Vietnam's food consumption with the world, it can be seen the potential for production and consumption of products. Vietnamese livestock products are still very potential: pork 62%, poultry meat 28%, cattle meat 9% and goat meat 1%.

2. NEW OPPORTUNITIES

The livestock production institution is increasingly perfected, interested in increasing enforcement, ensuring the harmonization of legal regulations and international economic integration.

The consumption market is large and will grow strongly when transitioning to the normal stage with Covid and the economy opening up tourism and services completely.

Vietnam has a well-developed agricultural background (agriculture-forestry-fishery) and increasing investment by large enterprises in animal husbandry.

Rapid development of science and technology (biotechnology, pharmaceutical chemistry, genetics, automation) will make important contributions to animal husbandry; investment programs and projects from private economic sectors

Digital transformation taking place in many fields and at all levels will effectively support connectivity – information sharing, linking market demand with production capacity.

3. CHALLENGES FOR ANIMAL PRODUCTION IN VIETNAM

The Covid epidemic in humans and emerging infectious diseases in livestock (DTLCP, Avian Influenza) are still complicated, not completely controlled, and meat consumption has been affected by the epidemic.

The increase in world energy prices and the conflict between Russia and Ukraine are pushing up the prices of feed materials in the world and in Vietnam, increasing input costs for production.

Climate change and unpredictable extreme weather (storms, floods, thunderstorms, droughts, severe cold) increasingly complex response

The level of food competition has increased between Vietnam and the world (17 free trade agreements, including new-generation trade agreements such as CPTTP, EVFTA)

4. OPPORTUNITIES FOR ANIMAL PRODUCTION IN VIETNAM

The livestock production institution is increasingly perfected, interested in increasing enforcement, ensuring the harmonization of legal regulations and international economic integration.

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Digital transformation taking place in many fields and at all levels will effectively support connectivity – information sharing, linking market demand with production capacity,

5. BASED FOR LIVESTOCK PRODUCTION IN VIET NAM

The Law on Livestock (2018) has opened up a legal corridor to develop the livestock industry towards exploiting comparative advantages, food safety, animal welfare, international integration and environmental protection.

Strategy for sustainable agricultural and rural development in period 2021 - 2030, with a vision to 2050 (Decision 150/QD-TTg)

Strategy for livestock industry development in period 2021-2030, with a vision to 2045 (Decision 1520/QD-TTg)

The orientation for the livestock industry to develop towards biosecurity, food safety, added value, sustainable development

6. ORIENTATION FOR LIVESTOCK DEVELOPMENT IN VIET NAM

Ensuring food demand for the domestic market (100 million people)

Developing potential products and markets such as poultry meat, eggs, milk; maintain pig sector and ruminal animal.

Developing native animals with high-value specialties, food safety, environmental sustainability, biosecurity, and disease control.

Developing the industrial farming methods applying high technology at large farms and enterprises

Encouraging household farming with improvements in the direction of professionalization and organic farming.

Setting the concentrated husbandry areas far away from places of concentrated population with convenient for environmental treatment and disease prevention.

Gradually develop and master technology of breeding stock, animal feed, veterinary medicine, processing livestock products,...

Efficient management and use of livestock waste, promoting circular economic models in livestock production.

Encourage the development of concentrated and industrial slaughtering activities;

Strengthen international cooperation in scientific research, training and trade in animal husbandry.

Improve competitiveness of livestock products

Accelerating the implementation of digital transformation in the livestock industry.

APPLICATION ON BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) REARING TECHNOLOGY AS A TOOL TO IMPROVE ENVIRONMENT SAFETY, SUSTAINABILITY AND RURAL DEVELOPMENT IN SOUTH OF VIETNAM: EMPHASIS ON AQUACULTURE PRODUCTION

Prof. Dr. Duong Nguyen Khang
Director, Research and Technology Transfer, NLU

BACKGROUND

Fishmeal feed ingredient is one of the main constraint to support the growth of livestock and aquaculture production in Vietnam. However, the price of aqua feed has increased 6-7 times since the beginning of 2021 up to now. Aqua feed cost has increased by 20% in year 2021 compared with year 2020. In the cost structure of aquatic products, feed accounts for about 50-70%, with an average of 60%. Therefore, when prices fluctuate, it will very quickly affect the market, increasing production costs, increasing risks for the aquaculture sector in reducing competition for products.

