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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
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ANIMAL GENETICS AND BREEDING

ANIMAL FEEDS AND NUTRITION

ANIMAL PRODUCTION

SCIENTIFIC NEWS

DIMENSIONS AND CARCASS CHARACTERISTICS OF VIETNAMESE MINH DU CHICKENS AT 90 DAYS OLD

Nguyen Thi Ngoc Linh[1], Nguyen Thi Thuy[1] and Le Thanh Phuong[1,2]*

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ABSTRACT

The major objective of this study was to determine body dimensions and carcass characteristic of hens and roosters Minh Du (MD) chickens at 90 days old. The animals were fed until slaughter age at commercial barn. A total of 66 chickens (33 hens and 33 roosters) were sellected to record body dimensions after that 12 chcikens (6 hens and 6 roosters) were slaughtered to analyse carcass yields. Most of evaluated traits and carcass paramesters also liver weight in males were significant ($P<0.05$) greater than those in females, though insignificant different ($P>0.05$) was found for organs cut-up parts. In contract, most of carcass propotions were ineffective ($P>0.05$) between gender except the smaller weight of thigh meat propotion ($P<0.05$) was shown in rooster compared with hens. In conclusion, the findings showned that MD roosters had better performances in live weight and carcass parameter than hens ones, those datas can be used for managerments, consumers and genetic cross-breeding improvement.

Keyworlds: *Minh Du chicken, dimensions, carcass traits.*

1. INTRODUCTION

Chicken industry in Vietnam has been growing rapidly in recent years. However, broiler chickens express great carcass performance and fast growing causes of genetic selection, improved nutrition, vaccination and well management therefore their meat defects to sensory and functional qualities (Fanatico *et al*., 2007). While, native chicken is considered healthy result of slow growing and natural environment whith non chemical antimicrobial growth promoters, their productions have no negative impact on human health. Nowaday, Vietnamese consumer has prefered healthy chicken meat and specific quality as taste, flavor, sweetness, toughness, stiffness.

Minh Du (MD) chicken is the result of crossing two purebred chickens Noi chicken and Ri chickens. Whereas, Noi chicken is classified as native chicken with larger body size, slow-growing, highly resistant to disease (Nguyen *et al*., 2010) and interested in food service markets in Mekong delta (Do *et al*., 2019; Nguyen *et al*., 2022b). Ri chicken has medium body size which is smaller compared to Dong Tao chicken, Mia chicken, Ho chicken and bigger than Ac chicken and Tre chicken, however Ri chicken account for 90% of the local in North Vietnam cause of highly environmental adaptation, premium price for production and good flavour and tasty meat (Nassim *et al*., 2011). Therefore, MD hybrid chicken has been obtained gaining weight fast in case of less feed consumption, its meat production is delicious similar to local native chicken with thick breast and lean and high resistance, adapting to climate conditions in Vietnam. Moreover, chickens' dimensions perform identification of those species (Liyanage *et al*., 2015) which could be investigated for prediction of live weight or chicken cages manufacture (Assan, 2013). Therefore, the objective of the present study aimed to evaluate dimensions and cut-up edible parts of MD chicken between genders at 90 days old under factory farm conditions.

[1] Department of Animal Sciences, Can Tho University, Can Tho City, Vietnam

[2] Vietswan Poultry Production Joint Stock Company, Binh Duong Province, Vietnam

* Corresponding Author: Dr. Nguyen Thi Ngoc Linh, Department of Animal Sciences, Can Tho University, Can Tho City, Vietnam. Tel: (84)983797177, Email: ntnlinh@ctu.edu.vn

2. MATERIALS AND METHODS

Study area: This study was conducted on Apr, 2022 on the farm. The sample was analyzed at the Department of Animal Sciences of Can Tho University.

Research protocol: A total of 66 chickens (33 males and 33 females) of MD strain of MD2.BĐ at slaughter stage (90 days old) were selected randomly to evaluate their dimensions as skull height (SH, mm), skull width (SW, mm), neck length (NL, mm), back length (BL, mm), wings length (WL, mm), thigh length (TL, mm), shank length (SL, mm), keel length (KL, mm), breast diameter (BD, mm), and thigh diameter (HD, mm) (FAO, 2012). The chickens were grown in commercial barn for one day old until 90 days old.

Carcass characteristics was examined on total of 12 chickens (6 males and 6 females) selected from 66 chickens for live weight (LW, g), killed weight (KW, g), de-feather weight (g), carcass weight (CW, g), head weight (HW, g), weight of breast meat (BW, g), weight of thigh meat (TW, g), wing weight (WW, g), drumstick weight (DW, g), shank weight (SW, g) (FAO, 1994; Lukaszewicz and Kowalczyk, 2014). Weight of internal organs (IOW, g), gizzard weight (GW, g), LW (g), HW (g), weight of abdominal fat (AFW, g); length of small intestine (SIL, mm); caeca length (CL, mm) (Kokoszyński *et al*., 2017).

The difference of parameters in male and female chickens were statistically analyzed using GLM procedures within Minitab 16 software. Data were expressed as Mean±SD for each sex. The means were considered significant when the P-value were less than 0.05 (P<0.05).

3. RESULTS

In this study, MD chickens demonstrated BW and all dimensions of roosters was significantly higher than hens (table 1). Roosters had BW (2,692g) were significantly heavier than hens did (1,892g). The skull of roosters were significant bigger than hen's skull which was shown in SW and SH (33.5 and 39.0mm compared to 29.9 and 35.5mm, respectively). While, hens tend to be more significant timid than roosters were indicated in neck length (NL), back length (BL), chest circumference (CC), wingspan leng (WsL) and wing length (WL) là 14.4, 36.4, 28.2, 52.9 and 24.4cm compared to 15.5, 40.5, 31.2, 59.9 and 28.2cm, respectively. In addition, other measurements of dimensions of hens reported significantly lower than roosters had in BL, TL, TC, DL and SL (17.9, 21.4, 9.42, 15.9 and 6.23cm compared to 19.4, 24.2, 11.8, 18.8 and 7.7cm, respectively)

Table 1. Dimensions of MD chicken in genders

Traits	Hens (n=6)	Roosters (n=6)	Mean (n=12)	P
BW, g	1,892±187a	2,692±278b	2,292±467	0.001
SW, mm	29.9±1.26a	33.5±1.59b	31.70±2.27	0.001
SH, mm	35.5±1.67a	39.0±1.96b	37.25±2.53	0.001
NL, cm	14.4±0.98a	15.5±1.39b	14.94±1.30	0.001
BL, cm	36.4±2.29a	40.5±2.75b	38.45±3.25	0.001
CC, cm	28.2±1.40a	31.2±2.33b	29.68±2.41	0.001
WsL, cm	52.9±2.38a	59.9±2.94b	56.44±4.39	0.001
WL, cm	24.4±1.58a	28.2±1.52b	26.30±2.43	0.001
BL, cm	17.9±2.15a	19.4±2.08b	18.6±2.23	0.008
TL, cm	21.4±1.44a	24.2±1.74b	23.03±2.25	0.001
TC, cm	9.42±1.84a	11.8±1.59b	10.61±2.08	0.001
DL, cm	15.9±0.73a	18.8±0.97b	17.38±1.69	0.001
SL, cm	6.23±0.64a	7.7±0.65b	6.99±0.99	0.001

Mean followed by different letter in the same row differ significantly (P<0.05)

Carcass part yields of roosters mostly predominated more than (P<0.05) hens have (Table 2), while there was none-significant (P>0.05) difference in carcass proportions between genders (Table 3). Roosters BLW significantly heavier than hens (2,780g versus 1,803g) caused of significant higher in KW, DFW, CW (2,586, 2,420 and 1,911g versus 1730, 1610 and 1284g, respectively), however their proportions related to LW and CW maked insignificant (P>0.05) differce between genders. Seces significantly effected on weight of cut-up parts as WW, BW meat, TW meat, DsW in roosters significant greater than hens

did (257, 382, 478, 335g versus 165, 226, 377, 200g, respectively), thought cut-up parts in both proportions only showed significance on TW meat, others had insignificant affect between seces. There was only rooster LW of IO cut-up items significant heavier than hen did (50.7 versus 33.3g, respectively), nevertheless percentage of organs in parts insignificantly differed between gender.

4. DISSCUSSIONS

In this study, the ALW of MD chickens at slaughter was higher than 3 breed groups of crossbred native chicken as (Broilers+Layers) xChee (1,512g), (Shanghai+Layer)xChee (1385g) and (Shanghai Road Bar+Layer) xChee (1,506g) at 12 weeks of age in Thailand (Doungnapa *et al.,* 2016). Additionally, genders BW of MD chickens were better-growing than Ho breed (hen 1,125g and rooster 1,297g) (Bui and Nguyen, 2006), Ninh Hoa Ri breed (hen 1,196g and rooster 1,572g) (Nguyen *et al*., 2017), Noi chickens (hen 1,293g and rooster 1,588g) at 91 days old (Nguyen *et al*., 2021), Hac Phong chickens (hen 1,158g and rooster 1,449g) at 20 weeks of age (Nguyen *et al*., 2022a). Moreover, MD rooster was weight greater than Kadaknath cocks (1,707g) at 27 weeks old and broiler (1,762g) at 5 weeks old in India (Haunshi *et al*., 2022), little higher than Noi chicken (2,472g) and slight lower than Noi-Asil (2,743g) at 6 months old (Nguyen *et al*., 2022b). Therefore, the overall average dimensions of MD chickens was far longer than those of Noi chicken reported by Do *et al*. (2019) at 84 days old. In the present study, back length and thigh length were higher while CC and SL were lower than that of Noi and Noi-Asil recorded by Nguyen *et al*. (2022) at 6 months old. Those evidence indirectly prove the advantages of carcass traits of MD chicken and their potential for domestic chicken meat production industry for future.

Carcass characteristics were significantly affected by seces. The significant impact of gender on cut-up parts were also researched by Do *et al*. (2019); Nguyen *et al*., 2022a). Moreover, hybrid advantage of MD chicken was shown in higher most of carcass characteristics than Noi chicken was slaughtered at the same age (Do *et al*., 2019). Similar to the present study, Nguyen *et al*. (2022a) reported the BW and TW meat of Hac Phong male chicken were heavier than females at 20 weeks old. The differences between genders caused of physiology which could lead high nutrition in FCR of rooster than hen in similar rearing system (Almasi *et al*., 2012), the significant liver observed in rooster could due to higher FI, digestion of nutrients and metabolism that were required for fast-growing. However, the non-significant differences in abdominal fat between genders reported in this research was also mentioned by Rondelli *et al*. (2003).

Table 2. Carcass characteristics of MD chicken

Traits	Hens (n=6)	Roosters (n=6)	Mean (n=12)	P
LW	1,803±123^{a}	2,780±160^{b}	2,292±550	0.004
KW	1,730±122^{a}	2,586±80.2^{b}	2,158±478	0.004
DFW	1,610±122^{a}	2,420±98.5^{b}	2,015±455	0.003
CW	1,284±61.9^{a}	1,911±184^{b}	1,598±364	0.005
HW	42.1±1.56^{a}	69.2±5.35^{b}	55.67±15.19	0.013
NW	92.7±13.5^{a}	148±6.38^{b}	120.6±32.0	0.023
WW	165±1.89^{a}	257±5.35^{b}	211.6±50.2	0.001
BW	226±46.4^{a}	382±62.3^{b}	304.4±98.7	0.040
TW	377±32.9^{a}	478±31.5^{b}	427.5±62.0	0.019
DW	200±17.4^{a}	335±26.7^{b}	267.8±77.0	0.005
SW	66.7±7.91^{a}	121±3.04^{b}	94.1±30.5	0.008
IOW	194±48.9	285±42.9	239.5±64.6	0.094
GW	28.0±5.41	35.2±2.02	31.58±5.36	0.165
HW	6.33±0.58	9.17±2.02	7.750±2.04	0.145
LW	33.3±6.05^{a}	50.7±5.01^{b}	42.0 ±10.71	0.032
AFW	42.8±20.9	58.7±18.1	50.75±19.53	0.395
SIL,cm	126±27.6	143±14.9	134.5±21.93	0.418
CL,cm	14.2±1.76	17.1±1.13	15.63±2.07	0.094

Carcass proportion of MD chicken in the present research was not clearly effected by seces. There was only thigh meat percentage in female significant greater than male had. Thigh meat was the highest ratio BW which was proving MD chicken belong to the active group similar research of those in Hac Phong chicken have been found by Nguyen *et al.* (2022a). Moreover, Nielsen *et al.* (2003) mentioned that slow develop mental chicken

would have lower BW and higher TW than fast-growing chicken. MD had a greater cut-up ratio of BW, TW, WW and AFW in this study was higher than 3 crossbred native chickens in Thailand (Doungnapa *et al*., 2016).

Table 3. Difference of cut-up parts

Traits	Hen (n=6)	Rooster (n=6)	Mean (n=12)	P
Relative to LW (%)				
KW	95.93±0.88	93.20±4.72	94.56±550	0.381
DFW	89.25±1.05	87.14±3.41	88.20±2.54	0.364
CW	71.36±3.41	68.64±3.25	70.0±3.33	0.374
HW	2.35±0.18	2.50±0.32	2.42±0.25	0.509
WW	9.23±0.66	9.27±0.39	9.25±0.49	0.923
BW	12.68±3.19	13.71±1.54	13.19±2.31	0.642
TW	20.93±1.13[a]	17.18±0.24[b]	19.05±2.18	0.005
DsW	11.09±0.60	12.07±0.38	11.58±0.70	0.075
SW	3.70±0.4	6.55±3.70	5.13±2.83	0.255
IOW	10.67±1.92	10.22±1.03	10.44±1.40	0.737
GW	1.56±0.36	1.27±0.08	1.42±0.28	0.231
HW	0.35±0.05	0.33±0.08	0.34±0.06	0.728
LW	1.84±0.25	1.82±0.15	1.83±0.07	0.917
AFW	2.33±0.97	2.09±0.56	2.21±0.72	0.729
Relative to CW (%)				
HW	3.29±0.24	3.66±0.60	3.47±0.45	0.378
WW	12.93±0.76	13.55±1.22	13.24±0.97	0.500
BW	17.76±4.43	19.93±1.40	18.84±3.17	0.463
TW	29.34±1.14[a]	25.07±1.32[b]	27.20±2.59	0.019
DsW	15.58±1.46	17.61±1.09	16.60±1.60	0.126
SW	5.20±0.74	6.41±0.81	5.81±0.96	0.130
IOW	15.02±3.13	14.86±0.85	14.94±2.06	0.936
GW	2.20±0.53	1.85±0.21	2.02±0.41	0.355
HW	0.49±0.05	0.49±0.12	0.49±0.09	0.917
LW	2.60±0.46	2.65±0.16	2.63±0.31	0.840
AFW	3.30±1.46	3.03±0.69	3.16±1.03	0.785

5. CONCLUSIONS

This study performed male chicken of MD breed had great LW, body dimensions and carcass performants. Males has bigger size with heavier cut-up parts parameters and liver. However, carcass proportions was non-significant difference between seces, while thigh meat ration was significant greater compared to the male one. This results were contributing in breeding programs for management selection and a niche market serving who prefer low-fat, chewy meat, more meat and growth fast.

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ESTABLISHMENT AND CRYOPRESERVATION OF SAANEN GOAT FIBROBLAST CELL LINES

*Hoang Thi Au[1], Vu Thi Thu Huong[1], Nguyen Thi Lan Anh[1], Giang Thi Thanh Nhan[1], Pham Doan Lan[2] and Nguyen Khanh Van[1]**

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ABSTRACT

This study aimed to establish the Saanen goat fibroblast cell bank and evaluate the pre- and post-frozen growth t of Saanen goat fibroblast. Saanen goat ear tissues were collected from 6 Saanen male goats and 14 Saanen female goats. Their ear tissues were cut into small pieces ($1mm^3$) and cultured in DMEM supplemented with FCS for insolation fibroblasts. The results showed that the successful isolation rate of Saanen goat fibroblasts from ear tissue was 96.43%. Fibroblasts were passaged when reaching 80-90% confluence. Fibroblasts at the third passage were used for freezing-thawing process. The rate of straws containing Saanen goat fibroblast viability after freezing-thawing was 94.37%. Although frozen-thawed fibroblast cells took longer to start grower than non-frozen group, the time required for monolayer formation with 80-90% confluent of these groups was similar (5 days). The concentration of adherent fibroblasts of frozen group at 48 and 120hrs after thawing was lower than that of non-frozen group ($1.52x10^5$ vs $1.71x10^5$ cells/ml, $4.16x10^6$ vs $4.42x10^6$ cells/ml, respectively). The results showed that Saanen fibroblasts were successfully isolated and cryopreserved.

Keywords: *Saanen goat, cryopreservation, isolation, ear tissue fibroblast.*

1. INTRODUCTION

Animal genetic resources are an important determinant of maintenance of biodiversity in farm livestock (Davood *et al.*, 2016). If these animal genetic resources are not protected and lost forever then the thorough explanation of biological mechanisms, genetic stability, replicative of donor cells and the subsequent epigenetic reprogramming of their nuclei in the oocytes reconstructed by somatic cell nuclear transfer have not been completed (Guan, 2002). At present preservation livestock genetic resources like semen, oocytes, embryos and somatic cells are practical options. Cryopreservation semen, oocytes, embryos that require species-specific techniques and only be performed for a limited number of species and needs customized techniques for each species (Woolliams and Wilmul, 1999). Meanwhile somatic cells cryopreservation is a technique used for all animals, so somatic cells cryopreservation is an alternative option for maintaining genetic diversity in endangered animals (Corley-Smith and Brandhorst, 1999). With development of animal cloning technology in farm livestock species, cryopreservation of somatic cells has opened a new option for conserving valuable livestock. The establishment of fibroblast banking of valuable livestock and endangered animals will provide valuable experimental materials for biological research, somatic cloning, genomics, postgenomic and other fields of life sciences in the future (Chunyu *et al.*, 2012).

Fibroblasts isolated from ear tissues or fetal skin were used to develop a fibroblast cell bank. The establishment of the fibroblast cell banks for some pig breeds such as lợn Hmong, Táp Ná pig, Hung pig, Ỉ pig and so onwas reported by the Key Laboratory of Animal Cell Biotechnology of National

[1] Key Laboratory of Animal Cell Biotechnology
[2] National Institute of Animal Science
* Corresponding Author: Dr. Nguyen Khanh Van, Director of Key Laboratory of Animal Cell Biotechnology, National Institute of Animal Science, Tel: (84)988447907; Email: cotihin@gmail.com

Institute of Animal Science but no fibroblast goat banks. The Saanen goat is a Swiss breed of domestic goat and takes its name from the Saanetal in the Bernese Oberland, in the western Switzerland. The Saanen goat is a highly productive dairy goat and is distributed in more than 80 countries worldwide. The Saanen goats have been imported in Vietnam since 2002 and now they are growing well and healthy and contributing to milk. To preserve this valuable genetic resource, the purpose of this study was to establish a Saanen goat fibroblast cells bank and *in vitro* evaluation of fibroblast Saanen goat frozen-thawed. This study has laid the foundation for genetic resource preservation of other valuable goat breeds.

2. MATERIALS AND METHODS

2.1. Materials

A total of 20 Saanen goats (6 males and 14 females) were used to for isolation fibroblasts. All chemical used in this study were from Sigma-Aldrich, St. Louis, MO, USA.