Lack of access to high-quality and competitive feed-priced means that farmers are unable to expand their production and reduce profits (even loss profits) in some cases. Black Soldier Fly (BSF) is an insect that can safely be reared because they are non-feeding adults, require only water and non-transmission diseases. Larvae of BSF feed on a large variety of organic matter, including plant material (Hillaire et al., 2007). They are capable of converting large amounts of waste biomass into stored protein (≥40%) and fat (≥20%). Hence using larvae as a replacement of protein source for fish will reduce dependence on commercial feed, it is estimated that the cost of 1kg feed with supplemented BSF larvae can be reduced by half compared to fully commercial feed. The waste residue post-harvesting larvae (BSF larvae manure) is also a valuable product, it can also be further processed and potentially sold or used as soil amendment with fertilizing properties.

Per weight gain, studies show that insects emit less greenhouse gases per weight gain e.g. carbon dioxide ($C0_2$), methane (CH_4), and nitrogen dioxide (NO_2), as well as less products responsible for soil eutrophication notably ammonia (NH_3) (Van Huis, 2012). Greenhouse gases emitted by insects are about 1% of ruminant's emissions (Oonincx et al., 2010). Insect rearing requires very little surface area by accepting high population densities as well as the possibility of being reared vertically. In 2006, FAO experts estimated the total land used to produce meat accounted for 23% to 30% of the entire global land surface. Insects are natural recyclers of organic matter and water requirements are low as water is provided by food and atmospheric humidity.

The proposed solution is to supply BSF larvae as a sustainable source of protein to livestock/and aquaculture farmers in order to raise rural farmers' income and improve farming practices.

OBJECTIVES

2.1. Capacity objective

The overall objective of the project is to establish the model of raising and using BSF larvae as a protein source for aquaculture and BSF larvae manure as a organic fertilizer source for crop production at the farms. This will contribute to recommendation of using BSF as aqua-feed to bring the benefit economically to farmers and mitigation for significant negative environmental impact.

2.2. Immediate objectives

(1) Analyze farmer's understanding on raising BSF and feeding aquaculture by BSF at localities of project, establishing potential solutions to improve farmer's techniques in raising system optimally.

(2) Guide the farmers on optimizing BSF larvae production by feeding the animal/ agricultural by-product wastes.

(3) Guide the farmers on using BSF larvae as aqua-feed for aquaculture production.

(4) Capacity-building of extension workers to be better positioned to advise farmers on techniques, management, and expansion of the combined model of fish farming and BSF.

DESCRIPTION OF THE PROJECT

3.1. Site of project

Four main activities categories will be conducted in this project during 1 year period (starting from early until late 2022). All activities will be conducted in Ho Chi Minh City, Long An and Tien Giang provinces.

Tien Giang and Long An are the first two provinces to raise black soldier fly as feed animals and two provinces also strongly developed the high aquatic surface for aquaculture at Mekong delta annually. However, feed cost issues are a hindrance to aquaculture development here. Raising BSF'sporadically, lack of the techniques for optimizing BSF productivity, and unknown how to successfully use BSF fed aquaculture are direct obstacles to realizing economic potential and improving the well-being of farmers.

In Ho Chi Minh City, with the coastal mangrove forest system, the advantage of Can Gio district is aquaculture development. The model of sustainable aquaculture farming by BSF-feeding under the forest canopy not only helps many households have stable incomes and get rich but also makes a significant contribution to protecting the "green lung" of the city.

3.2. Activities of project

a) Survey to farmer's understanding of raising model for black soldier fly based-aquaculture feed supply

The survey focus on the farming scale, yield, farming techniques, and organic waste supply for BSF production which offers the analysis of the actual situation, from there providing potential solutions to improve farming techniques towards optimal production and nature-friendly.

Survey some typical techniques of farmers in fish farming based on BSF, yield, and obtained quality of fish from this model. This helps provide informations for the project to analyze how to support the solution for farmers in building a model combining black soldier fly farming with fish farming in the most effective way.