2.2. Methods

2.2.1. Ear tissues collection, isolation and cell culture

Saanen goat ear tissues were collected from Saanen goats (6 males and 14 females) that were provided by the Thuc Thiem Goat Breeding Farm, Ba Vi, Hanoi, Vietnam. The ear tissues were cleaned with 70% alcohol, carefully dissected free from hair, fat. After that these samples were collected into separate tubes which contained DPBS (Sigma-Aldrich, St. Louis, MO, USA) supplement with 100 IU/ml penicillin (Sigma-Aldrich, St. Louis, MO, USA) and 100 µg/ml streptomycin (Sigma-Aldrich, USA) and aseptically brought to the laboratory within 2-3hrs. The skin samples were washed 5 times with DPBS then chopped into 1 mm^3 pieces, which were seeded onto the bottom surfaces of a tissue culture flasks containing DMEM and 10% fetal bovine serum (FBS) and cultured at 37°C in an incubator with 5% CO_2 and saturated air humidity. The culture flasks were left undisturbed for 24hrs to avoid the tissue from dislodging. Any culture flask found with microbial contamination was immediately discarded. When the cells reached 80-90% confluency, they were harvested using 0.25% Trypsin EDTA (Sigma-Aldrich, St. Louis, MO, USA) and divided into prepared culture flasks at 1:2 or 1:3 ratios.

2.2.2. Frozen Saanen goat fibroblast

Saanen goat fibroblasts were collected from culture flasks and counted with a hemocytometer before freezing. The harvested fibroblasts were re-suspended in freezing medium containing DMEM (Sigma-Aldrich, St. Louis, MO, USA) and 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) and 10% FBS (Sigma-Aldrich, St. Louis, MO, USA), to a final concentration between $3x10^6$ -$4x10^6$ viable fibroblasts/ml. Single fibroblasts were dispensed into sterile 0.25ml French straws labeled with species, gender, date. The straws were sealed with hot forceps. The sealed straws were initially kept at 4°C for 20-30min to allow the DMSO to equilibrate. Then the straws were kept at -80°C for 24hrs and subsequently transferred to liquid nitrogen.

2.2.3. Thawing Saanen goat fibroblast

To thawing Saanen goat fibroblasts, the straws were removed from liquid nitrogen and immediately thawed in a 37°C water bath for 10sec and transferred to 0.5M sucrose medium. The fibroblasts were collected by centrifugation at 200xg for 5min, and washed with culture medium and fibroblasts were transferred into flasks gently blown into uniform single cell suspension and cultured at 37°C and 5% CO_2. The medium was changed after 24hrs of thawing.

2.2.4. Recovery fibroblast by Trypsin solution

When the fibroblasts were 80-90% confluence, they were dissociated from culture flasks surface using 0.25% Trypsin EDTA solution (Sigma-Aldrich, St. Louis, MO, USA). The fibroblasts in flasks were carefully

washed 4 times using DMEM (Sigma-Aldrich, St. Louis, MO, USA). The fibroblasts were rinsed by gently shaking the flask, and the wash solution was discarded. The fibroblasts were incubated with 1ml Trypsin EDTA 0.25% for 5min at room temperature. When the fibroblasts were completely detached, 2ml culture medium was added to the flask, and the fibroblasts dispersed gently by pipetting up and down 2-3 times. These fibroblasts were purified and expanded by serial passaging.

2.2.5. Cell counting by Thoma cell counting chamber

The frame of the counting chamber contains a large central square and this large central square is divided into 16 medium squares, each with 25 small squares inside (9 of them are divided in half). When we put the sample under the coverslip, the cell suspension reaches a height of 0.1mm. Taking these data into account, and considering one of the large squares, the volume will be $1x1x0.1=0.1mm^3=10^{-4}ml$. All the cells in the 16 medium squares must be counted according to the following criteria: All the cells within each medium square and those that are over the top and right sides of the square (even when they are partially out) are counted. Following this approach, in the figure the cells in green will be counted, but not the cells in red. If we have counted N cells in one of the large squares (that is, in 16 medium squares), the concentration of our sample will be: $Nx10^4$ cells/ml. When prior to counting we concentrated or diluted the initial sample, we must take into account the concentration-dilution factor (f): $Nx10^4xf$ cells/ml.

2.3. Data analysis

The data was analyzed using ANOVA test.

3. RESULTS AND DISCUSSION

3.1. Isolation of the fibroblasts from Saanen goat ear tissues

Fibroblasts were observed sprouting from the margins of Saanen goat ear tissue pieces at about 5-7 days after these tissue explants adhered to the bottom of flasks and then these ear tissue pieces were removed and the medium was changed. After the piece of tissue was removed, the fibroblasts covered the bottom of the flasks within 3–5 days and formed a monolayer. The fibroblasts grew rapidly and gradually replaced the epithelial cells in the subculture. Morphology is the important qualitative parameter of epidermal tissue reconstitution was evaluated by light microscopy. In our study, the fibroblasts showed typical morphology such as rhomboid or stellate with centrally located oval nuclei (Figure 1), that is the characteristic shape of fibroblasts (Davood *et al.*, 2016). The result of Saanen goat fibroblasts isolation was shown in Table 1.

In this study, at Day 7 after the ear tissue adhered to the bottom of flasks, Saanen goat fibroblasts were developed from the ear tissues. The fibroblast isolation time in our study was shorter than that Liu *et al.* (2008). According to Liu *et al.* (2008), the Luxi cattle fibroblast could be seen migrating from the ear tissue pieces 12 days after the ear tissue adhered to the bottom of flasks. The difference between experimental results most likely due to the origin of fibroblast used in the study, experimental conditions, fibroblast culture environment.

Table 1. The result of Saanen goat fibroblasts isolation from ear tissue

Number of isolated flasks	Flasks with fibroblasts sprouting from ear tissue (n, %)	Flasks were infected or no fibroblasts sprouting from ear tissue (n, %)
84	81 (96.43%)	3 (3.57%)

Table 1 showed that the rate of flasks with fibroblasts sprouting from ear tissues was 96.43%. There are many reasons for the infection or absence of fibroblasts during fibroblast isolation, such as: experimental conditions, technician manipulation, the tissue sample itself was contaminated before isolation and culture.

To prevent this problem, during isolation we changed culture medium at Day 1 (24h

after the ear tissue adhered to the bottom of flasks. The purpose of changing culture medium was to stimulate cell growth, detect the bacterial and fungal contamination, removed dead cells and inhibitors and toxicity of dead cells secreted into culture medium. However, the change of medium on the Day 2 after the ear tissue adhered to the bottom of flasks probably led to the ear tissue being removed from the bottom of culture flask surface after addition of culture medium. In our study, we observed the Saanen goat fibroblast growth only around the adhered ear tissues. None of the floating ear tissue ever exhibited growth of Saanen goat fibroblasts in our study. According to Singh and Sharma (2011), 20-33% of the skin explants dislodged (floated) after addition of culture medium and growth of fibroblast could not be seen. The number of fibroblasts migrating around the individual ear tissues was not uniform. In some ear tissues, it was more than others.

3.2. *In vitro* growth performance of goat fibroblasts after frozen-thawed

In this study, *in vitro* growth performance of Saanen goat fibroblasts after frozen-thawed was evaluated based on the number straws has viable fibroblast, the time of growth initiation, the time of monolayer formation about 80-90% confluent and the concentration of adherent fibroblasts at these stage (80-90% confluency). In our study, Saanen goat fibroblasts were frozen-thawed as described by Gao *et al.* (2013), the fibroblast density was $1x10^6$ fibroblast/ml before cryopreservation, these fibroblast density is suitable for freezing (Singh and Sharrma, 2011). The Saanen goat fibroblasts were frozen at 3rd passage. The medium was changed at 24hrs after thawing to remove dead cells.

In our study, we evaluated 102 straws that had viable fibroblast at time 24h after frozen-thawed based on the fibroblasts attached to the bottom of the flask, growing and not infected by bacteria or fungus. The results showed that the percentage of straws that had viable fibroblast after frozen-thawed was 94.37%. This percentage was higher than those reported by Gajda *et al.* (2007). According to Gajda *et al.* (2007), the percentage of samples that had viable bovine fibroblast after frozen-thawed was 87.5%. The cause likely due to the quality of fibroblast and the origin of fibroblasts. Gajda *et al.* (2007) found that with same concentration of cells before freezing (1×10^6 cells/ml), the viability after thawing of cumulus cells from matured oocytes group was higher than that of cumulus cells from immature oocytes group and fibroblast group (100% vs 62.5 and 87.5%, respectively). The harvested viable fibroblasts after frozen-thawed were cultured in the culture medium DMEM and FBS at 37°C with 5% CO_2 in a humidified incubator for evaluation of development *in vitro*. The control group was fresh fibroblast. These results were shown in Table 3.

In this study, within 9-10hrs of primary culture setup, the Saanen goat fibroblast frozen-thawed started growing and on Day 5 the 80-90% confluence formation occurred (Table 3). The results in Table 2 showed that the time of fibroblast started growing of frozen-thawed group was longer than that of control group (9-10hrs and 7hrs, respectively, Fig 2). There was no difference in the morphology of fibroblast goat of frozen-thawed group and control group. The Saanen goat fibroblasts morphology was indistinguishable from fibroblasts observed prior to cryopreservation, and they proliferated normally and made monolayer cultures. Although the time of growth initiation of frozen-thawed group was later than that of control group but no difference in the time of monolayer formation about 80-90% confluent between these groups (5 days, Table 2).

In vitro growth performance of Saanen goat fibroblasts after thawing was evaluated based on the concentration of adherent fibroblasts (viable fibroblasts) at 48 and 120hrs after thawing. These results were shown in Table 3.

Table 2. Growth performance of Saanen goat fibroblasts frozen-thawed and fresh fibroblast

Goat fibroblasts	Morphology of fibroblast goat	Growth performance	
		Growth initiation	Monolayer formation about 80-90% confluent
Frozen	Normal	9-10 hours	5 days
Non frozen	Normal	7 hours	5 days

Table 3. The *in vitro* growth ability of Saanen goat fibroblast after thawing

Fibroblast	The concentration of adherent Saanen goat fibroblast after thawing	
	48hrs	120hrs
Frozen	$1,52x10^5$	$4,16x10^6$
Fresh	$1,71x10^5$	$4,42x10^6$

In this study, the concentration of Saanen goat fibroblast before culture of frozen-thawed group and control group were similar, at a density of $2.5x10^4$ fibrolblasts/ml, this density is suitable for *in vitro* culture (Singh and Sharrma, 2011). The results in Table 3 showed that at 48hrs after thawing, the density of adhered fibroblasts (viable fibroblast) of frozen group and control group reached the confluency stage. According to our results, at 120hrs after thawing, the density of adhered fibroblasts of the frozen group was lower than that of control group ($1.52x10^5$ fibroblasts/ml and $1.71x10^5$ fibroblasts/ml, respectively). The Adherent fibroblasts reason of this decrease is freezing-thawing resulted in damage in the fibroblasts viability. At 120 hours after thawing, Saanen goat fibroblasts of frozen-thawed fresh group ($4.16x10^6$ fibroblasts/ml and $4.42x10^6$ fibroblast/ml, respectively). This finding was similar to the results reported by Davood *et al.* (2016). According to Davood that *et al.* (2016), at the 5 days after thawing, the concentration of goat fibroblast was lower than of the control group ($6x10^5$ fibroblasts/ml vs $8x10^5$ fibroblasts/ml, respectively). In this study, although at 120hrs after thawing, the concentration of adhered fibroblasts of the frozen group was lower than that of the fresh group, but these density is suitable for cryopreservation (Singh and Sharrma, 2011).

Cryopreservation fibroblasts is an option for *in vitro* conservation of genetic animal resources (Corley-Smith and Brandhorst, 1999). Fibroblasts cryopreservation for every species is a cheap and fast way, and the method of choice for the rapid creation of gene banks. When explanting ear tissues to derive new primary cultures, epithelial and fibroblast cells would initially grow together. Fibroblasts can be trypsinized and adhered more easily to the bottom of the culture flasks and more readily than epithelial cells (Ren *et al.*, 2002). Because of these characteristics, fibroblast cells would quickly outgrow their epithelial counterparts. That's why according to Li *et al.* (2009), a culture of pure fibroblasts may be obtained at 3rd passage or more. According to Davood *et al.* (2016), the genetic characteristics of the fibroblasts can be changed by *in vitro* culture conditions after many passages, so a minimal number of passages are recommended to conserve them. Therefore, in our study, we used the Saanen goat fibroblasts at 3rd passage for cryopreservation Saanen goat fibroblast.

These results showed that Saanen goat fibroblasts successfully cryoconservation using a freezing medium containing 10% DMSO. DMSO, cell penetrating cryoprotectant, is commonly used to cryopreserve animal cells and protect them from intracellular formation of ice crystals (Chaytor *et al.*, 2012). Dimethyl sulfoxide is frequently used in cell banking applications as a cryoprotectant. DMSO was first used in cryopreservation of bovine embryos in 1973 (Wilmult và Rowson, 1973). When added to media, DMSO prevents intracellular and extracellular crystals from forming in cells during the freezing process. Without DMSO, these crystals cause cell death,

thus rendering the cells useless for transplant. For most cryopreservation applications, DMSO is used at 10% concentration and is usually combined with FBS.

4. CONCLUSION

In conclusion, a fibroblasts culture was established from ear tissue of the Saanen goat using standard tissue adherent culture and continuous passaging following trypsinization. This simple method of isolating and culturing highly proliferative fibroblasts from small samples of Saanen goat outer skin biopsies and their efficient cryopreservation as demonstrated here, along with the Saanen goat fibroblasts lines established in our study, should be useful in future.

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IMPROVING RABBIT SPERM QUALITY DURING COLD STORAGE PRESERVATION THROUGH CYSTEINE SUPPLEMENTATION

Tran Thi Thanh Khuong[1], Tran Thi Nga[1], Nguyen Lam Khanh Duy[1] and Truong Thanh Trung[2]*

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ABSTRACT

The role of a cold storage preservative is crucial in maintaining the quality of sperm by protecting and reducing damage caused during cold storage. The aim of this study was to determine

[1] Institute of Food and Biotechnology, Stem Can Tho University, Can Tho City
[2] College of Agriculture, Can Tho University, Can Tho City
* Corresponding Author: Tran Thi Thanh Khuong, Stem cell Laboratory, Institute of Food and Biotechnology, Can Tho University, Can Tho City, Viet Nam. Tel: (84)901002285; Email: tttkhuong@ctu.edu.vn

the appropriate concentration of cysteine to supplement the preservation environment for the best quality sperm, as well as to identify the optimal cold storage time for sperm at a temperature of 15°C. The experiments were conducted on three local crossbred male rabbits, with each experiment repeated nine times. Rabbit semen was collected using an artificial vagina and diluted with TCG preservation medium supplemented with cysteine at four concentrations (0, 1.25, 2.5 and 5mM) and stored at 15°C. The experiments were observed at intervals of 0, 6, 12, 24, 48, and 72hrs. The results showed that rabbit sperm achieved the best quality when supplemented with 2.5mM cysteine during cold storage. The results of the progressive motility of sperm at each time were as follows 0, 6, 12, 24, 48, and 72hrs were 63.88, 60.99, 55.42% 47.52, 34.29, and 16.67%, respectively. The sperm survival rate at each time were as follows: 0, 6, 12, 24, 48, and 72hrs were 84.33, 80.82, 77.17, 73.04, 67.24 and 58.81%, with statistically significant differences compared to the remaining experimental groups (P<0.05). The study results indicate that supplementing the TCG preservation medium with 2.5mM cysteine significantly improves the quality of sperm during cold storage.

Keywords: *Liquid storage, sperm, rabbit, Cysteine, artificial insemination.*

1. INTRODUTION

Rabbit farming has become increasingly important in the Mekong Delta of Vietnam due to its potential to provide a sustainable source of income for farmers in the region. This is especially important considering the challenges faced by the agriculture sector, such as climate change, soil degradation, and population growth (Silagadze, 2022). Rabbits are ideal livestock for small-scale farmers in the delta, as they are able to digest up to 70% of green roughage, making them a cost-effective and sustainable source of protein. Additionally, rabbits are easy to raise and adaptable to different climates. As a result, the initial costs of rabbit farming are low, and the breeding cycle allows for quick returns on investment (Lukefahr, 2007). Rabbit meat is also a highly competitive product in the market, with low cholesterol levels and suitability for all age groups (Thu and Dong, 2011). Moreover, rabbit products have potential in the pharmaceutical industry, such as producing vaccines, surgical sutures, and antibodies (Ros *et al.*, 2020). In order to ensure the success of artificial insemination in rabbit breeding, it is crucial to understand the factors that affect the fertility of bucks. Sperm quality, especially sperm motility, is one of the most important factors affecting artificial insemination success. Liquid semen strorage is a useful method capable of preserving sperm for short periods at low temperatures (15-17^{0}C). However, the quality of the spermatozoa is extremely affected during liquid storage. Spermatozoa contain large amounts of unsaturated fatty acids in the plasma membrane, making them highly susceptible to reactive oxygen species (ROS) stress (Wang *et al.*, 1997). Unfortunately, the process of liquid sperm storage lead to accumulation of ROS. ROS may cause lipid peroxidation (LPO) in cell membranes (Sharma and Agarwal, 1996), which is associated with damage such as extensive structural alterations, irreversible loss of motility, a profound change in metabolism, and a high rate of leakage of intracellular cell constituents (Jone and Mann, 1977). Therefore, addition of antioxidants to storage extender may prevent spermatozoa from oxidative stress.

Cysteine, one component of glutathione, contains thiol groups and may penetrate the plasma membrane easily (Coyan *et al.*, 2011). Uysal and Bucak (2007) reported that Cysteine enhanced intracellular glutathione (GSH) biosynthesis and protected the proteins, DNA and membrane lipids because of the direct radical-scavenging ability of GSH. It has been shown that Cysteine protects spermatozoa against cryo-damage in boars (Kaeoket *et al.*, 2010) bulls (Tuncer *et al.*, 2010), buffalos (Topraggaleh *et al.*, 2014) goats (Memon *et al.*, 2012), cats (Thuwanut *et al.*, 2008), and rams (Sharafi *et al.*, 2015). Besides that, cystein was added to semen diluent and evaluated

semen quality parameters (Khan *et al.*, 2021). In addition, Cysteine was added to freezing extender for protecting rabbit spermatozoa against reactive oxygen species-induced damages (Zhu *et al.*, 2017). However, there are no research on the effects of Cysteine on liquid storage of local rabbit semen in the Mekong Delta of Vietnam. Therefore, this study was carried out to find out the optimal concentration of Cysteine added to the storage medium for the best sperm quality and to determine the optimal storage time at 15^0C.

2. MATERIALS AND METHODS

2.1. Animals

The study was carried out on 3 local crossbreeding does weighing between 2.5-3.4kg at the animal experiment farm of the Stem Cell Laboratory, Can Tho University. The rabbit were fed with a recommended nutritional diet from the Department of Animal Sciences, College of Agriculture, Can Tho University. The animals were individually housed in flat-floor cages at the animal experimental farm and exposed to a photocyclic cycle of 16hrs of light and 8hrs of darkness. They were provided with a standard diet and a sufficient supply of drinking water. All animals were fully vaccinated against hemolytic diseases and parasites, and the study was conducted with ethical approval for animal care, housing, and semen collection procedures under the Animal Welfare Assessment (BQ2022-02/VCNSHTP).

2.2. Experimental design

Semen samples were collected from 3 healthy male rabbits between the ages of 8 and 12 months old at the animal experiment farm of the Stem Cell Laboratory, Can Tho University.

Semen samples were diluted with TCG medium supplemented with Cysteine at 4 concentrations of 0mM; 1.25mM; 2.5mM; 5mM in a dilution ratio of 1:10 and stored at 15^0C. The samples were evaluated for quality (mobility, viability, and membrane integrity) according to each time point of 0, 6, 12, 24, 48 and 72hrs. The experiment had 4 treatments, each treatment was repeated 9 times.

2.3. Rabbit semen collection

Rabbit semen was collected using an artificial vagina, stimulated by a doe. Samples were incubated at 37°C and evaluated within 15 minutes for macroscopic characteristics (pH and volume) and semen quality (sperm concentration, motility, viability, and membrane integrity). Semen was collected twice a week for four months using a warmed (40-42°C) artificial vagina lubricated with gel. The collected semen was evaluated for macroscopic characteristics (pH and volume) and semen quality, including sperm concentration, motility, viability, and membrane integrity (Cathy *et al.*, 2013).

2.4. Sperm concentration

The counting chamber was fixed at room temperature for 4 minutes after drawing up 9µL of the sample. Using a microscope with a magnification of 40x, at least 200 intact spermatozoa were counted per counting chamber. To prevent duplicate counting of spermatozoa in adjacent squares, spermatozoa lying on the dividing line between 2 squares were counted once, and spermatozoa with heads lying on the dividing line above and to the left of the square were counted. The WHO guidelines were followed to calculate the sperm count (Ros *et al.*, 2020).

2.5. Sperm motility

Two wet mounts with a depth of about 20µm were prepared on a counting chamber for each sample. The motility of all spermatozoa in the same location in the field was evaluated based on three types: progressive motility (PR), non-progressive motility (NP), and immotility (IM). A random counting area was chosen, avoiding areas where only motile spermatozoa were visible. A quick assessment was performed on any field without waiting for spermatozoa to swim into the evaluation area. At least 200 spermatozoa in at least 5

fields in each wet mount were counted. The count was performed twice on two different wet mounts, and the results of the two wet mounts were compared. If the difference in the percentage of samples was within the acceptable range, the average was calculated for each motility classification (PR, NP, and IM) (Fumuso *et al.*, 2018).

2.6. Sperm viability

The viability of sperm was measured by the Eosin-Nigrosin method (Agha *et al.*, 2014). To evaluate sperm viability, 50µl of a semen sample was mixed with 50µL of Eosin-Nigrosin solution and allowed to stand for 30 seconds. The sample was then placed on a glass slide and allowed to air dry. Using a microscope, 100 spermatozoa were counted and evaluated. Live spermatozoa appeared white or only stained red or dark pink in part of the neck region, while the rest of the head was unstained. Dead spermatozoa had a reddish or dark pink head region. The percentage of live spermatozoa was calculated.

2.7. Sperm membrane integrity

The test was assessed using the Hypo-Osmotic Swelling Test (HOS Test). A total of 20µl of semen sample was mixed with 80µL of HOS solution in an Eppendorf tube and placed in an incubator at 37°C. The samples were evaluated after 40 minutes of incubation. After incubation, 10µl of the mixed sample was placed on a glass slide and observed under a microscope. Spermatozoa with intact membranes showed swelling in the tail, while those with damaged membranes did not show swelling (Luong and Thu, 2005).

2.8. Statistical analysis

Data analysis was performed using Excel (2016) and the Minitab (2016). The main factor examined was the effect of Cysteine concentration. ANOVA was used for analysis, with mean comparison between treatments analyzed using the Turkey method in the Minitab (2016). Results are presented as Mean ± standard error (SE). Statistical significance was set at $P<0.05$.

3. RESULTS

3.1. Fresh semen quality

The quality of fresh sperm is an important factor determining the quality of samples during testing and storage. At each collection, sperm samples were evaluated for sperm volume, pH, and concentration.

The results of evaluateing the quality of fresh sperm after collection are shown in Table 1. The data in Table 1 shows that the pH and volume of the semen remained consistent at 6.91±0.05 and 0.78±0.07ml, respectively. The concentration of cells/ml was 1379.4±20.7*10^6 cells/ml. The value of pH, volume and concentration of sperms in between quality was according to WHO criteria (2021). If the semen has a pH lower or higher than about 6.8 to 7.2, it is abnormal semen, not good for the vitality and ability of sperm to fertilize. Similarly, the concentration also affects the sperm's ability to fertilize, the measured concentration has a normal value in accordance with the experimental survey criteria.

Table 1. Quality of fresh sperm (Mean±SEM)

Evaluation criteria	Result
pH	6.91±0.05
Volume (ml)	0.78±0.07
Concentration (*10^6 cells/ml)	1379.4±20.7

3.2. Effect of Cysteine concentrations (0, 1.25, 2.5, 5mM) on rabbit sperm quality stored at 15^0C

Sperm samples were stored with TCG medium supplemented with Cysteine at 15^0C. Sperm quality was assessed at each specific time (0, 6, 12, 24, 48 and 72hrs).

3.2.1. Sperm motility

The results of evaluating the effect of Cysteine concentrations on the sperm motility over time are shown in Table 2. The data in Table 2 showed that the overall motility and the progressive motility of sperm decreased gradually with storage time. Specifically, after 24hrs of storage, the overall motility of sperm was reduced by 22.19, 22.38, 10.96 and 20.38%

in the treatments supplemented 0, 1.25, 2.5 and 5mM Cysteine, respectively. After 72hrs of storage, the overall motility of sperm was reduced by 41.28, 40.22, 29.94 and 38.72%, in the treatments supplemented 0, 1.25, 2.5 and 5mM Cysteine, respectively.

The treatment supplemented 2.5mM Cysteine showed the best overall and progressive motility of rabbit spermatozoa and significantly different from other treatments during the storage (P<0.05). The overall motility and the progressive motility of sperm in the treatment supplemented 1.25 and 2.5mM Cysteine were not statistically significant when compared with the treatment without Cysteine (P>0.05). According to WHO standard (2021), the sperm motility ratio at the treatment supplemented 2.5mM Cysteine for 24hrs still ensures the standard quality.

Table 2. Sperm motility (Mean±SEM)

Evaluation criteria	Time point	Cysteine concentration			
		0mM	1.25mM	2.5mM	5mM
Overall motility (%)	0	76.88^{ab}±0.533	76.6^{b}±0.580	78.51^{a}±0.376	75.48^{b}±0.276
	6	69.68^{b}±1.017	70.15^{b}±1.099	75.77^{a}±0.757	69.64^{b}±0.978
	12	63.52^{b}±1.133	64.60^{b}±1.124	72.01^{a}±0.788	63.76^{b}±1.564
	24	54.69^{b}±1.282	54.22^{b}±1.537	67.55^{a}±0.886	55.10^{b}±1.262
	48	45.50^{b}±1.341	44.99^{b}±1.502	58.42^{a}±1.408	46.01^{b}±1.498
	72	35.60^{b}±0.820	36.38^{b}±0.847	48.57^{a}±0.706	36.76^{b}±0.817
Progressive motility (%)	0	61.31^{a}±0.862	61.87^{a}±0.847	63.88^{a}±1,236	60.53^{a}±1.080
	6	55.57^{b}±0.982	55.63^{b}±1.043	60.99^{a}±1.186	54.49^{b}±1.192
	12	48.65^{b}±1.056	48.99^{b}±1.039	55.42^{a}±1.034	46.05^{b}±1.185
	24	39.23^{b}±0.948	39.74^{b}±1.033	47.52^{a}±1.662	37.28^{b}±0.980
	48	26.57^{b}±1.428	28.09^{b}±1.413	34.29^{a}±1.488	25.80^{b}±1.759
	72	9.15^{c}±0.266	11.41^{b}±0.608	16.67^{a}±0.686	8.63^{c}±0.427

Note: In the same row, values followed by lowercase letters are significantly different (P<0.05).

3.2.2. Sperm viability

The results of evaluating the effect of Cysteine concentrations on the sperm viability over time are shown in Table 3. The data in Table 3 showed that the viability rate of sperm decreased gradually with the storage time. Specifically, after 24hrs of storage, the viability rate of sperm was reduced by 19.37, 19.69, 11.29 and 18.68% in the treatments supplemented 0, 1.25, 2.5 and 5mM Cysteine, respectively. After 72hrs of storage, sperm viability was reduced by 44.65, 37.66, 25.52 and 35.01% in the treatments supplemented 0, 1.25, 2.5 and 5mM Cysteine, respectively.

The treatment supplemented 2.5mM Cysteine showed the best viability rate of sperm and was statistically different from the rest of the treatments during storage (P<0.05). The viability rate of sperm in the treatment supplemented 1.25 and 5mM Cysteine concentrations was not statistically significant when compared with the treatment without adding cystein to the TCG storage medium (P>0.05). According to WHO standards (2021), the viability rate of sperm at treatment supplemented 2.5mM Cysteine for 24hrs still ensures the quality meets the standards.

Table 3. Sperm viability over time (Mean±SEM)

Time point	Cysteine concentration			
	0mM	1.25mM	2.5mM	5Mm
0	81.51^{b}±0.633	82.30^{ab}±0.846	84.33^{a}±0.634	80.65^{b}±0.678
6	75.37^{b}±0.580	75.53^{b}±0.573	80.82^{a}±0.721	74.47^{b}±0.691
12	68.55^{b}±0.804	68.83^{b}±0.879	77.17^{a}±0.687	67.80^{b}±1.130
24	62.14^{b}±1.277	62.61^{b}±1.587	73.04^{a}±1.163	61.79^{b}±1.427
48	54.1^{b}±1.159	53.78^{b}±1.387	67.24^{a}±1.110	53.30^{b}±1.278
72	44.65^{b}±1.047	44.64^{b}±0.897	58.81^{a}±1.047	45.64^{b}±1.259

3.2.3. Cell membrane integrity

The results of the evaluation of the effect of Cysteine concentrations on the cell membrane integrity of the sperm over time are shown in Table 4. The data in table 4 showed that the sperm cell membrane integrity rate gradually decreased with the storage time. Specifically, after 24hrs of storage, sperm cell membrane

integrity was reduced by 11.5, 11.93, 6.52 and 11.1% in the treatment supplemented 0, 1.25, 2.5 and 5mM Cysteine, respectively. After 72hrs of storage, the percentage of sperm with HOS reaction was reduced by 20.27, 20.89, 13.01 and 14.55% in the treatment supplemented 0, 1.25, 2.5 and 5mM cystein, respectively.

The treatment supplemented 2.5mM Cysteine showed the best sperm cell membrane integrity rate and was statistically different from the rest of the treatments during the storage ($P<0.05$). The sperm cell membrane integrity in the treatment supplemented 1.25 and 5mM Cysteine concentrations was not statistically significant when compared with the treatment without adding cystein to the TCG storage medium ($P>0.05$). According to WHO standards (2021), the cell membrane integrity of sperm at the treatment supplemented 2.5mM for 24hrs still ensures the quality meets the standards.

Table 4. Membrane integrity over time
(Mean±SEM)

Time point	Cysteine concentration			
	0mM	1.25mM	2.5mM	5mM
0	53.40^{b}±1.426	53.96^{ab}±1.291	58.76^{a}±1.221	52.10^{b}±1.312
6	50.31^{b}±1.349	50.12^{b}±1.411	56.97^{a}±1.301	48.85^{b}±1.454
12	46.64^{b}±1.349	46.87^{b}±1.395	55.03^{a}±1.280	45.42^{b}±1.067
24	41.96^{b}±0.868	42.03^{b}±1.129	52.24^{a}±1,212	41.00^{b}±0.774
48	37.55^{b}±0.961	38.07^{b}±1.080	49.20^{a}±0.938	33.45b±0.708
72	33.13^{b}±0.660	33.07^{b}±0.650	45.75^{a}±0.729	37.55^{b}±0.413

4. DISCUSSION

In general, the quality of rabbit sperm decreased gradually with storage time. The results show that the storage medium supplemented Cysteine can help improve sperm quality after storage. The treatment supplemented 2.5mM Cysteine after 24hrs of storage, the rate of overall sperm motility was 67.55% and the rate of progressive sperm motility was 47.52%. When increasing the Cysteine concentration to 5mM, the rate of sperm motility was significantly reduced, which proves that too high Cysteine concentration will adversely affect the motility of rabbit sperm. The research results show that the addition of Cysteine concentration 2.5mM into TCG medium will significantly improve sperm quality, which is completely consistent with the study of Chanapiwat and Kaeoket (2021). The treatment supplemented 2.5mM Cysteine had the most positive effect on liquid storage of rabbit sperm. The treatment supplemented 2.5mM Cysteine improved the viability and cell membrane integrity of the sperm, specifically the viability of sperm in the treatment supplemented 2.5mM Cysteine after 72hrs of storage decreased only about 25% and the rate of membrane integrity decreased 13%. While in other treatments, after 72hrs of storage, sperm viability was reduced by 35-45% and membrane integrity was reduced by 15-20%. The results of this study are also completely consistent with the study of Gungor *et al.* (2017) when studying the effect of Cysteine and trehalose on sheep sperm.

This study provides evidence that exposure of rabbit spermatozoa to Cysteine improved sperm motility, sperm viability and membrane integrity, and that this protective role was related to the prevention of ROS accumulation. As a key antioxidant, glutathione protects cellular components from ROS attack (Pompella *et al.*, 2003). The glutathione content increased when spermatozoa were exposed to Cysteine, suggesting that Cysteine enhances the ability of spermatozoa to synthesize glutathione during oxidation stress (Zhu *et al.*, 2017).

Mammalian sperm plasma membranes contain an extraordinarily high concentration of polyunsaturated lipids, making them extremely susceptible to free radicals, which leads to lipid peroxidation (LPO) (Sharma and Agarwal, 1996). LPO results in loss of membrane integrity and fluidity increased permeability (Ohyashiki *et al.*, 1988). This can lead to impaired motility, and abnormal morphology. Peroxides are the most pernicious of the metabolic free radicals among which H_2O_2 is formed in highest quantities. H_2O_2 can move easily through different compartments and attack molecules

within the cells. It has been shown that H_2O_2 is the primary ROS responsible for the human sperm damage (Aitken *et al.,* 1993) and ROS was major generated in sperm mitochondria (Zhu *et al.,* 2015). The addition of Cysteine to TCG extenders solution scavenged ROS and H_2O_2. Taken together, Cysteine may enhance the synthesis of glutathione which is an antioxidant, and also stimulate the activity of glutathione peroxidase. Therefore, addition of Cysteine protects spermatozoa from ROS attack during liquid storage.

The study on liquid storage of rabbit sperm had both strengths and limitations. On one hand, the study showed the effects of Cysteine through baseline assessments, thereby confirming the role of Cysteine in improving the motility, viability and integrity of the rabbit sperm membrane. On the other hand, the limitation of this study is that other indicators such as acrosome activity status and DNA fragmentation are needed to provide a more detailed view of the effects of Cysteine. Besides, it is necessary to expand the experimental animal population to more closely evaluate the influence of Cysteine on the sperm quality of other rabbit breeds when refrigerated for a long time.

In conclusion, the study highlights the importance of storage time and Cysteine on the quality of rabbit sperm stored at 15^0C. The optimum Cysteine concentration was 2.5mM, which helped sperm retain the best motility, viability rate and membrane integrity when compared with other concentrations ($P<0.05$). The treatment supplemented 2.5mM Cysteine also helps sperm to maintain quality according to WHO standards (2021) after 24hrs of storage. However, to ensure the best rate of insemination on rabbits, it should be carried out as soon as possible after semen collection to avoid damage and injury caused by storage.

5. CONCLUSION

TCG storage medium supplemented with 2.5mM Cysteine showed a significant beneficial effect on improving sperm quality during storage. The optimal storage time is 24hrs.

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EFFECTS OF PRE-TREATMENT AND DRYING TEMPERATURE ON PHYSICO-CHEMICAL PROPERTIES OF CARROT PEEL POWDER

Nguyen Tuyet Giang[1,2] and Le Thi Thuy Hang[1,2]*

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ABSTRACT

The study was conducted to evaluate the effects of pre-treatment (treated and un-treated) and drying temperature (50, 60 and 70°C) on some physico-chemical properties and color values of carrot peel powder. The six treatments yielded dehydrated products with different carotenoids contents and color attributes. It was seen that all treatments at higher temperature were dried faster than those at lower ones. However, there is a non-significant difference in drying yield among the six treatments, which was about 10% in average. It is also noted that the carotenoids content was strongly affected by the treatments, particularly the drying temperature. The highest carotenoids content was found in treated sample dried at 50°C (713.33 µg/gDW) while the lowest value was shown in un-treated sample dried at 70°C (86.89 µg/gDW). Regarding the color changes, the results shows that all color values are significantly different among the treatments. Due to the increased drying temperature, the L^* increased significantly, while reductions in the a^* and b^* values were observed in both treated and un-treated samples. In addition, the lowest ΔE^* value was noted for treated samples when dried at 50°C (10.42), followed by un-treated samples dried at 50°C (11.56) and highest value was noted for un-treated sample dried at 70°C (18.61). Consequently, it showed that treated carrot peel samples dried convectively at 50°C were preferable. Overall, these samples retained quality attributes of carrot peel recording minimum drying time, higher total carotenoids and minimum color changes.

Keywords: *Carotenoids, carrot peel, color change, hot-air drying, pre-treatments.*

1. INTRODUCTION

In recent years, abundant agicultural by-products, residues or wastes from the food processing industry has been considered because of their potential biological properties and applicability (Nguyen and Scarlett, 2016). Carrot (*Daucus carota*), classified as a root vegetable, has been known as one of popular vegetables due to it is rich in carotenoids and anthocyanindins, which display potential biological properties, particularly antioxidant and anticancer activities (Bozalan and Karadeniz, 2011; Al-Amin *et al*., 2015). Annually, about 5.3 million tonnes carrot were produced for fesh consumption or processing industry, also indicating that there is a huge amount of carrot peel-the main residue from carrot root (Nguyen and Scarlett, 2016). This waste is still rich in organic compounds which associates with increased risks of environment pollution when it is directly discarded to the landfills. Therefore, carrot peel could be used as potential source for extraction of valuable bioactive compounds and applied in functional food manufactures or other industries, including cosmetic, medicines and pharmaceuticals (Wanna, 2019). The cull carrot or carrot waste (such as carrot peel, carrot top and carrot pomace) that cannot be used for human consumption can be utilized as feed for animal (Wadhwa and Bakshi, 2013; Bakshi *et al*., 2016; Yitbarek, 2019). Particularly, the color of egg-yolk can be enhance by adding carrot with carotenoids, a natural pigment source (Spasevski *et al*., 2018). However, with high moisture content, fresh carrots are sensitive to microbial spoilage,

[1] An Giang University, An Giang, Vietnam

[2] Vietnam National University Ho Chi Minh City, Vietnam

[*] Corresponding Author: Dr. Nguyen Tuyet Giang, Department of Animal Science and Veterinary medicine, An Giang University, An Giang province, Vietnam. Tel: (84)902719021, E-mail: ntgiang@agu.edu.vn.

even when refrigerated and under cold storage conditions; therefore, drying is the oldest and most popular processing technique to prolong the shelf life of carrots (Lau *et al.,* 2018; Nguyen and Le, 2018; Alam *et al.,* 2018).

In freshly vegetable, drying process reduces water content, making the material becomes easier to handle and prevents the microbial degradation. However, over drying might result in a loss of flavor, color and bioactive compound whereas inadequate drying gives rise to microbial infection (Zhang *et al.,* 2017). Different materials require different drying temperatures, depending on their desired quality attributes. The most commonly temperatures applied for convective hot air drying were reported to be in the range of 50-90°C (Krokida and Maroulis, 2000). Minimizing quality losses of carrots during drying is nessesary for quality control of the final product. Such studies have analyzed convective drying of different varieties of carrots with diverse pre-treament conditions, which have lead to result variation (Al-Amin *et al.,* 2015; Lau *et al.*, 2018; Saleh *et al.*, 2020). To date, there has been few findings on the optimal drying conditions for carrot peel. Therefore, the aim of this study was to investigate the effect of pre-treatment and drying temperature on drying yield and dynamic quality changes, such as color and total carotenoids content. The results could be fundamental in the quality-oriented innovative strategy for the processing of carrot peel.

2. MATERIALS AND METHODS

2.1. Determination of mass proportion of carrot root

Carrot roots (cultivated in Lam Dong-Da Lat) were purchased from the local market and immediately transported to the laboratories at the An Giang University. Ten carrot roots were randomly selected to determine their total weight prior to separation into the head, peel and flesh. Each part was individually weighed by digital scale to calculate their proportions.