This activity will be carried out in provinces in Mekong delta (Long An, Tien Giang, Ben Tre, Vinh Long, Tra Vinh, Can Tho, Hau Giang, Dong Thap, An Giang, Soc Trang, Bac Lieu, Kien Giang, Ca Mau and Ho Chi Minh City) as representatives in Vietnam that are high potential on BSF's farming and fish raising. There are 200-300 data sampling from farmer smallholders approximately will be collected in this survey.

b) Building capacity activities

* Training/improving BSFs' production: support adoption of new techniques among farmers, the training on best practices on BSF's will be provided for both extension officers at provincial/districts and smallholder farmers. This activity will be purposed to improve both quantity and quality of BSF's at smallholder farms levels. These productions will be contributed and supported the gap between demand and supply of BSFs' productions in the combination of fish and BSF model. The best demonstration pilots will be done for the pieces of training on new techniques of BSF production in project areas.

* Improving quality of extension/ veterinary services: the training and set up group of extension/veterinary officers will be organized. These purposed will be for increasing their building capacities, sharing information and support each other's. The extension/veterinary officers at national level, provincial and district levels will work together for strongly network and better support smallholder farmers in both project target areas and outsides project areas.

Mentoring visits, evaluations, and learning will be done monthly by NLU staff mainly, while other partners will join in every quarterly.

*Set up farmer group and farmer association will be the next activities after farmer training. Based on policy of government, farmer groups/associations can easily access new technologies via the training by NLU researchers and province extension staff; farmers can exchange their knowledge, experiences, and information among their group members; farmers can access the materials needed, investment cost or/and funding resources in case they would like to expand their activity.

* Demonstrations/model farms will be set up at least one plot in each target village with 12 plots in total of two provinces and Ho Chi Minh city. This model farms will be including the best practices techniques on BSFs farming such as technical feeding/house system for BSF larvae health care, improving the yield of BSF larvae production with consumption of residues/animal wastes by BSF effectively, feeding BSF to fish (feeding norms the BSF larvae in growth stages of fish), yield and quality of fish.

3.3. Stakeholder workshop

At the beginning and the end of the project, stakeholder workshop will be organized. The output from the project activities under agreement of stakeholders on BSF and fish production will be included in the activities, particularly at small holdder farms. As well as, monitoring and mentoring planning and outputs, the stakeholder workshop will be organized two times during the project period.

3.4. Communications activity

Table 1. Maping to activities and the output

Activities	The expected outcomes	Outputs/Deliverables
Baseline survey	The group of project has the overview of actual circumstances connected with the farmer's understanding of raising model for black soldier fly based-aquaculture feed supply. Propose an appropriate solution for building an optimal and sustainable model on fish and BSF production.	Report.
Building capacity farmers	The farmers have increased their knowledge and know how to make the best practice on BSF production and capable of building and managing a farming model that combines BSF and fish production.	Report from training. Number of farm families learned and applied of the BSF and understand the important of BSF for aqua-feed production by end of 2022.
Extension/ veterinary officers	The extension workers have improved their knowledge in communicating and training farmers to expand the model.	Number of extension/veterinary workers are able to multiple their knowledge by training more farm families.
Mentoring	Mentor increases the understanding of farmers' needs in terms of technical and management models that are in accordance with location, thereby offering suggestions and solutions to aid farmers.	Number of mentors are able to analyse the feedback from field results in communicating and training farmers to expand the model.
Set up farmer groups	Farmer groups/associations have a positive perception of how applying new technologies of BSF for aqua-feed production will bring about economic efficiency and sustainability.	Number of farmer groups/associations have belief and accept apply technologies of the BSF for aqua-feed production by end of 2022.
	Farmers groups/associations are easily accessed the materials, investment cost for applying their activities.	Number of farmer groups are accessed to investment cost (via banks) for applying their activities.
Set up model farms	Farmers can practice techniques fluently and manage model farms effectively, creating the effect of expanding the model locally.	Number of model farms have set up the plot in the best practices techniques on BSFs farming.
Stakeholder workshop	Increasing the engagement and insight of developing a model of combining BSF and aquaculture by stakeholders, to seek their input on the project (if any) and expect to achieve consensus.	Number of agreements of stakeholders has signed.
Communic-ation	Disseminating and enhancing the exchange of experiences on project-related issues, maintaining the model even after the project ends.	A website page at Research and Technology Transfer Center, Nong Lam University.