2.2. Pre-treatment and drying process and measurements

The carrot peel was divided into two portions, where half was treated by 2 minute-soaking in salt solution (5% NaCl) followed by blanching in hot water for 5min, and the other half was left un-treated. After blanching, the samples were drained to remove excess water. Each peel portion was subdivired into three parts and subjected to drying at 50, 60 and 70°C under hot air condition in a Yamato DKN812 forced convection oven (Yamato Co., Ltd., Japan). About 300g of the carrot peel was evenly spread on a perforated wire tray (50×35cm). The moisture contents of the samples were determined at 30 minute-interval until they attained a final moisture content of about 5-7%, as suggested by Al-Amin *et al.* (2015). The drying time was recorded for each experiment using a timer.

The experiment with these six treatments was completely randomized designed and run in triplicate. The dried samples were then milled and stored in plastic containers under refrigeration conditions (2-4°C) for further analysis.

After drying, the samples were reweighed to determine their drying yield (%). Moisture of fresh carrot peel and the peel powder during drying were determined based on the Association of Official Analytical Chemists (AOAC, 2005) official methods of analysis using a Yamato DX602 hot-air oven (Yamato Co., Ltd., Japan) at 105◦C, until reaching 7% or below.

The total carotenoids were analyzed at 436nm by using spectrophotometric method, followedAOACOfficialMethod941.15(AOAC, 2000). Samples were prepared and analyzed in triplicate. Color measurements were done using a Minolta chroma meter (Minolta, CR-400, Japan). The chroma meter was calibrated against a standard white reference tile D65 (L^*=94.20, a^*=-0.02 and b^*=9.77) prior to sample measurements. L^* (lightness), a^* (redness) and b^* (yellowness) values from the CIE

(Commission Intemationale de 1'Eclairage) color scale (CIE, 1978) were determined. The color values of sample were the average of five readings on different sites in the surface. The total differences in color (ΔE^*) was calculated according to the following equation.

$$\Delta E^* = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b)^2}$$, *where,* L_0^*, a_0^*, b_0^* *represent the respective readings of fresh peel sample.*

2.3. Data analysis

All data were subjected to analysis of variance two-way (ANOVA), using GLM procedure of Minitab software (version 16.0, Minitab Inc., State College, PA, USA). Significant differences among the treatments were assessed by the Tukey test at P<0.05.

3. RESULTS AND DISCUSSIONS

3.1. Mass proportion of carrot root

The study results showed that carrot root consisted of three individual parts in terms of head, peel and flesh with their mass proportion accounted for 1.20±0.26, 11.81±1.23 and 87.07±2.08% by fresh weight. Of these, carrot peel is the main residue, given about 118g carrot peel could be obtained from a kg of carrot root.

3.2. Physico-chemical properties

The moisture of fresh carrot peel was determined at 87.56±2.03%, which was in the range (80-90%) reported by Tadesse et al. (2015). After soaking and blanching, the moisture increased to 91.32±2.03%. Table 1 describes the drying time, drying yield, moisture content, residual carotenoids and color attributes of the carrot peel powder. It showed that the drying time was affected by the treatment. Treated samples dried in higher temperatures had lower drying time compared to other un-treated samples. It is also seen that all treatments at higher temperatures were dried faster than those at lower ones (70>60>50°C). Oven drying took 6.0-7.0h to dry carrot peel at the lowest temperature (50◦C), while drying at 70◦C took only 4-4.5h. The drying time in this study was shorter than that in the research of Nguyen and Le (2018) when applying different thermal drying (50 and 100◦C). According to El-Shehawy and El-Mashad (2010), pre-treatment may change the physical properties of the tissue, by damaging the cell membrane structure.

Table 1. Effect on drying time and some physicochemical properties of carrot peel powder

Pre-treatment	T (°C)	Drying time (h)	Moisture (%)	Drying yield (%)	Carotenoids (µg/gDW)
Treated	50	6.00	6.12^{abc}	10.57	713.33^{a}
	60	5.00	5.50^{c}	9.90	253.48^{b}
	70	4.00	6.47^{ab}	9.93	89.51^{c}
Un-treated	50	7.00	5.75^{bc}	9.85	683.50^{a}
	60	6.00	6.60^{a}	10.64	316.74^{b}
	70	4.50	5.99^{abc}	10.30	86.89^{c}
SEM		-	0.14	0.26	15.52
P		-	0.010	0.251	0.000

Note: Means with different superscript letters within a column indicate significant differences (P<0.05).

As compared to the moisture content of the fresh carrot (87.56%), rehydrated samples contained significantly lower moisture content (5.50-6.60%). The moisture reduction due to the loss of water content during drying process may lead to irreversible damage in the plant tissues (Al-Amin *et al.*, 2015). Regarding drying yield, there is a non-significant difference

among the six treatments (P>0.05), which was about 10% in average. In comparison with another study, Nguyen and Le (2018) indicated that the drying yield obtained from the carrot peel was higher under heat treatment at 100◦C (11.75%), compared to that at 50°C (9.60%).

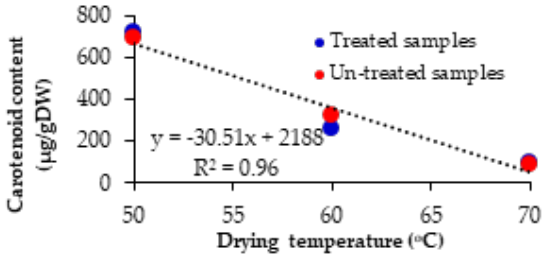

Fig 1. Effect of drying temperature on the carotenoids retention carrot in peel powder

As can be seen, the carotenoids content was strongly affected by the treatments (P<0.05). The carotene losses were observed mainly by drying temperature. The highest carotenoids content was found in treated sample dried at 50°C (713.33 µg/gDW) while the lowest value was noted in un-treated sample dried at 70°C (86.89 µg/gDW). Figure 1 shows the relationship between the drying temperature and total carotenoids retention in carrot peel powder. The carotenoids contents decreased constantly by rising the drying temperature so at 70°C it reached the lowest value. This finding is in agreement to that of Cherrat *et al.* (2019) who elucidated the inverse relationship between the total carotenoids content and high temperature processing.

Color is one of the most important attributes of dried food products since it influences consumer acceptability. The color of the dehydrated product could be observed by the most common parameters measured in the final product. The effect of pre-treatment and drying temperature on the color attributes of carrot peel powder was shown in Table 2 and Photo 1.

The higher drying temperature the more visible changes in color were noticed (Photo 1). The results show that all color values are significantly different (P<0.05). The initial color L^*, a^* and b^* values of the fresh carrot peel were 65.92±0.19, 31.44±0.29 and 46.86±0.32, respectively. Under the effect of increased temperature during hot-air drying, the carrot peel powder became lighter, corresponding to a rise in L^* value, highest for un-treated samples dried at 70°C (66.82) and lowest for the treated samples dried at 50°C (63.59). In addition, significant reductions in the a^* and b^* values were observed when drying temperature increased from 50°C to 70°C, in both treated and un-treated samples. Similar findings were reported by More and Khodke (2023).

Table 2. Effect on the color of carrot peel powder

Treatment	T (°C)	L^*	a^*	b^*	ΔE^*
Treated	50	63.59^{c}	25.69^{a}	38.51^{a}	10.42^{d}
	60	65.11^{b}	24.22^{b}	37.40^{b}	11.99^{c}
	70	64.91^{b}	22.61^{c}	35.86^{c}	14.16^{b}
Un-treated	50	64.69^{bc}	24.80^{ab}	37.50^{b}	11.56^{c}
	60	64.85^{bc}	22.10^{c}	36.21^{c}	14.21^{b}
	70	66.82^{a}	18.46^{d}	33.56^{d}	18.61^{a}
SEM		0.29	0.22	0.18	0.20
P		0.000	0.000	0.000	0.000

Differences in the ΔE^* values (total differences in color) among the treatments (P<0.05) were also shown in Table 2. Pre-treatment and drying temperature significantly affected the values of ΔE^*, which ranged of 10.42-18.61. The lowest ΔE^* value was observed for treated samples when dried at 50°C (10.42), followed by un-treated samples dried at 50°C (11.56) and highest value was noted for un-treated sample dried at 70°C (18.61). Drying at 50°C led to lower color changes for both treated and un-treated sample despite the drying temperatures. This is most likely due to the higher drying temperature leading to an increase in color degradation (Nguyen *et al.*, 2021). As reported by other authors (Zielinska and Markowski, 2012; Garba *et al.*, 2015), the quality of dehydrated carrots does not only depend on the drying techniques and drying conditions but also on the other processing methods applied before and after drying. In addition,

an initial weak chemical treatment followed by blanching could minimize the changes in tissue structure, increase drying rate and improve the overall acceptability of the final products by avoiding color deterioration.

4. CONCLUSION

The results from this study showed that treated carrot peel samples dried convectively at 50°C were preferable. Overall, these samples retained quality attributes of carrot peel recording minimum drying time, higher total carotenoids content and minimum color changes. Future research are suggested to focus on maximizing the quality retention through different drying techniques as well as minimizing the production cost of dried carrot peel.

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EFFECT OF MEATTIDE ON PRODUCTION AND ITS OPTIMAL SUPPLEMENTATION IN DIET FOR DUROC×F_1(LANDRACE × YORKSHIRE) FATTENING PIGS

Do Duc Luc[1], Duong Van Toi[2], Le Van Khoa[2], Do Thi Phuong[3] and Ha Xuan Bo[1]*

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ABSTRACT

This study aimed to (1) evaluate the effects of MeatTide (MT–myostatin inhibition product) on production performance and carcass traits and (2) estimate optimal requirement of MT in the diet for improving average daily gain (ADG), lean meat percentage (LMP) of fattening pigs from D boars and L×Y sows (DLY). A total of 75 DLY pigs (35 gilts and 40 barrows) aged 82 days with body weight (BW) of 26.11±2.90kg were used. Animals by gender were randomly allocated to 5 pens (7 gilts and 8 barrows per pen). One pen was assigned to one of 5 treatments. One was a control group where piglets were fed a basal diet. Four other pens were treatment groups where animals were fed basal diet supplemented with 0.3, 0.4, 0.5 and 0.7‰ of MT product. Experiment was divided into two phases: (1) Grower from beginning to 45 experiment days and (2) Finisher from 45 days to the end of the experiment. Effects of MT and gender on BW, ADG, back fat thickness (BF) and LMP were evaluated using ANOVA. Break points (MT requirement) were estimated using two-slope linear broken-line model. The results shown that increasing MT from 0 to 0.4‰ in basal diet led to an increase of BF1 and BF2 while to a decrease of LMP (P<0.05). When MT in the basal diet increased to 0.5 and 0.7‰, BF decreased and LMP increased (P<0.05). MT affected studied traits in different ways for gilts and barrows. For gilts, ADG from during grower phase (ADG2) and BF at the first point (BF1) were different between treatments (P<0,05). For barrows, MT affected BF1, BF at the second point (BF2), *longissimus* muscle depth (LMD), and LMP (P<0.05). The fitting model to quadratic did not observe for pooling sexes (P>0.05). For the gilt model, BW2 was fitted to the quadratic model (P<0.05) and break point was 0.42‰. Inversely, BW3, ADG2, BF1, BF2, LMP were observed quadratic fitting (P<0.05) except ADG3 had a quadratic tendency (P=0.056). Break points of BW3, ADG2, ADG3 BF1, BF2 and LMP were 0.33, 0.40, 0.39 0.40, 0.36 and 0.40‰ respectively. To improve growth rate, 0.40‰ of MT is recommended for gilts in grower phase and barrows during fattening period. If the demand of market for more lean meat, 0.7‰ of MT could be recommended for barrows. Further studies should be implemented to estimate optimal MT requirement to improve LMP for fattening pigs.

Keywords: *Myostatin inhibition, fattening pigs, growth rate, lean meat percentage.*

1. INTRODUCTION

In pig production, lean meat percentage has been an important trait (Martinsen *et al.*, 2016). Myostatin is a protein and is considered as a negative regulator of skeletal muscle mass (Lee, 2004). This author confirmed that the muscle mass of mice with targeted deletion of the myostatin gene increased widely. Inhibition of myostatin could improve muscle mass and was reported in the previous study (Hill *et al.*, 2002; Tsuchida, 2008; Lee *et al.*, 2022). Besides selection based on the targeted genes, the alternative solution is finding additional ingredients to inhibit myostatin to improve muscle mass. MeatTide is a product from Enriching Innovation Biotech. It is a bioactive peptide extracted from animal tissue by using the newest gene editing technique (Enriching

[1] Vietnam National University of Agriculture
[2] Anh Duong Khang Nutrition Company, Ho Chi Minh city
[3] Asia Livestock Innovation Center, Sunjin Vina Co., Ltd, Ha Nam Branch, Ha Nam
* Corresponding Author: Assoc. Prof. Dr. Do Duc Luc, Faculty of Animal Science, Vietnam National University of Agriculture, Hanoi, Vietnam. Tel: (84)912370193; Email: ddluc@vnua.edu.vn

Innovation Biotech, 2022a). This peptide will weaken the effect of inhibiting the growth of muscle cells, thereby promoting muscle cell growth and differentiation (Enriching Innovation Biotech, 2022b). It could also enhance energy conversion efficiency to reduce fat accumulation, improve growth rate, and reduce fattening time to market weight (Enriching Innovation Biotech, 2022a). According to GSO (2022) GSO of Vietnam, 4539.2 tons of living weight of pig was produced in 2022 equivalent to 62% of living weight of the livestock sector. Improvement of lean meat helps to increase the economic value of pig production in our country. Fattening pigs (DLY) from Duroc boars (D) and hybrid sows between Landrace (L) and (Yorkshire) have been used widely in swine production due to their high growth performance and meat quality (Trinh Hong Son and Nguyen Thi Huong, 2019). This study aimed to determine the effect of MeatTide (MT) on growth performance and carcass traits of DLY fattening pigs from D boars and F_1(L×Y) sows under farming condition in Southern Vietnam.

2. MATERIALS AND METHODS

The experiment was conducted at a commercial pig farm in Dong Nai province, South Vietnam, from February to June 2023. A total of 75 DLY fattening pigs (35 females and 40 castrated males) aged 82 days with body weight (BW) of 26.11±2.90 (±SD) kg born from D boars and F_1(L×Y) sows were used. The animals by gender were randomly allocated to 5 pens (7 females and 8 castrated males per pen). One pen was assigned to one of 5 treatments. One was a control group where piglets were fed a basal diet (T0). Four other pens were treatment groups where animals were fed a basal diet supplemented with 0.3‰ (T3), 0.4‰ (T4), 0.5‰ (T5) and 0.7‰ (T7) of MT. This product was produced by Enriching Innovation Biotech and provided by Anh Duong Khang Company, Vietnam. The experiment was divided into two phases: (1) Grower from the beginning to 45 experiment days and (2) Finisher from 45 days to the end of the experiment.

The feed was produced at the farm according to grower and finisher pigs. For the first two weeks of the experiment, animals were provided feed for grower and then for finisher to the end of the fattening. The nutrition compositions in Table 1 are on an as fed basis. The pigs were provided with feed ad libitum and free access to the water by drinking nippers.

Table 1. Nutrition compositions of basal diet

Parameter	Grower	Finisher
ME (Kcal/kg)	3,100	3,000
Protein (%)	17	15.6
Fiber (%)	4	6
Methionine (%)	0.67	0.35
Lysine (%)	1.05	1.00
Ca (%)	0.7-1.0	0.6-1.0
P (%)	0.6-1.0	0.6-1.0

At the beginning of the experiment, each animal was identified using ear tags and weighed to obtain initial body weight (BW1). During the experiment, body weights were recorded individually two times: (1) at 45 days of the experiment (BW2) and (2) at 103 days (the end) of the experiment (BW3) when the animals reached approximately 100kg. Average daily gain (ADG, g) was calculated for the periods from (1) beginning to 45 experiment days (ADG1), (2) from 45 days to the end of the experiment (ADG2) and (3) from the beginning to the end of the experiment (ADG3) based on BW1, BW2, BW3 and the duration according to the periods.

At the end of the experiment, back fat thickness and depth of *longissimus* muscle were measured on the live animals using ultrasound device Piglog 105 (Carometec A/S, Herlev, Denmark) in the same time when BW3 was weighted. Measurements of back fat thickness between the third and fourth last lumbar vertebrae (BF1) and back fat thickness and the muscle thickness between the third and fourth last rib (BF2 and LDT). The lean meat percentage (LMP) was calculated by the

in-coded Piglog 150 formula based on BF1, BF2, and LDT.

The effect of MT supplementation on growth performance and carcass traits was evaluated using the statistical model as follows: $y_{ijk}=\mu+T_i+S_j+e_{ijk}$, *where* y_{ijk} *= observation of studied traits,* μ *= overall mean,* T_i *= effect of treatment* i^{th}, S_j *= effect of gender* j^{th} *and* e_{ijk} *= residual error.* The effect of MeatTide was also analyzed separately for gilts and barrows using the above model without gender effect. The pairwise comparisons were made using Ducan test.

To estimate MT requirement, data were analyzed using a two-slope linear broken-line model according to method of Robbins *et al.* (2006) with the model y=L+U×(R-x)+V×(x-R); *where y = studied traits, L = asymptote for the first segment, U = slope 1 for the first segment, V = slope 2 for the second segment, x = MT supplementation level, R = break point x value*.

A number of observations (n), arithmetic mean (Mean), root square mean error (RSME), standard error (SE) and coefficient of determination (R^2,%) are presented in the result section. Statistical significance level was preset at P<0.05 while a trend was confirmed at 0.05≤P<0.1. All data were performed SAS® OnDemand for Academics.

3. RESULTS

The effects of MT supplementation (T), gender (S) and interaction between these factors (T×S) on growth performance and carcass traits are presented in Table 2. BW, ADG and LMD were similar between T (P>0.05). However, BF1, BF2 and LMP were significantly different between T (P<0.05). Increasing MeatTide from 0 to 0.4‰ in basal diet led to an increase of BF1 and BF2 while to a decrease of LMP (Table 3). When MeatTide in the basal diet increased to 0.5 and 0.7‰, BF decreased and LMP increased (P<0.05). At the beginning and 45 days of experiment, BW1 and BW2 were not significantly different between gilts and barrows (P>0.05), whereas BW3 was found differently between S (P=0.009). The barrows were heavier and grew faster than gilts (Tables 4 and 5).

Table 2. Effect of Treatment (T), gender (S), T×S interaction on production performance of DLY and fitted models to linear (L), quadratic (Q)

Trait	ANOVA			Pooling fitted model				Gilt fitted model			Barrow fitted model		
	T	S	T×S	R^2	L	Q	R^2	L	Q	R^2	L	Q	R^2
BW1	0.892	0.914	0.855	3.63	0.077	0.814	98.97	0.661	0.501	38.62	0.814	0.838	16.19
BW2	0.661	0.253	0.989	6.05	0.747	0.986	21.88	0.012	0.029	98.58	0.174	0.240	71.94
BW3	0.364	0.009	0.654	18.74	0.561	0.914	45.98	0.189	0.234	66.97	0.037	0.016	98.23
ADG1	0.327	0.093	0.915	12.00	0.936	0.784	12.31	0.203	0.562	87.30	0.058	0.105	91.98
ADG2	0.228	0.007	0.308	23.18	0.226	0.648	89.53	0.331	0.346	44.80	0.409	0.045	98.29
ADG3	0.479	0.005	0.509	20.30	0.814	0.760	36.37	0.162	0.221	73.30	0.129	0.056	94.18
BF1	0.007	0.020	0.338	29.78	0.875	0.757	14.01	0.456	0.435	31.88	0.013	0.009	98.30
BF2	0.013	0.001	0.683	31.26	0.778	0.573	39.16	0.475	0.388	41.49	0.005	0.004	99.29
LMD	0.548	0.210	0.222	14.78	0.773	0.505	50.65	0.342	0.518	58.95	0.390	0.314	50.38
LMP	0.005	<0.001	0.296	35.87	0.951	0.655	31.89	0.540	0.488	27.18	0.045	0.033	93.81

The fit to quadratic models to studied traits according to pooling sexes, gilts and barrows were presented in Table 2. The fitting model to quadratic did not observe for pooling sexes (P>0.05). For the gilt model, only BW2 was fitted to the quadratic model (P<0.05). Inversely, BW3, ADG2, BF1, BF2 and LMP were observed quadratic fitting (P<0.05) while ADG3 had a quadratic tendency (P=0.056).

In our study, MeatTide had correlated effects on growth performance and carcass traits depending on the supplementation

amount in the diet. With the limited amounts, the inhibiting myostatin was not detected whereas, this limited amount was as a growth promotor for the fattening pigs. The BW2, BW3, ADG2 and ADG3 at T3 seem to be highest but this difference was not significant (Table 3). However, the effect of myostatin inhibition was found when 0.5‰ and 0.7‰ of MeatTide were added to the diets for BF1, BF2 and LMP (Table 3).

Table 3. Production performance of DLY according to MeatTide supplement

Variable	T0		T3		T4		T5		T7		RSME
	n	Mean	n	Mean	n	Mean	n	Mean	n	Mean	
BW1 (kg)	15	26.03	15	26.81	15	26.04	15	25.93	15	25.73	3.03
BW2 (kg)	15	59.4	14	62.25	14	61.71	15	62.13	15	61.8	5.82
BW3 (kg)	15	105.33	14	111.57	13	108.77	15	107.2	14	105.36	9.39
ADG1 (g)	15	741.63	14	781.43	14	797.14	15	804.44	15	801.48	92.19
ADG2 (g)	15	791.95	14	850.37	13	807.69	15	777.01	14	752.46	113.89
ADG3 (g)	15	769.97	14	820.25	13	801.64	15	789	14	773.93	82.77
BF1 (mm)	15	14.73^{b}	14	18.43^{a}	13	18.08^{a}	15	15.27^{b}	14	14.36^{b}	3.66
BF2 (mm)	15	12.40^{bc}	14	15.21^{a}	13	14.23^{ab}	15	12.80^{bc}	14	11.57^{c}	2.94
LMD (mm)	15	56.67	14	57.36	13	55.92	15	58.40	14	59.29	5.68
LMP (%)	15	58.33^{a}	14	55.59^{b}	13	55.55^{b}	15	58.07^{a}	14	59.14^{a}	3.04

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

Growth rate and carcass traits of DLY were calculated separately for gilts, barrows, and are presented in Tables 4 and 5 respectively. MeatTide affected studied traits in different ways for gilts and barrows (Tables 4 and 5). ADG2 and BF1 of gilts were different between treatments (Table 4). For barrows, MT affected BF1, BF2, LMD and LMP (Table 5).

Table 4. Production performance of DLY gilts according to MeatTide supplement

Variable	T0		T3		T4		T5		T7		RSME
	n	Mean	n	Mean	n	Mean	n	Mean	n	Mean	
BW1 (kg)	7	26.11	7	26.43	7	26.74	7	25.40	7	25.66	2.75
BW2 (kg)	7	58.36	6	60.58	7	61.43	7	61.43	7	61.21	6.27
BW3 (kg)	7	98.56	6	109.50	7	106.00	7	104.00	7	104.71	9.80
ADG1 (g)	7	716.51	6	746.30	7	770.79	7	800.64	7	790.16	103.24
ADG2 (g)	7	698.28^{b}	6	843.39^{a}	7	768.47^{ab}	7	733.99^{ab}	7	750.00^{ab}	98.27
ADG3 (g)	7	706.24	6	800.97	7	769.49	7	763.11	7	767.54	80.83
BF1 (mm)	7	13.29^{ab}	6	18.00^{a}	7	17.14^{ab}	7	12.57^{b}	7	14.57^{ab}	4.18
BF2 (mm)	7	11.43	6	14.17	7	12.86	7	10.57	7	11.14	3.07
LMD (mm)	7	56.29	6	57.17	7	59.00	7	61.00	7	58.57	6.72
LMP (%)	7	59.54	6	56.75	7	57.21	7	60.66	7	59.17	3.36

The ADG2 and BF1 of gilts increased from T0 to T3 and then decreased to T4, T5 and T7 (Table 4). For barrows, BF1, BF2 had a similar tendency as for gilts but LMD and LMP were in reverse direction. These values of barrows decreased from T0 to T4 and increased after this point (Table 5)

For the gilt, only BW2 was fitted to the quadratic model (P<0.05). Inversely, BW3, ADG2, BF1, BF2, and LMP of barrows were observed quadratic fitting (P<0.05). Based on these significant levels of fitting model to quadratic, the break points (R) were estimated for BW2 of gilts, BW3, ADG2, BF1, BF2 and LMD of barrows. The coefficient of determination of these dependent variables was high and ranged from 98.22 to 100% (Table 6).

Table 5. Production performance of DLY barrows according to MeatTide supplement

Variable	T0		T3		T4		T5		T7		RSME
	n	Mean	n	Mean	n	Mean	n	Mean	n	Mean	
BW1 (kg)	8	25.95	8	27.15	8	25.43	8	26.40	8	25.80	3.26
BW2 (kg)	8	60.31	8	63.50	7	62.00	8	62.75	8	62.31	5.40
BW3 (kg)	8	111.00	8	113.13	6	112.00	8	110.00	7	106.00	9.00
ADG1 (g)	8	763.61	8	807.78	7	823.49	8	807.78	8	811.39	81.60
ADG2 (g)	8	873.92	8	855.60	6	853.45	8	814.66	7	754.93	126.40
ADG3 (g)	8	825.73	8	834.71	6	839.16	8	811.65	7	780.31	84.49
BF1 (mm)	8	16.00^{ab}	8	18.75^{a}	6	19.17^{a}	8	17.63^{ab}	7	14.14^{b}	3.12
BF2 (mm)	8	13.25^{ab}	8	16.00^{a}	6	15.83^{a}	8	14.75^{ab}	7	12.00^{b}	2.82
LMD (mm)	8	57.00^{ab}	8	57.59^{ab}	6	52.33^{b}	8	56.13^{ab}	7	60.00^{a}	4.54
LMP (%)	8	57.28^{ab}	8	54.71^{bc}	6	53.62^{c}	8	55.80^{bc}	7	59.11^{a}	2.73

To improve BW2 of gilts, 0.42‰ of MT supplementation was required in the diet. BW2 at this break point was 61.52kg. For barrows, to improve BW3, ADG2, ADG3, BF1 and BF2, MT requirements were 0.33, 0.40, 0.39 0.40 and 0.36‰ respectively. Of these requirements, BW3, ADG2, ADG3, BF1 and BF2 were 113.40kg, 850.00, 837.40g, 19.35 and 16.50mm respectively. MT supplementation of 0.40‰ was break point for LMP of barrows. Around 0.40‰ of MT, the maximization of growth rate and back fat thickness was observed but LMP was the lowest (53.83%).

Table 6. Estimated parameters of DLY gilts according to MeatTide supplementation

Dependent variable		Asymptote (L±SE)	Slop 1 (U±SE)	Slop 2 (V±SE)	Break point (R±SE)	R^2 (%)
Gilts	BW2	61.52±0.08	-7.61±0.22	-1.10±0.46	0.42±0.01	99.94
Barrows	BW3	113.40±1.75E-14	-7.10±9.47E-14	-20.00±9.34E-14	0.33±1.16E-15	100.00
ADG2		850.00±8.36	59.83±23.17	-320.00±22.74	0.40±0.03	99.73
ADG3		837.40±7.36	-29.93±29.83	-190.5±29.30	0.39±0.05	98.22
BF1		19.35±0.22	-8.21±0.99	-17.45±2.07	0.40±0.02	99.51
BF2		16.50±0.13	-9.17±0.74	-12.91±0.73	0.36±0.01	99.79
LMP		53.82±0.22	8.65±0.98	17.79±2.04	0.40±0.02	99.55

Myostatin is considered a transcriptional growth factor that inhibits growth of skeletal muscle (Li *et al.*, 2020)2020. These authors confirmed that myostatin has become an important way for improving lean meat in livestock production. Inhibition or deficiency of myostatin in animals led to an increase in skeletal muscle mass known as hyper-muscling (Hill *et al.*, 2002; Tsuchida, 2008; Li *et al.*, 2020; Lee *et al.*, 2022). Selection based on the genotype of target genes (Li *et al.*, 2020, Pei *et al.*, 2022) or genome-editing technology (Kang *et al.*, 2017; Dilger *et al.*, 2022) reduced myostatin in animals. However, these approaches are not easy ways to practice for farmers due to the costs of analysis and laboratory equipment. Another approach is the application of biological products to inhibit myostatin in animals to increase lean meat. In our study, MT was used as a feed additive for myostatin inhibition. MT affected growth rate and carcass traits at different levels. With the low dose of MT, ADG, and BF increased. ADG and BF reduced when the MT dose in the diet increased to 0.5 and 0.7‰ consequently LMD and LMP improved. There was a negative correlation between BF and LMP. Increasing BF led to a decrease in LMD and LMP (Hoa *et al.*, 2021). Sulforaphane - an alternative bioactive compound as a myostatin inhibitor in porcine satellite cells was reported in the study of Fan *et al.* (2012). The authors

also concluded that bioactive products such as myostatin inhibitors could be used in livestock production improvement.

From observed results, to maximize the growth rate in the grower phase, the recommendation of MT supplementation is 0.42‰ and 0.40‰ for gilts and barrows respectively. For barrows, the recommendation of MT for barrows in the diet depends on the production goals. If growth rate is the priority, 0.40‰ of MT could be recommended for both phases. If the demand of the market for more lean meat, 0.7‰ of MT without effect on growth rate is the appropriate dose based on Table 5. However, the optimal MT requirement for LMP was not estimated in this study. Additionally, our study was repeated once time. Therefore, further studies should be conducted separately for males and females, evaluating effect of MT on feed conversion ratio and predicting optimal MT requirement for LMP.

4. CONCLUSIONS

MeatTide affected production performance and carcass traits of fattening pigs. To improve the growth rate, the recommendation of MeatTide supplementation in the diet is 0.40‰ for gilts in the grower phase and barrows during the fattening period. If the demand of the market for more lean meat, 0.7‰ of MeatTide could be recommended for barrows. Further studies should be implemented to estimate the optimal MeatTide requirement to improve LMP for fattening pigs.

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STUDY ON GROWTH PERFORMANCE AND FEED INTAKE OF FATTENING CROSSBRED CATTLE BASED ON TOTAL MIXED RATION FOR FEED FORMULATION TOOL APPLICATION TO MEET BETTER PRODUCTION

Nguyen Minh Quoc[1], Diep Tan Toan[2], Le Thuy Binh Phuong[1] and Duong Nguyen Khang[1,2]*

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ABSTRACT

The study was undertaken to assess the effect of total mixed ration (TMR) on the growth and availability of feed intake in crossbred Zebu cattle for feed formulation tool application to meet better production and methane mitigation. Thirty crossbred Zebu male cattle (9 months, 205.5+/-46.8kg BW) divided in to 3 equal groups were offered T1: A ration consisting of typical traditional feed regimen of rice straw, mombasa grass, urea and rice bran; T2: An adequate ration based on locally available feed of rice straw, mombasa grass, brewery waste and rice bran; and T3: A ration including high quality feed of rice straw, mombasa grass, brewery waste and concentrate feed. All diets contained mineral premix 0.1% and had about 16% CP and 2.36 Mcal/kg DM metabolisable energy. The amount of each feed ration will be estimated by the tool application to meet standard requirement for beef cattle. The experimental period lasted for 84 days. Results shown that animals on T2 and T3 had higher ($P \leq 0.05$) intake of supplementary diets than those on T1. Growth rates of animals on T2, T3 and T1 did not differ ($P > 0.05$) with 603.6, 596.4 and 591.7 g/day, respectively. Feed conversion ratio for animals on T1, T2 and T3 did not differ ($P > 0.05$) with 9.03, 8.90 and 8.95 respectively. The highest gross margins per animal were observed from animals on T2, followed by those on T3 and T1. It is concluded that the total mixed rations in crossbred Zebu cattle in this study could be used for feed formulation tool application to meet better production.

Keywords: *Zebu cattle, total mixed ration, feed intake, feed conversion ratio, profit.*

1. INTRODUCTION

Feeding a total mixed ration (TMR) helps cattle achieve better performance (Kolver and Muller, 1998; Bargo *et al*., 2002; Tozer *et al*., 2003; Boadi *et al*., 2004). Since 1950s, it is now the most adopted method for feeding high producing, such as intensive dairy cows or feedlot beef cattle. This is accomplished by feeding a nutritionally balanced ration at all times, allowing cattle to consume as close to their actual energy requirements as possible and maintaining the physical or roughage characteristics for better rumen function (Bargo *et al*., 2002; Hassanat *et al*., 2013). Moreover, it should be to achieve the methane reduction in cattle production systems. This TMR feeding system has a advantages and disadvantages (Boadi *et al*., 2004), therefore, must be studied, tested and weighed before choosing a better TMR system.

Good feeding management practices must be followed to achieve better performance and methane reduction from cattle (Soriano *et al*., 2001; Robertson and Waghorn, 2002). First, monitor the forage and feed inventory on a regular basis and mange to allocate to the appropriate animal groups. Second, analyses the nutrient composition of forages and feeds several times throughout the year. Lastly, update ration formulations based on performance production, body weight and body condition scores, dry matter changes in forages or high moisture feed ingredients, and prices of feeds. One important thing, checking

[1] Nong Lam University of Ho Chi Minh City

[2] Center of Animal, Seeds and Aquatic Breeds of Ho Chi Minh City

* Corresponding Author: Prof. Dr. Duong Nguyen Khang, Nong Lam University of Ho Chi Minh City; Tel: (84)989390179; Email: duongnguyenkhang@gmail.com.

the forage moisture on a frequent basis is critical to implementing a successful TMR system.

Separate TMR can be developed for different animal groups such as fresh cows, early lactation cows, mid- and late-lactation cows, far off and close-up dry cows. Dairy cows can be grouped based on actual or fat-corrected milk, days in milk, reproductive status, age, nutrient requirements, and health. The same models could be applying to the beef cattle system. Such multi-group strategies are particularly helpful for meeting the needs of dairy cow or beef (Tozer *et al*., 2003; Vibart *et al*., 2008), particularly methane mitigation from ruminant production (Boadi *et al*., 2004; Hassanat *et al*., 2013).

The aim of this study to facilitate and carry out the effect of total mixed ration on cross-bred male Sindhi cattle based on rice straw and grass diets for better performance. The effects expected from total mixed ration such as the treatments to be compared, the parameters to be measured, the analysis to be performed as well as the statistical needs are presented. The results from this study could be used the data for feed formulation tool application to meet better production and methane reduction in cattle diets.

2. MATERIALS AND METHODS

2.1. Location

The experiment was conducted in the cattle farm of the Research and Technology Transfer Center of Nong Lam University from Dec 2022 to Mar 2023.

2.2. Treatments and experimental design

Thirty crossbred Sindhi male cattle were allocated to three pens according to live weight (LW) and fed a basal diet of rice straw and sulphur-rich minerals (0.1% of diet DM). Each pen received one of the following treatments according to a completely randomized design:

T1: A ration consisting of typical traditional feed regimen of rice straw (RS), mombasa grass (MG), urea and rice bran (RB).

T2: An adequate ration based on locally available feed and by-products of RS, MG, brewery waste, and RB.

T3: A ration including high quality feed of RS, MG, BW and concentrate feed (CF).

2.3. Animals and housing

The crossbred Sindhi male cattle had an initial weight in range of 205.5±46.8kg and were allocated to 3 pens so that mean LW within each pen were similar. Vaccination was done against epidemic diseases and the cattle were drenched against internal parasites before the commencement of the experiment. The cattle were weighed before morning feeding at the begin and at the end of 84-days trial.

2.4. Feeding and management

Crossbred Sindhi male cattle were adapted gradually to experimental feeds for two weeks prior to starting experiment. The BW was bought from Intermalt Vietnam Co, LTD factory in Ba ria Vung Tau province and provided for each treatment. Feeds were offered two times a day, at 7.30am and 2.30pm. Feeds offered and refused were recorded daily. Water was supplied all day.

2.5. Data collection and measurements

Cattle were weighed at the begin and the end of 84-days trial, using an electronic balance. Feeds offers were weighed before giving them to cattle. Feed refusals were collected each morning prior to offering fresh feed and weighed to measurea feed intake (FI). Samples of feeds offered and refused were collected every 14 days to determine DM, Ash, CP, CF, lignin, EE, NDF and ADF according to AOAC methods (2005). The morbidity rate was recorded using the diagnosis of treatment condition ie, pneumonia, fever, diarrhea for each animal determined to be sick. Provide the information on all medications given including vaccines, de-wormers, and therapeutic antimicrobial treatments. Specific medications and dosages administered to each animal.

2.6. Chemical analysis

All samples were analyzed for dry matter (DM), ash, CP, CF, lipid (EE), NDF and ADF

according to AOAC (2005). While NDF and ADF analysis was followed the Van Soest *et al.* (1991); GE, DE, ME, NEM, NEG, TDN, NEL and NFC was calculated following the formula suggested by Sauvant *et al*. (2004).

2.7. Statistical analysis

The data were subjected to analysis of variance using the General Linear Model procedure of Minitab software version 17.00. Tukey's pairwise comparisons (P<0.05) were applied to determine the differences between dietary treatments. Response curves were fitted to the data using linear and quadratic equations in Microsoft Office Excel software, with different total mixed rations as the independent variable (X) and the response component: FI, WG as dependent variable (Y).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of feed, nutrients in diets

There were major differences in crude protein, crude fiber, NDF, DNF, lignin with higher values for BW, RB, CF than for RS or MG (Table 1).

Table 1. Composition of diet ingredients
(% in DM)

	BW	RB	CF	RS	MG
DM, %	86.2	89.2	90.1	89.2	20.2
CP, %	14.96	8.36	18.4	4.16	11.84
CF, %	15.25	16.64	5.8	27.49	29.02
Lipid, %	1.98	4.01	4.2	0.89	1.76
Ash, %	4.21	9.96	6.4	12.99	9.32
Ca, %	0.10	0.23	1.68	0.39	0.60
P, %	0.41	0.46	0.73	0.10	0.26
TDN, %	72.22	66.75	74.06	39.99	58.14
NDF, %	41.51	35.02	22.1	69.92	63.17
ADF, %	16.30	20.54	11.9	40.81	34.13
Lignin, %	1.14	6.36	3.5	2.66	3.04
NFC, %	37.35	42.64	48.9	11.04	14.42
Starch, %	4.73	29.63	5.12	0.90	2.06
GE, Mcal/kg	4.09	3.70	3.26	3.67	4.21
DE, Mcal/kg	3.18	2.94	2.67	1.76	2.56
ME, Mcal/kg	2.61	2.41	1.76	1.44	2.10
NEM, Mcal/kg	1.70	1.53	1.33	0.60	1.24
NEG, Mcal/kg	1.27	1.09	1.69	0.13	0.79
NEL, Mcal/kg	1.65	1.52	18.4	0.86	1.30

The amount of each feed ration will be estimated by the tool application to meet standard requirement for beef cattle to present in Table 2.