An information sharing and network expansion will be implemented during the project period. The experiences on the best practice on BSF's farming and the combination of fish and BSF into the project activities will be shared online (website page such https://rttc.hcmuaf.edu.vn/ of Research and Technology Transfer Center, Nong Lam University, linked to others pages).

4. DURATION

One year (from January 2022 to February 2023).

5. TARGET AREA

Residues from agricultural by-products and manures from animal/bird production are more pollution in Vietnam. We could train the farmers how to clasify these residues/wastes, how to use these residues/wastes for BSF production, how to use BSF manure for crop organic fertilizer. By this way, we will reduce (1) post-harvest loss from agricultural residues (2) environemntal polution from wastes (3) improve the quality of aquaculture products.

Use the larvae to supplement as aqua-feed diets for aquaculture production, make organic biofertilizer for crops: the advantage of this approach is that farmers will have access to high quality aqua-feed at affordable cost, feed cheapest cost, easier to access markets and less aqua-feed import.

Improve the feed products from BSF meat, then provide the best human food quality.

6. PROJECT TEAM

a, Nong Lam University staff

Name and surname	Department	Mail	Role of Project
Prof. Dr. Duong Nguyen Khang	Director, Research and Technology Transfer, NLU. Senior lecturer, Veterinary Bio-Science Department, Faculty of Animal Science and Veterinary Medicine, NLU.	duongnguyenkhang@gmail.com duongnguyenkhang@hcmuaf.edu.vn	Coordinator, consultant, researcher, lecturer and trainer
Dr. Le Thuy Binh Phuong	Lecturer, Veterinary Bio-Science Department, Faculty of Animal Science and Veterinary Medicine	phuong.lethuybinh@hcmuaf.edu.vn	Lecturer and trainer
MSc. Dang Hoang Dao	Lecturer, HUTECH Institute of Applied Sciences, Ho Chi Minh City University of Technology	dh.dao@hutech.edu.vn	Assistant
Dr. Nguyen Ngoc Thuy	Head of International Relations Office. Lecturer, Faculty of Economics.	nnthuy@hcmuaf.edu.vn	Lecturer and trainer
Assoc. Prof. Dr. Nguyen Nhu Tri	Dean of Faculty. Senior lecturer, Faculty of Aquaculture, NLU.	nntri@hcmuaf.edu.vn	Lecturer and trainer
Assoc. Prof. Dr. Nguyen Quang Thieu	Deputy of Faculty. Senior lecturer, Nutrition Department, Faculty of Animal Science and Veterinary Medicine, NLU.	nguyen.quangthieu@hcmuaf.edu.vn	Lecturer and trainer
Assoc. Prof. Dr. Nguyen Phu Hoa	Head of Scientific research Management Office, NLU. Senior lecturer, Aquaculture and Fisheries Resource Management industry. Faculty of Aquaculture, NLU.	phuhoa@hcmuaf.edu.vn	Lecturer and trainer
Assoc. Prof. Dr. Nguyen Bao Quoc	Director, Nong Lam University Library, NLU. Senior lecturer, Biological Sciences Department, Faculty of Biological Sciences, NLU.	baoquoc@hcmuaf.edu.vn	Organizer at Library, lecturer and trainer
Dr. Nguyen Duc Xuan Chuong	Lecturer, Plant Microbiology, Faculty of Agronomy, NLU	ndxchuong@gmail.com	Lecturer and trainer
Assoc. Prof. Dr. Le Trung Thien	Senior lecturer. Faculty of Chemical Engineering and Food Technology, NLU.	le.trungthien@hcmuaf.edu.vn	Lecturer and trainer
Assoc. Prof. Dr. Nguyen Tri Quang Hung	Senior lecturer. Faculty of Environment and Natural Resources, NLU.	quanghungmt@hcmuaf.edu.vn	Lecturer and trainer

b, Partners

AgriFoSe2030 II Swedish team.

c, Stakeholders

Farmers association

Agricultural private company

Extension center

Veterinary service

Local authorities

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