Table 2. Formulation and nutrients in diets
(cattle/day)

Item	T1	T2	T3
Mombasa grass, kg	18.6	16.5	16.6
Rice straw, kg	1.1	1.6	2.1
Rice bran, kg	2.3	1.0	0.0
Concentrated feed, kg	0.0	0.0	1.0
Brewery waste, kg	0.0	1.2	1.1
Mineral premix, kg	0.1	0.1	0.1
Urea, kg	0.07	0.0	0.0
DMI, %	2.82	2.82	2.82
ADG, kg	0.87	0.87	0.87
Cost, $/day	2.5	2.35	2.35
ME, Mcal/kg	2.41	2.43	2.39
Methane, % NE	5.55	5.55	5.56

3.2. Dry matter intake, live weight gains and FCR

DM intake, LWG and FCR in T1, T2 and T3 were not differ with 4.94, 4.97 and 4.93 kg/cattle/day for DM intake; with 592, 604 and 596 g/cattle/day for LWG; with 8.35, 8.24 and 8.27 for FCR, respectively (Table 3, Fig 2 and 3). Growth rate was similar with more supplementation of concentrate in diets. The similar on the rate of response in LWG when using supplementation of CF was not in accordance with studies in which protein-rich supplements were fed in increasing quantities in diets rich in carbohydrates as fish meal and molasses-urea (Preston and Leng, 1987) cotton seed cake and ammoniated wheat straw (Weixian *et al*., 1994).

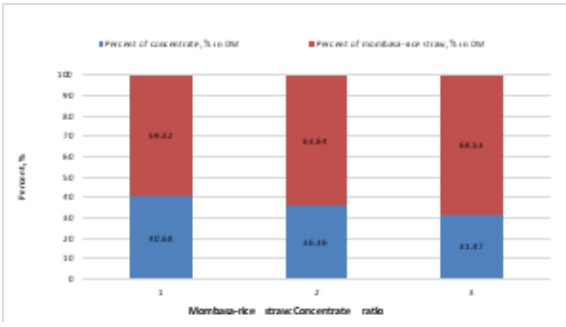

Fig 1. Proportion of intake as mombasa -rice straw and concentrate according to treatments

Table 3. Live weight, DM intake, feed conversion rate

Item		T1	T2	T3	SEM	P
LW, kg	Initial	203.0	201.6	201.9	7.72	0.998
	Final	252.7	252.3	252.0	7.09	0.999
	Total WG, kg	49.7	50.7	50.1	1.68	0.973
	DWG, kg/day	0.592	0.604	0.596	0.02	0.973
DM intake, kg/day	FI, kg in DM	4.94	4.97	4.93	0.17	0.995
	DMI, %	2.17	2.19	2.17	-	-
	CF,kgDM	2.01[a]	1.81[ab]	1.55[b]	0.07	0.020
	Roughage, kgDM	2.93	3.17	3.38	0.11	0.269
	Roughage, %	59.32	63.64	68.53	-	-
FCR	Total	8.35	8.24	8.27	-	-
	CF	3.40	3.00	2.60	-	-
	Roughage	4.95	5.25	5.67	-	-

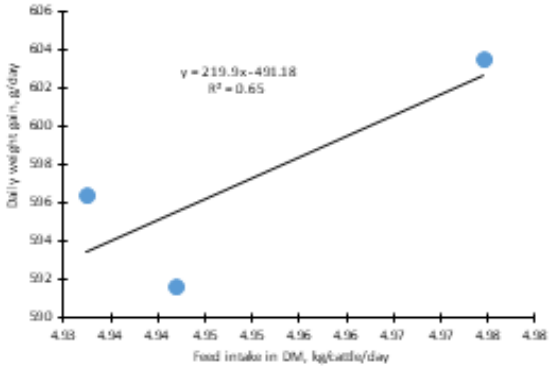

Fig 2. Effect of FI on DG of cattle

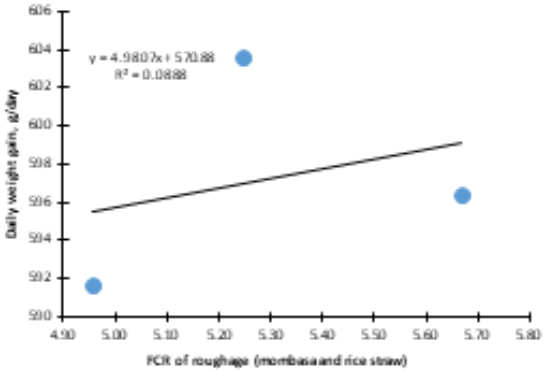

Fig 3. Effect of FCR of roughage on DG

3.3. Cost and profit

Table 4. Cost and profit of the trial

Item	1	2	3
IW, kg	203.0	201.6	201.9
FW, kg	252.7	252.3	252.0
WG, kg	49.7	50.7	50.1
DWG, g/day	591.7	603.6	596.4
TFI, kg	971	828	895
CF, kg	189	174	147
MG-RS, kg	782	655	748
Cost			
Feed mix/kg WG	22,776	20,536	17,546
Feed mix/all WG	1,131,960	1,041,180	879,060
MG-RS/kg WG	39,337	32,277	37,330
MG-RS/all WG	1,955,050	1,636,450	1,870,250
Labor/day	75,000	75,000	75,000
Cattle	18,270,000	18,144,000	18,171,000
Total	219,870,100	214,516,300	215,503,100
Price/kg WG	90,000	90,000	90,000
Price for all WG	227,430,000	227,070,000	226,800,000
Benefit	7,559,900	12,553,700	11,296,900

Note: Price of concentrate (in average) is 6,000 VND/kg, price of roughage (in average) is 2,500 VND/kg.

Cost and profit of the trial were recorded and calculated based on the experimental results (Table 4).

4. CONCLUSIONS

Growth rate and FCR were similar in treatments which has higher supplementation of CF in a basal diet of RS or MG. It is concluded that the total mixed rations in crossbred Zebu cattle could be used for feed formulation tool application to meet better production based on RB diets.

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GROWTH PERFORMANCE AND FEED INTAKE OF CROSS-BRED SINDHI CATTLE FED MICROORGANISM FERMENTED RICE STRAW

*Ba Thanh Huy[1], Dang Hoang Dao[2], Huynh Vu Duy Khang[3], Le Thuy Binh Phuong[1] and Duong Nguyen Khang[1,2]**

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ABSTRACT

The study was undertaken to assess the effect of micro-organism fermented rice straw (FRS) or unfermented rice straw (UFRS) based complete diets (Total mixed ration, TMR) on the growth and availability of feed intake of Cross-bred Sindhi cattle. Fifty female Cross-bred Sindhi cattle (8 months, 134+/-26.12 kg BW) divided in to 5 equal groups were offered (1) Micro-organism fermented rice straw *ad libitum* supplemented with soya meal and rice polishing, (2) Micro-organism fermented rice straw *ad libitum* supplemented with dried brewers' grain and rice polishing, (3) Micro-organism fermented rice straw *ad libitum* supplemented with soya meal, coconut meal and rice polishing, (4) Micro-organism fermented rice straw *ad libitum* supplemented with dried brewers' grain, soya meal, coconut meal and rice polishing, (5) Unfermented rice straw *ad libitum* supplemented with soya meal, palm meal and rice polishing as a control treatment. All diets contained mineral premix 0.1% and had about 15% CP. Results shown that the dry matter

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

intake were higher (P<0.01) in animals fed microorganisms fermented rice straw as compared to those fed unfermented rice straw. The study conclusively revealed that rice straw could be fed to ruminants after microorganism fermentation with no adverse effects on rumen profile and improvement the digestibility of nutrients, lead to increase feed intake and weight gain of animals. This practice would not only prevent burning straws in the field, but also add to the ever depleting feed resources.

Keywords: *Sindhi cattle, microorganism fermented rice straw, feed intake, feed conversion ratio, profit.*

1. INTRODUCTION

Rice straw is a common agricultural by-product after rice harvesting in many tropical countries (Gummert *et al.,* 2020). The farmers store and use it as ruminant feed, especially during the dry season. Rice straw has low protein content (3-6% in DM), high neutral detergent fiber (NDF) and acid detergent fiber (ADF) consisted of degradable carbohydrate fractions such as starch, cellulose and hemicellulose (Preston and Leng, 1987). It also contains a high indigestible phenolic substance called lignin. Because of its high lignification which makes the less palatable and digestible and low nutritional content, it is classified as a sub-maintenance feed for ruminants (Kabir *et al*., 2022). When used as fodder, rice straw primarily serves as bulk or filler to meet the dry matter requirement of ruminants (Preston and Leng, 1987). This contains 80% substances which are potentially degradable and a source of energy (Shen *et al*., 1998). The poor nutritive quality can be improved and voluntary consumption of straw can be maximized by using different treatments, supplementing with some concentrate, or combining both techniques to improve the palatable and digestible (Chaudhry and Miller, 1996). By pre-treatment of rice straw with enzymes and/or micro-organism, the microbial degradation is becoming a preferred alternative since it breaks down cellulosic polymers into cellulose digested by microbial cellulases and hemicellulases (Wu *et al*., 2004; Hughes *et al*., 2008). Higher feed intake and improvement the nutritional value of rice straw by micro-organism fermentation for cattle feeding will be choices the best methods. The use of probiotics (micro-organisms) in rice straw fermentation benefits both pre-treatment and normal digestion in the rumen (Agarwal *et al*., 2002; Selim *et al*., 2019). Many studies shown that fermented rice straw treated by using of enzymes (Wu *et al*., 2004) and/or different micro-organisms, such as bacteria (Agarwal *et al*., 2002; Selim *et al*., 2019) and/or fungi (Nguyen *et al*., 2019). Different fungi strains have the capacity to act on the cell wall contents of the straw thereby improving the degradation rates and making other nutrients available to the animal (Jalc, 2002). Nevertheless, its current use in developing countries is still a big question due to limitation in technical skills and the availability of resources to produce and handle large quantities of fungi or their enzymes for practical and field application (Schiere and Ibrahim, 1989). For example, fungi require an environment for them to grow and reproduce, such as pH, temperature, pressure, and O_2, and CO_2 concentrations before, during, and after the treatment period. Zadrazil (1977) shown that the species of fungi acting on the lignin content is the most recommended for rice straw treatment because of its peculiarity to break and degrade structural carbohydrates presented in the rice straw. It is suggested that the new selected fungal strains are essential with desired characteristics to efficiently improve the nutritive value of rice straw. However, data on the use of probiotic fermented rice straw in complete rations/TMR and its effects on cattle are limited. Therefore, the study was carried out to determine the effect of feeding either rice straw-based or probiotic/prebiotic fermented rice straw-based total mixed rations (TMRs) versus the conventional feeding system on feed intake, growth performance, rumen biochemical parameters, and faecal microflora in fattening cattle.

The aim of this study to facilitate and carry out the effect of micro-organism fermented rice straw on cross-bred Sindhi cattle fed different total mixed rations. The effects expected from micro-organism fermented rice straw such as the treatments to be compared, the parameters to be measured, the analysis to be performed as well as the statistical needs are presented.

2. MATERIALS AND METHODS

2.1. Location

The experiment was conducted in the cattle farm of the Research and Technology Transfer Center of Nong Lam University from February to June 2023.

2.2. Treatments and experimental design

Fifty cross-bred Sindhi female cattle were allocated to five pens according to live weight (LW) and fed a basal diet of rice straw (RS) and sulphur-rich minerals (0.1% of diet DM). All diets had about 15% CP. Each pen received one of the following treatments according to a completely randomized design:

T1: Microorganisms fermented RS supplemented with soya meal (SM) and rice polishing (RP).

T2: Microorganisms fermented RS supplemented with dried brewers' grain and RP.

T3: Microorganisms fermented RS supplemented with SM, coconut meal (CM) and RP.

T4: Microorganisms fermented RS supplemented with dried brewers' grain, SM, CM and RP.

T5: Unfermented RS supplemented with SM, palm meal (PM) and RP.

2.3. Animals and housing

The cross-bred Sindhi female cattle had an initial weight in range of 134±26.12kg and were allocated to 5 pens so that mean LW within each pen was similar. Vaccination was done against epidemic diseases and the cattle were drenched against internal parasites before the commencement of the experiment. The cattle were weighed before morning feeding at the beginning of the trial and the end 84-days trial.

2.4. Micro-organism fermented rice straw, feeding and management

Micro-organism fermented rice straw: Dissolve 1 litter of Digest One product containing the *Latobacillus spp., Bacillus spp.* and *Saccharomyces cerevisiae* bacterial strains in 20l of clean water and 0.5kg molasses into a container, make up to 22l. Spray uniformly on the rice straw surface according to the recommendations. Dosage and dilution may vary depending on the intended application and sprayer capacity. It should follow technical recommendations before use.

Cross-bred Sindhi female cattle were adapted gradually to experimental feeds for two weeks prior to starting experiment. The barley dust by-product was bought from Intermalt Vietnam Co, LTD factory in Ba ria Vung Tau province and fed the amount in each diet. Rice straw, rice bran, soya meal, coconut meal and palm meal was bought from the feedstuff companies.

Feeds were offered two times a day, at 7.30am and 2.30pm. Feeds offered and refused were recorded daily. Water was supplied all day.

2.5. Data collection and measurements

Cattle were weighed at the begin and the end of 84-days trial, using an electronic balance. Feeds offers were weighed before giving them to cattle. Feed refusals were collected each morning prior to offering fresh feed and weighed to measurea feed intake. Samples of feeds offered and refused were collected every 14 days to determine DM, Ash, crude protein, crude fiber, lignin, EE, NDF and ADF according to AOAC methods (2005). The morbidity rate was recorded using the diagnosis of treatment condition ie, pneumonia, fever, diarrhea for each animal determined to be sick. Provide the information on all medications given including vaccines, de-wormers, and therapeutic antimicrobial

treatments. Specific medications and dosages administered to each animal.

2.6. Chemical analysis

All samples were analyzed for dry matter (DM), ash, crude protein (CP), crude fiber (CF), lipid (EE), NDF and ADF according to AOAC (2005). While NDF and ADF analysis was followed the Van Soest *et al.* (1991); GE, DE, ME, NEM, NEG, TDN, NEL and NFC was calculated following the formula suggested by Sauvant *et al.* (2004).

2.7. Statistical analysis

The data were subjected to analysis of variance using the General Linear Model procedure of Minitab software version 17.00. Tukey's pairwise comparisons ($P<0.05$) were applied to determine the differences between dietary treatments. Response curves were fitted to the data using linear and quadratic equations in Microsoft Office Excel software, with different total mixed rations as the independent variable (X) and the response component, eg: feed intake (FI), weight gain (WG) ... as dependent variable (Y).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of feeds

There were major differences in crude protein, crude fiber, NDF, DNF, lignin with higher values for brewery waste (BW), rice bran (RB), soya meal (SM), coconut meal (CM), palm meal (PM) than for rice straw (RS) (Table 1). The proportion of intake as fermented RS and concentrate according to treatments for cattle to present in Figure 1 and 2.

Table 1. Composition of diet ingredients (% in DM)

	RS	BW	RB	SM	CM	PM
DM, %	89.11	86.20	89.2	89.01	86.10	87.02
CP, %	4.16	14.96	8.36	22.17	18.89	15.13
CF, %	27.49	15.25	16.64	18.3	9.61	13.80
Lipid, %	0.89	1.98	4.01	11.77	8.73	6.46
Ash, %	12.99	4.21	9.96	4.01	6.30	7.20
Ca, %	0.39	0.10	0.23	0.68	0.15	0.39
P, %	0.10	0.41	0.46	0.29	0.58	0.50
TDN	47.45	72.22	66.75	81.19	83.12	75.53
NDF, %	69.92	41.51	35.02	34.16	46.51	49.87
ADF, %	40.81	16.30	20.54	24.7	27.14	28.56
Lignin, %	2.66	1.14	6.36	3.43	3.73	7.36
NFC, %	11.04	37.35	42.64	27.89	19.58	21.35
Starch, %	0.90	4.73	29.63	2.79	12.30	5.25
GE, Mcal/kg	3.67	4.09	3.70	4.51	4.37	4.12
DE, Mcal/kg	2.09	3.18	2.94	3.57	3.66	3.32
ME, Mcal/kg	1.71	2.61	2.41	2.93	3.00	2.73
NEM, Mcal/kg	0.87	1.70	1.53	1.97	2.03	1.80
NEG, Mcal/kg	0.41	1.27	1.09	1.55	1.62	1.38
NEL, Mcal/kg	1.04	1.65	1.52	1.87	1.92	1.73

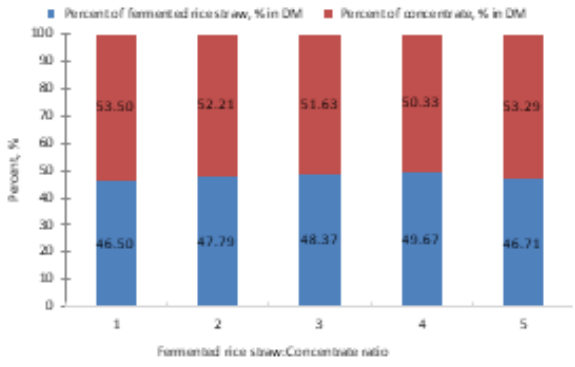

Fig 1. Proportion of intake as fermented rice straw and concentrate according to treatments

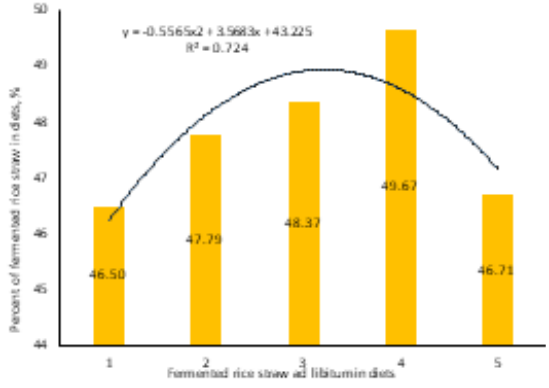

Fig 2. Rice straw and concentrate ratio in diets was differ according to treatments

3.2. Feed intake, feed conversion rate and live weight

DM intake, live weight gains and FCR in T1, T2, T3, T4 and T5 were not differ with 4.32, 4.43, 4.48, 4.60 and 4.34 kg/cattle/day for DM intake; with 462, 510, 538, 543 and 451 g/cattle/day for LWG; with 9.62, 8.92, 8.55, 8.68 and 9.89 for FCR; respectively (Table 3, Figure 3 and 4). Growth rate was similar with more supplementation of concentrate in diets. The similar on the rate of response in LWG when using supplementation of concentrate in fermented RS diets in this study was not in accordance with studies in which protein-rich supplements were fed in increasing quantities in diets rich in carbohydrates; e.g.: FM and molasses-urea (Preston and Leng, 1987), cotton seed cake and ammoniated wheat straw (Weixian *et al*., 1994). Oppositly, Kabir *et al.* (2022) found that the feeding of RS fermented with micro-organism along with concentrate mixture in the form of TMR produced a better response in fattening cattle than the conventional system of feeding concentrate and straw separately.

Table 2. Mean values for changes in live weight, DM intake and feed conversion rate in diets

Item		T1	T2	T3	T4	T5	SEM	P
LW, kg	Initial	134.2	134.2	134.1	134.2	134.1	2.97	1.000
	Final	189.6	195.4	198.6	199.4	188.2	4.44	0.914
	Total weight gain, kg	55.4	61.2	64.5	65.2	54.1	2.13	0.357
	Daily weight gain, kg/day	462	510	538	543	451	17.7	0,357
DM intake, kg/cattle/day	Total feed intake, kg in DM	4,32	4,43	4,48	4,60	4,34	0.17	0,985
	Concentrate	2,31	2,31	2,31	2,31	2,31	0.09	1,000
	Rice straw	2,01	2,11	2,17	2,28	2,03	0.08	0,796
	Percent of rice straw	45.26	46.54	47.12	48.43	45.46	-	-
FCR	Total	9,36	8,68	8,33	8,46	9,62	-	-
	Concentrate	5.01	4.53	4.30	4.26	5.13	-	-
	Rice straw	4.35	4.15	4.03	4.20	4.50	-	-

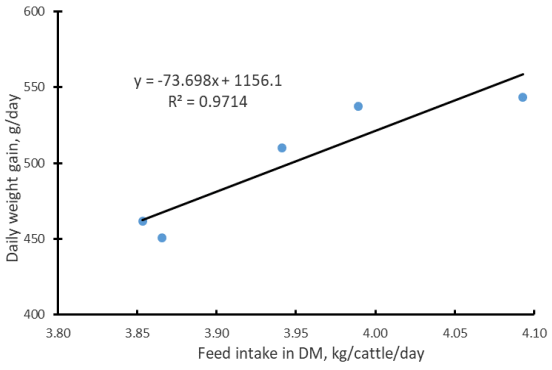

Figure 3. Effect of feed intake on daily weight gain of cattle

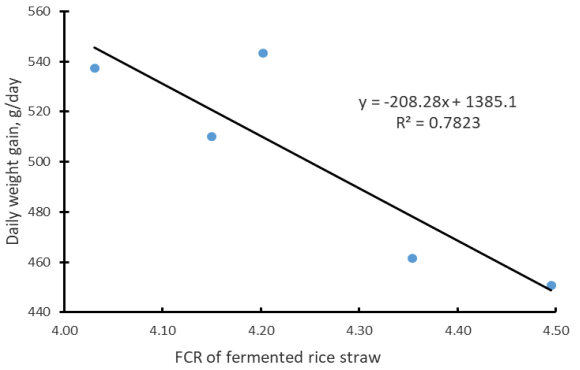

Figure 4. Effect of feed conversion rate of fermented rice straw on daily weight gain of cattle

3.3. Cost and profit

Cost and profit of the trial were recorded and calculated based on the experimental results (Table 3).

Table 3. Cost and profit of the trial

Item	1	2	3	4	5
IW, kg	134.20	134.20	134.10	134.20	134.10
FW, kg	189.60	195.40	198.60	199.40	188.20
WG, kg	55.4	61.2	64.5	65.2	54.1
DWG, g/day	462	510	538	543	451
TFI, kg in fresh	5187	5315	5375	5516	5207
Concentrate, kg	2775	2775	2775	2776	2775
RS, kg	2412	2540	2600	2740	2432
Cost, VNĐ					
RS for 1 kg WG	10,884	10,376	10,078	10,506	11,238
RS for all WG	6,030,000	6,350,000	6,500,000	6,850,000	6,080,000
Concentrate for 1kg WG	30,054	27,206	25,814	25,548	30,776
Concentrate for all WG	16,650,000	16,650,000	16,650,000	16,657,200	16,650,000
Labor/day	80,000	80,000	80,000	80,000	80,000
Buying cattle	12,078,000	12,078,000	12,069,000	12,078,000	12,069,000
Total	153,060,000	153,380,000	153,440,000	153,887,200	153,020,000
Price for 1 kg LWG, VNĐ	90,000	90,000	90,000	90,000	90,000
Price for all WG, VNĐ	170,640,000	175,860,000	178,740,000	179,460,000	169,380,000
Profit	17,580,000	22,480,000	25,300,000	25,572,800	16,360,000

Note: Price of concentrate in average is 6,000 VND/kg, price of roughage in average is 2,500 VND/kg.

4. CONCLUSIONS

Growth rate and FCR were similar in treatments which has higher supplementation of concentrate in a basal diet of micro-organism FRS. It is concluded that the FRS with micro-organism for crossbred Zebu cattle could be used better production based on RS diets.

ACKNOWLEDGEMENTS

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INVESTIGATION OF GROWTH PERFORMANCE AND FEED INTAKE OF CROSS-BRED CALVES BETWEEN SINDHI AND BBB, CHAROLAISE, WAGYU OR BRAHMAN

Nguyen Van Nghia[1], Dang Hoang Dao[2], Le Duc Ngoc[3], Huynh Vu Duy Khang[4] and Duong Nguyen Khang[1]*

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ABSTRACT

The aim of this study was to evaluate the growth performance of high-yielding crossbred calves between Sindhi female with the semen of Wagyu (Wag), Charolaise (Cha), BBB or Brahman (Br) breed from neonatal to 9 months of age at farm of Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh city, Vietnam from April 2022 to February 2023. The experiment was carried out into a completely randomized design with the groups of crossbred calves and lasted 9 months. The results shown that birth weight of crossbred BBB was the highest with 29.60 kg, followed by crossbred Cha and Wag with 24.04 and 22.64kg, the lowest in crossbred Br with 19.65kg (P<0.001). At the age of 3, 6 and 9 months, body weight of crossbred BBB was the highest with 85.5; 150.22 and 219.67kg, respectively; followed by crossbred Cha with 70.04, 115.54 and 159.27kg, respectively; by crossbred Wag with 67.14, 109.64 and 147.17kg, respectively; and the lowest in crossbred Br with 53.69, 83.90 and 117.55kg, respectively (P<0.001). Average daily gain at 5 to 9 month was the highest with crossbred BBB with 0.78 kg/head/day, followed by crossbred Cha and Wag with 0.48 and 0.43 kg/head/day, and the lowest in crossbred Brahman with 0.36 kg/head/day (P<0.001). Feed conversion ratio for the crossbred BBB, Cha, Wag and Br breed was significantly differ (P<0.001) with 5.60, 7.22, 7.82 and 8.25, respectively.

Keywords: *SindhixBBB, SindhixCharolaise, SindhixWagyu, SindhixBrahman, growth, feed conversion rate.*

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

1. INTRODUCTION

Recently, there are many crossbred of cow breed in Vietnam based on the local yellow cattle (Dung *et al.*, 2013). The selling price of the local yellow beef is lower than that of crossbred beef. This is mainly because the beef of crossbred beef becomes a marbling beef which is generally preferred by Vietnamese consumers. In the beef production of crossbred, therefore, it is important to not only reduce the feeding and management costs but also enhance the lean beef quality. Crossbred cattle, as well as other Vietnamese beef cattle, is generally fattened indoors with a concentratebased diet until they are slaughtered. Thus, a grazing system seems to be possible way to reduce the feeding and management costs. On the other hand, it is necessary to investigate enough the characteristic of beef of pasture-fed crossbred cattle to improve better beef production for future development.

The increasing consumption of meat in developing countries related to rising household income and rapid urbanisation has been well documented, particularly in Asia, Latin America and Africa (McDermott *et al*., 2010). This trend is also observed in Vietnam (Dung *et al*., 2013). While much of this increase can be attributed to increased consumption of pork, consumption of beef has been predicted to almost double between 2001 and 2020 (Stur *et al*., 2013). The Vietnamese domestic production of beef meat for markets however remains far below the demand. At present 106,500 tons of beef meat and 550,000 cattle are imported in year 2020 (Customer Statistical, 2021). The increasing demand for beef meat creates opportunities for farmers to move from "cattle keepers" to "producers of beef meat", pariticularly in Vietnam. It provides a potential pathway from poverty for smallholder farmers (Stur *et al*., 2013).

To date, knowledge on sustainable domestic beef production in Vietnam is scarce. Traditionally cattle are kept as asset accumulation or as draught animals which are only culled and used as meat animals at the end of their useful working lives. The indigenous cattle in Vietnam are small in size and have low performance in meat and milk production, but they can adapt well with harsh conditions and have good reproduction capacity (FAO, 2003). Genetic improvement within a cow breed is relatively slow process due to the long generation interval, range about 0.5-3% per year (Smith, 1984). Substantial improvement can only be achieved after 10-20 year (Kosgey *et al*., 2011). Terminal cross-breeding of indigenous breed (Bos indicus) with high performance exotic breeds (Bos taurus) deserves more attention as a mean to increase the output of beef cattle in the subtropics and tropics. Promising results are already obtained in Africa and Southeast Asia (Scholtz and Theunissen, 2010; Waritthitham *et al*., 2010). An added advantage of the system of terminal cross-breeding utilizing indigenous breeds is that the conservation of these breeds is ensured, because a constant stream of purebred females will be required (Scholtz and Theunissen, 2010).

The system of cross-breeding could be successful if it is accompanied by appropriate herd and health management at the farm level as well as by sufficient forage quality and supply. One of the challenges for extending the application of this cross-breeding has been to identify the documenting current practices of crossbreed's beef cattle in southern Vietnam. Through this knowledge, guidelines will be developed to modify current practices towards a more sustainable and profitable production by to raising the per head performance significantly. In the research farm, different breeding and feeding strategies will be evaluated with different breed such as BBB, Charolise, Wagyu and Brahman. The aim of this study was to evaluate the growth performance of high-yielding crossbred calves between Sindhi with BBB, Charolaise, Wagyu or Brahman from neonatal to 9 months of age.

2. MATERIALS AND METHODS

2.1. Location

The experiment was conducted in the cattle farm of the Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City from April 2022 to February 2023.

2.2. Treatments and experimental design

From neonatal to 5 months of age, the calves were kept with their mothers. From 6 to 9 months of age, calves were separated and divided to the groups.

Sixty female calves crossed from the Sindhi (Sind) female cattle with the semen of BBB, Cha, Wag or Br breed were allocated to four pens according to breed group and fed the same basal diet for all treatments. Each pen received one of the following treatments according to a completely randomized block design with different breeds of Sind×BBB, Sind×Cha, SindxWag and SindxBr.

2.3. Animals and housing

For the feeding trial, the cross Sind×BBB, Sind×Cha, SindxWag and SindxBr female calves had an initial weight in range of 126.67±3.31, 101.73±2.93, 95.260±3.86 and 74.08±3.97kg, respectively and were allocated to 4 pens. Vaccination was done against epidemic diseases and the cattle were drenched against internal parasites before the commencement of the experiment. The cattle were weighed before morning feeding at the begin and the end of the trial.

2.4. Feed, feeding and chemical analysis

Crossbred Sindhi female calves were adapted gradually to experimental feeds for two weeks prior to starting experiment. The barley dust by-product (as concentrate feed) was bought from Intermalt Vietnam Co, LTD factory in Ba Ria Vung Tau province and fed *ad libitum* for each treatment. Mombasa grass was cut and carry from the grass field of Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City. Feeds were offered two times a day, at 7.30am and 2.30pm. Feeds offered and refused were recorded daily. Water was supplied all day.

All samples of feed and residues were analyzed for dry matter (DM), ash, crude protein (CP), crude fiber (CF), lipid (EE), NDF and ADF according to AOAC (2005). The NDF and ADF analysis was followed the Van Soest *et al.* (1991). GE, DE, ME, NEM, NEG, TDN, NEL and NFC were calculated following the formula suggested by Sauvant et al. (2004).

2.5. Data collection and measureaments

Cafves were weighed at beginning and monthly individual record, using an electronic balance. Feeds offers were weighed before giving them to cattle. Feed refusals were collected each morning prior to offering fresh feed and weighed to measurea feed intake. Samples of feeds offered and refused were collected every 14 days to determine DM and CP according to AOAC methods (1990). The morbidity rate was recorded using the diagnosis of treatment condition ie, pneumonia, fever, diarrhea for each animal determined to be sick. Provide the information on all medications given including vaccines, de-wormers, and therapeutic antimicrobial treatments. Specific medications and dosages administered to each animal.

2.6. Statistical analysis

The data were subjected to analysis of variance using the General Linear Model procedure of Minitab software version 17.00. Tukey's pairwise comparisons ($P<0.05$) were applied to determine the differences between different breeds or dietary treatments. Response curves were fitted to the data using linear and quadratic equations in Microsoft Office Excel software, with different total mixed rations as the independent variable (X) and the response component, different breeds, feed intake, weight gain... as dependent variable (Y).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of feeds

There were major differences in the protein and lipid contents with higher values for barley dust by-product than for Mombasa grass (Table 1).

Table 1. Composition of diet ingredients
(% in DM)

Items	Barley dust by-product	Mombasa grass
DM, %	86.6	20.4
CP, %	26.04	11.84
CF, %	12.07	29.02
Lipid, %	7.83	1.76
Ash, %	4.40	9.32
Ca, %	0.30	0.60
P, %	0.38	0.26
TDN, %	83.01	58.14
NDF, %	44.88	63.17
ADF, %	19.21	34.13
Lignin, %	5.23	3.04
NFC, %	16.86	14.42
Starch, %	4.98	2.06
GE, Mcal/kg	4.83	4.21
DE, Mcal/kg	3.65	2.56
ME, Mcal/kg	2.99	2.10
NEM, Mcal/kg	2.03	1.24
NEG, Mcal/kg	1.61	0.79
NEL,Mcal/kg	1.91	1.30

3.2. Dry matter intake, FCR and live weight

Proportion of dietary intake betwen mombasa and barley dust by-product was differ according to the breeds (Figure 1). DM intake decreased with a curvilinear trend as crossed with BBB, Cha, Wag and Br breeds (Table 2). Feed conversion ratio was improved by BBB and Cha breeds, reflecting the high rates of live weight gain with 0.78 and 0.48 kg/day (Table 2, Figure 2 and 3). Growth rate increased with the cross-breeding of indigenous breed with high performance exotic breeds as BBB and Cha. The increasing rate of response in live weight gain when cross-breeding with high performance exotic breeds is in accordance with similar studies in which promising results are already obtained in Africa and Southeast Asia (Scholtz and Theunissen, 2010; Waritthitham *et al*., 2010). An advantage of the system of terminal cross-breeding by utilizing indigenous breeds is that the conservation of these breeds is ensured, because a constant stream of purebred females will be required for genetic develpment (Scholtz and Theunissen, 2010).

Table 2. Mean values for changes in live weight, DM intake, feed conversion rate in breeds

	Treatments	BBB	Charolaise	Wagyu	Brahman	SEM	P
LW, kg	New-born	29.60^{a}	24.04^{b}	22.64^{b}	19.65^{c}	0.52	0.001
	3 month of ages	85.05^{a}	70.04^{b}	67.14^{c}	53.69^{d}	1.48	0.001
	Initial (5 month of ages)	126.67^{a}	101.73^{b}	95.26^{c}	74.08^{d}	2.28	0.001
	6 month of ages	150.22^{a}	115.54^{b}	109.64^{c}	83.90^{d}	3.10	0.001
	Final (9 month of ages)	219.67^{a}	159.27^{b}	147.27^{c}	117.55^{d}	4.88	0.001
	Total weight gain, kg	93.00^{a}	57.55^{b}	51.91^{c}	43.47^{d}	2.51	0.001
	Daily weight gain, kg/day	0.78^{a}	0.48^{b}	0.43^{b}	0.36^{c}	0.021	0.001
DM intake, kg/cattle/day	Total feed intake, kg in DM	4.34^{a}	3.43^{b}	3.37^{b}	2.96^{c}	0.07	0.001
	Concentrate	1.71^{a}	1.70^{a}	1.60^{b}	1.71^{a}	0.007	0.001
	Mombasa	2.63^{a}	1.73^{b}	1.77^{b}	1.25^{c}	0.066	0.001
	Percent of Mombasa	60.52	50.51	52.48	42.36	-	-
FCR	Total	5.60^{c}	7.22^{b}	7.82^{ab}	8.25^{a}	0.15	0.001
	Concentrate	2.21^{c}	3.58^{b}	3.72^{b}	4.75^{a}	0.124	0.001
	Mombasa	3.39^{b}	3.64^{b}	4.10^{a}	3.50^{b}	0.05	0.001

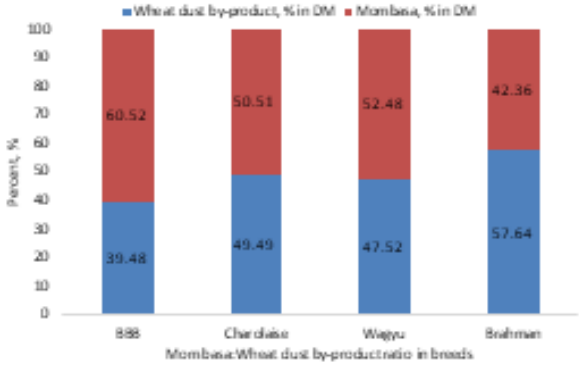

Fig 1. Proportion of dietary intake as mombasa and barley dust by-product according to breeds

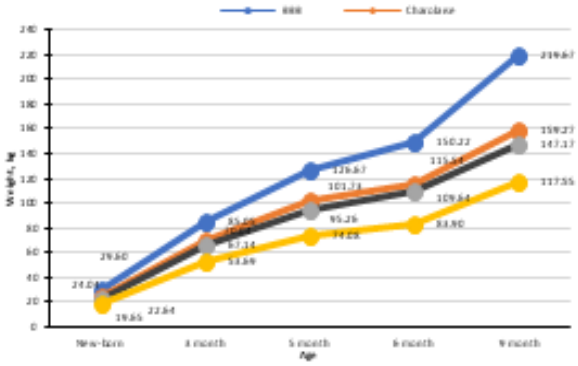

Fig 2. Live weight according to breeds

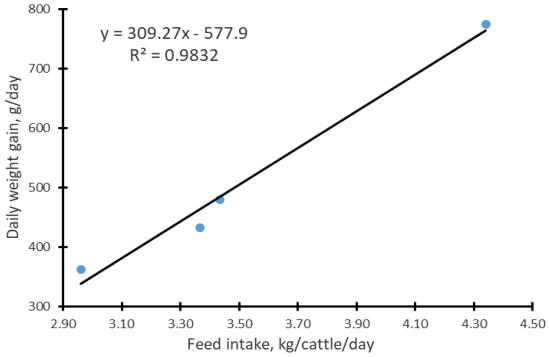

Fig 3. Effect of feed intake on daily weight gain of cattle breeds fed mombasa as basal diet

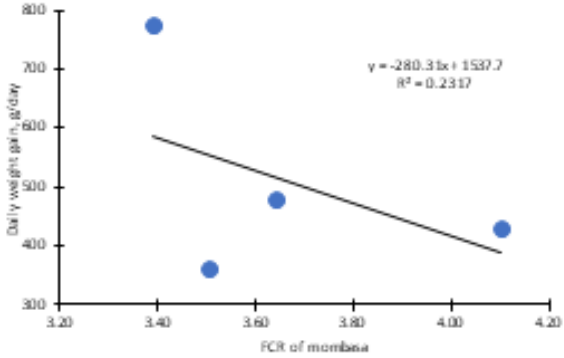

Fig 4. Effect of FCR of mombasa on daily weight gain of cattle breeds fed mombasa as basal diet

3.3. Cost and profit

Table 4. Cost and profit of the trial

Item	BBB	Charolaise	Wagyu	Brahman
IW, kg	126.67	101.73	95.26	74.08
FW, kg	219.67	159.27	147.17	117.55
WG, kg	93.0	57.5	51.9	43.5
DWG, g/day	775	480	433	362
TFI, kg in fresh	26,874	18,894	18,974	14,627
Concentrate, kg	3,465	3,437	3,235	3,451
Mombasa, kg	23,409	15,457	15,739	11,176
Cost, VNĐ				
Mombasa for 1kg WG	41,951	44,771	50,533	42,849
Mombasa for all WG	58,522,008	38,641,708	39,347,153	27,939,728
Concentrate for 1kg WG	14,904	23,894	24,927	31,754
Concentrate for all WG	20,791,327	20,622,539	19,409,056	20,705,056
Labor/day	80,000	80,000	80,000	80,000
Buying cattle	12,667,000	10,173,000	9,526,000	7,408,000
Total	278,918,335	221,459,247	211,246,210	169,364,784
Price for 1kg WG, VNĐ	100,000	100,000	100,000	100,000
Price for all WG, VNĐ	329,505,000	238,905,000	220,755,000	176,325,000
Benefit	50,586,665	17,445,753	9,508,790	6,960,216

Note: Price of concentrate in average is 6,000 VND/kg, price of roughage in average is 2,500 VND/kg.

Cost and profit of the trial were recorded and calculated based on the experimental results (Table 4).

4. CONCLUSIONS

Growth rate was increased and feed conversion was improved in treaments which had cross-breeding of indigenous breed with high performance exotic breeds deserves more attention as a mean to increase the output of beef cattle in the tropics in a basal diet of mombasa grass.

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EFFECTS OF SUPPLEMENTATION OF HERB MIXTURE ON THE INCREASING RATES OF LIVE WEIGHT AND BODY DIMENSIONS OF NOI CHICKENS FROM 28 TO 98 DAYS OLD

Le Thi Thuy Hang[1,2] and Nguyen Tuyet Giang[1,2]*

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ABSTRACT

The study was conducted to investigate the effect of dietary herb mixture (mixed powder of turmeric, lemongrass and garlic) on the increasing rates of live weight and body dimensions of Noi chickens from 28 to 98 days of age. There was a factorial experimental design with 2 sexes of the chickens and 5 levels of herbal powder in the diet (0.0, 0.25, 0.5, 0.75 and 1.0%). Each treatment was replicated 3 times with 8 birds/cage, giving a total of 240 chickens. Research results showed that the male chickens had relatively higher mean values for most of the measured traits as compared to the females ($P<0.05$). Chickens fed a diet supplemented with herbal powder had higher values of wing length, wingspan, thigh length and shank length compared to those fed the control diet. Birds supplemented with 0.75 and 1.0% herbal powder showed highest increasing rates in wing length, wingspan and shank length. The combination of the sex and herb level did not lead to significant difference in most of the indicators ($P>0.05$).

Keywords: *Body dimension, increasing rate, live weight, Noi chicken, herb mixture.*

1. INTRODUCTION

Chickens have increased in relevance as the most commonly farmed species, with over 90 billion tons of chicken meat produced annually, making them one of the top food industries worldwide. Since then, a large diversity of antibiotics was also used in broiler production to enhance growth rate and reduce mortality. However, the abuse of antibiotics as agent of growth promoter in poulrty farming, leading to antibiotic resistance and increasing treatment failures, is the biggest threat to global health, food security, and sustainable agriculture (Mund *et al.*, 2017, Agyare *et al.*, 2018). Accordingly, strategies aimed to discover antibiotic alternatives have been encouraged. Among the solutions, herbal medicines are a possible alternative to antibiotic growth promoters because they are natural, readily available, non-toxic and residue-free (Abdelli *et al.*, 2021).

The flora in Vietnam comprises more than 12,000 plant species, with about one-third (3,948 species) has been used as common medicinal herbs (Phan and Otsukab, 2018). In animal production, herbs or phytogenics with varied biologically active compounds have attracted increased interest as feed additives, due to the ability to enhance enzyme secretion and nutrient absorption, promote immune status and inhibit the growth of pathogenic microbes in the gut. This in turn suggests that medicinal herbs are able to improve animal performance (Abdelli *et al.*, 2021). However, better understanding about the interaction of phytogenics with poultry growth is crucial to design. The objectives of this study were to determine the influence of herb levels on the increasing rates of live weight and some body dimensions of Noi chickens.

2. MATERIALS AND METHODS

The experiment was conducted from February to April 2022, on an experimental

[1]An Giang University, An Giang, Vietnam

[2]Vietnam National University Ho Chi Minh City, Vietnam

* Corresponding Author: Dr. Le Thi Thuy Hang, Department of Animal Science and Veterinary medicine, An Giang University, An Giang province, Vietnam. Tel: (84)919458 223, E-mail: ltthang@agu.edu.vn.

farm in Long Xuyen city, An Giang province. A total of 240 Noi chickens were acclimatized for seven days before starting the experiment at 28 days of age, in a factorial designed study with 2 sexes and 5 levels of herb mixture in the diet (0, 0.25, 0.5, 0.75 and 1.0%). The treatments were replicated 3 times with 8 birds/cage.

The herb powder was mixed from three different ingredients of turmeric, lemongrass and garlic with an equivalent ratio of 1:1:1, before added into the basal diets. The basal diets were formulated to meet the nutritional requirements of broiler chickens as indicated by NRC (1994) for two growth phases, as shown in Table 1. Water and mash feed were offered *ad libitum* according to chickens' requirements.

Table 1. Ingredients, chemical composition 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 vit A; 350,000IU vit D_3; 1,000mg vit E; 1,500,000mg B_1; 2,500,000mg vit B_2; 8,000mg vit B_5; 650mg vit B_6; 9,000mg vit 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. **The ME value was estimated according to database of McDonald et al. (2011). The chemical composition was analysed following the standard methods of AOAC (2005).*

Two birds (marked with a numbered wing tag) in each cage were used to measure body weight and body dimensions according to earlier descriptions of FAO (2012), Do *et al.* (2019), and Nguyen *et al.* (2020). The criteria were live weight (LW, g), body length (BoL, cm), neck length (NL, cm), back length (BaL, cm), wing length (WL, cm), wingspan (WS, cm), chest girth (CG, cm), breast width (BW, cm), keel length (KL, cm), thigh length (TL, cm), drumstick length (DL, cm), drumstick circumference (DC, cm), shank length (SL, cm) and shank circumference (SC, cm). The LW of the chickens were measured using a 0.01g electronic weighing scale. The linear measurements were performed using a tailor's tape, acording to Rachma *et al.* (2013). Scheduled vaccines against contagious diseases such as Newcastle, Gumboro, fowl pox and avian influenza disease were administered.

The numerical data were statistically analyzed using GLM procedure of Minitab 16.0 and differences among the individual means were compared by the Tukey test. Differences were considered significant at $P<0.05$. Pearson's coefficients of correlation were calculated to determine the relationships among measured traits.

3. RESULTS AND DISCUSSION

3.1. Live weight and body dimensions of Noi chickens at 28 days of age

Mean values for LW and body measurements with respect to sex and herb level are presented in Tables 2. At 28 days of age, Noi chickens had an average weight of 330 g/bird in male chickens and 282 g/bird in female chickens, lower than that of Tam Hoang chickens in the study of Nguyen *et al.* (2020). At the beginning, LW and most of the measurements were similar, the significant difference ($P<0.05$), was mainly due to the sex difference. Particularly, male birds have a higher LW than the females, leading to differences in other dimensions, as reported by Do *et al.* (2019). The supplementation of herb mixture resulted in significant differences in WL and TL. In the same pattern, the combination of sex and herb level (S×H) did not lead to any significant difference in all indicators ($P>0.05$) at the beginning of the experiment.

3.2 Live weight and some body dimensions of Noi chickens at 98 days of age

The development of the body dimensions in chickens is synonymous with the growth performance and reflects the phenotypic characterization of sex and traits (FAO 2012). The effects of experimental factors on LW and body measurements in 98-day-old chickens are presented in Table 3. Similar to the results obtained in Table 2, in 98-day-old chickens, almost all parameters were significantly different (P<0.05) due to the influence of sex. The LW and other variables of male birds were higher than those of the females with the exception of back length (BaL). The results of this study are similar to findings of previous reports (Azmal *et al*., 2006, Do *et al*., 2019), showing that roosters are always heavier than hens, leading to a significant difference in most of the body measurements.

The herb level (H) did not affect the LW but had an impact on some measures of Noi chickens. Herbal supplementation at 0.75 and 1.0% showed higher results of NL, WL, WS, BW and SL than other levels. For DL, chickens fed the control diet had a value of 13.5cm, which was significantly lower (P<0.05) than those fed diet supplemented with herb. Table 3 also shows that the interaction between sex and herb only affected the BW (P<0.05). The male birds consumed diets with herb supplemented at 0.75 and 1.0% had significantly higher BW than the others. The results further showed a positive effect of herb mixture (turmeric, lemongrass and garlic) on the dimensional traits of Noi chickens at 98 days of age, particularly breast, the most valuable part of the chicken carcass.

Table 2. Live weight and some body dimensions of Noi chickens at 28 days of age

Factors			LW	BoL	NL	BaL	WL	WS	CG	BW	KL	TL	DL	DC	SL	SC
Sex (S)	Male		330^{a}	23.6^{a}	6.01	13.0	12.5^{a}	27.0	14.7	8.14	5.94^{a}	5.56	6.31	6.09	4.99^{a}	2.64
	Female		282^{b}	23.0^{b}	6.03	12.8	12.0^{b}	26.8	14.4	7.90	5.72^{b}	5.48	6.21	5.85	4.75^{b}	2.54
Herb level, %	0.0		293	23.3	5.96	12.8	12.4^{ab}	26.8	14.6	8.37	5.77	5.28^{b}	6.31	6.03	4.74	2.48^{b}
	0.25		316	24.1	6.16	13.2	12.6^{a}	27.6	14.6	7.72	5.95	5.90^{a}	6.54	5.90	4.88	2.57^{ab}
	0.5		300	22.9	6.03	12.9	12.0^{b}	26.4	14.4	7.92	5.64	5.35^{ab}	6.03	5.84	4.84	2.51^{ab}
	0.75		306	22.7	5.82	12.9	12.1^{ab}	26.7	14.7	8.31	5.87	5.45^{ab}	6.26	5.93	5.03	2.58^{ab}
	1.0		315	23.4	6.13	12.5	12.2^{ab}	26.8	14.3	7.81	5.93	5.61^{ab}	6.18	6.16	4.84	2.80^{a}
S×H	Male	0.0	309	24.0	5.97	13.1	12.4	26.6	14.4	7.98	5.95	5.50	6.45	6.13	4.73	2.52
	Male	0.25	343	24.4	6.12	13.8	13.1	28.0	14.8	7.97	6.20	6.03	6.75	6.07	5.13	2.67
	Male	0.5	313	23.3	6.03	12.8	12.1	26.4	14.5	8.00	5.50	5.12	6.00	5.77	4.83	2.47
	Male	0.75	332	23.0	5.73	13.0	12.5	27.0	15.3	8.88	6.13	5.45	6.33	6.07	5.22	2.67
	Male	1.0	352	23.4	6.18	12.2	12.6	26.7	14.3	7.88	5.92	5.68	6.03	6.43	5.02	2.87
	Female	0.0	277	22.7	5.95	12.5	12.4	27.0	14.8	8.75	5.58	5.07	6.17	5.92	4.75	2.43
	Female	0.25	288	23.7	6.20	12.7	12.2	27.2	14.3	7.47	5.70	5.77	6.33	5.73	4.63	2.47
	Female	0.5	287	22.5	6.03	13.0	11.9	26.4	14.4	7.83	5.78	5.58	6.05	5.92	4.85	2.55
	Female	0.75	280	22.4	5.90	12.8	11.7	26.5	14.2	7.73	5.60	5.45	6.18	5.80	4.85	2.50
	Female	1.0	279	23.5	6.08	12.8	11.8	26.8	14.3	7.73	5.95	5.53	6.33	5.88	4.67	2.73
SEM	S		4.47	0.22	0.08	0.14	0.09	0.20	0.19	0.20	0.07	0.09	0.08	0.10	0.06	0.05
	H		7.06	0.35	0.13	0.22	0.14	0.31	0.30	0.31	0.12	0.15	0.12	0.17	0.10	0.08
	S×H		9.99	0.50	0.18	0.31	0.20	0.44	0.42	0.44	0.17	0.21	0.17	0.23	0.14	0.11
P	S		0.000	0.044	0.820	0.215	0.000	0.538	0.319	0.398	0.046	0.567	0.356	0.107	0.012	0.152
	H		0.122	0.079	0.348	0.273	0.021	0.103	0.877	0.479	0.334	0.039	0.056	0.695	0.372	0.039
	S×H		0.138	0.681	0.961	0.056	0.155	0.708	0.469	0.317	0.072	0.272	0.253	0.673	0.255	0.718

LW=live weight (g), BoL=body length (cm), NL=neck length (cm), BaL=back length (cm), WL=wing length (cm), WS=wingspan (cm), CG=chest girth (cm), BW = breast width (cm), KL=keel length (cm), TL=thigh length (cm), DL=drumstick length (cm), DC=drumstick circumference (cm), SL=shank length (cm), SC=shank circumference (cm). Means in the same column with different superscripts are significantly different (P<0.05).

3.3. Increasing rate of live weight and body dimensions Noi chickens 28-98 days of age

It can be seen that LW and all body measurements of Noi chickens increased with age (Table 4). Linear LW of the chickens reflect the structure increase which is mostly dependent on the skeletal and muscle growth. This completely followed the normal growth pattern of poultry from the time of hatching to maturity. From 28 to 98 days of age, LW showed the highest increasing rate (>300%), the other parameters also had high rates of increase (>50%). Differences in the increasing rates were shown mainly between the males and females (P<0.05), proving that roosters have higher growth performance than hens. It may reflect a positive impact of the combined product from turmeric, lemongrass and garlic on BW, DL and DC, leading to differences in the increasing rates of these traits. Significant differences between treatments with and without herbal supplements are also shown in the increasing rate of WL, WS, DL and SL.

The chickens receiving diets with herbs had a higher growth rate than those fed the control diet. For SL, chickens fed 1.0% herb had the highest increasing rate (118%), compared to the lowest of the control treatment (86.5%). For DL, the birds supplemented with 0.5% and 1.0% herb had the highest rates (141 and 140%, respectively) compared to the others. Interaction between sex and herb level did not affect the increasing rate of LW and other variables (P>0.05).

The present findings suggest that herbal supplementation had a positive effect on the growth rate of several dimensions of the Noi chickens, as indicated in previous studies that relative body weights and size of the chickens increased in response to increasing phytogenic compounds (Kwiecień and Winiarska-Mieczan, 2009, Abdelli *et al.*, 2021). The beneficial active ingredients such as allicin in garlic, curcumin in turmeric and citral in lemongrass might act as growth-promoting agents to improve performance of the chickens during their growth period (Ratika *et al.*, 2018, Alagbe and Oluwafemi, 2019).

Table 3. Live weight and body dimensions of Noi chickens at 98 days of age

Factors		LW	BoL	NL	BaL	WL	WS	CG	BW	KL	TL	DL	DC	SL	SC
Sex (S)	Male	1,824^{a}	46.2^{a}	14.7^{a}	25.5	23.7^{a}	52.2^{a}	26.6^{a}	16.8^{a}	12.1^{a}	11.0^{a}	15.1^{a}	12.4^{a}	10.4^{a}	4.87^{a}
	Female	1,488^{b}	43.0^{b}	12.3^{b}	26.1	21.8^{b}	48.1^{b}	25.0^{b}	14.1^{b}	11.2^{b}	10.3^{b}	13.6^{b}	11.1^{b}	8.87^{b}	4.28^{b}
Herb level, %	0.0	1,618	43.7	13.7^{ab}	24.2	20.1^{b}	48.3^{b}	25.1	13.5^{b}	11.7	10.3	13.2^{b}	11.2^{b}	8.83^{c}	4.42
	0.25	1,614	44.9	12.8^{ab}	25.2	23.3^{a}	48.2^{b}	25.1	15.2^{a}	11.2	10.3	14.9^{a}	11.8^{ab}	9.50^{bc}	4.33
	0.5	1,638	44.3	12.7^{b}	26.2	23.2^{a}	49.8^{ab}	25.7	16.0^{a}	11.2	10.5	14.5^{a}	12.0^{ab}	9.17^{c}	4.62
	0.75	1,702	44.8	14.4^{a}	28.3	23.7^{a}	52.2^{a}	26.3	16.0^{a}	11.7	11.1	14.3^{a}	11.5^{ab}	10.2^{ab}	4.87
	1.0	1,709	45.3	14.1^{ab}	25.2	23.5^{a}	52.0^{a}	26.8	16.4^{a}	12.4	10.8	14.7^{a}	12.5^{a}	10.5^{a}	4.65
S×H	Male 0.0	1,670	44.7	14.0	23.3	20.8	49.0	26.0	13.9^{d}	12.2	10.5	14.0	12.0	9.50	4.50
	Male 0.25	1,819	46.8	14.0	26.2	23.7	50.7	26.8	16.8^{abc}	11.3	10.7	15.7	12.6	10.3	4.70
	Male 0.5	1,834	46.2	14.0	26.7	24.3	52.8	26.8	17.2^{ab}	11.3	10.8	15.3	12.5	9.83	4.90
	Male 0.75	1,839	46.3	15.8	25.3	25.2	54.5	26.2	17.7^{a}	12.0	11.3	14.5	11.8	10.9	5.10
	Male 1.0	1,958	47.0	15.8	26.0	24.3	54.0	27.1	18.3^{a}	13.5	11.5	15.8	13.2	11.5	5.17
	Female 0.0	1,566	42. 7	13.3	25.0	19.3	47.7	24.2	13.2^{d}	11.2	10.1	12.3	10.3	8.17	4.33
	Female 0.25	1,408	43.0	11.7	24.3	22.9	45.8	23.4	13.7^{d}	11.0	10.0	14.2	11.0	8.67	3.97
	Female 0.5	1,440	42.5	11.3	25.7	22.0	46.8	24.5	14.8^{bcd}	11.2	10.2	13.7	11.4	8.50	4.33
	Female 0.75	1,565	43.3	13.0	31.3	22.3	50.0	26.5	14.3^{d}	11.3	11.0	14.2	11.2	9.50	4.63
	Female 1.0	1,461	43.7	12.3	24.3	22.7	50.0	26.5	14.5^{cd}	11.4	10.2	13.7	11.8	9.50	4.13
SEM	S	41.4	0.36	0.25	0.84	0.31	0.51	0.44	0.21	0.21	0.16	0.15	0.17	0.15	0.10
	H	65.4	0.57	0.39	1.33	0.48	0.80	0.69	0.33	0.33	0.26	0.24	0.28	0.23	0.17
	S×H	92.5	0.81	0.56	1.88	0.69	1.13	0.98	0.47	0.47	0.36	0.34	0.39	0.33	0.23
P	S	0.000	0.000	0.000	0.600	0.000	0.000	0.020	0.000	0.010	0.009	0.000	0.000	0.000	0.001
	H	0.735	0.328	0.019	0.268	0.000	0.003	0.349	0.000	0.094	0.149	0.000	0.030	0.000	0.211
	S×H	0.286	0.807	0.165	0.225	0.606	0.366	0.375	0.029	0.300	0.624	0.128	0.708	0.815	0.460

Table 4. Increasing rate (%) of live weight and body dimensions Noi chickens 28-98 days of age

Factors			LW	BoL	NL	BaL	WL	WS	CG	BW	KL	TL	DL	DC	SL	SC
Sex (S)	Male		455	95.8	147^{a}	96.7	89.0	93.7^{a}	82.1	110^{a}	103	98.9	139^{a}	105^{a}	109^{a}	85.9
	Female		429	87.8	105^{b}	104.7	82.4	79.5^{b}	74.0	80.6^{b}	96.4	89.1	119^{b}	91.6^{b}	87.4^{b}	69.7
Herb level, %	0.0		454	87.1	130	89.1	62.1^{b}	80.4^{bc}	72.3	63.7	102	95.7	109^{b}	85.9	86.5^{b}	78.4
	0.25		410	86.6	108	91.0	84.8^{a}	74.5^{c}	73.6	102	88.3	76.3	128^{ab}	100	94.5^{ab}	68.7
	0.5		445	93.2	110	103	93.3^{a}	88.6^{abc}	78.2	107	100	97.6	141^{a}	106	89.8^{b}	84.6
	0.75		460	97.5	151	119	96.0^{a}	95.4^{a}	79.0	92.9	99.2	105	129^{ab}	95.1	102^{ab}	88.8
	1.0		441	94.5	131	101	92.2^{a}	94.2^{ab}	87.1	111	110	94.9	140^{a}	103	118^{a}	68.4
S×H	Male	0.0	442	85.9	135	77.5	67.7	84.2	81.2	76.8	105	91.3	118	96.9	101	78.7
	Male	0.25	431	91.8	129	89.6	81.4	80.8	82.3	116	82.8	78.8	133	108	101	76.5
	Male	0.5	488	97.9	132	109	101	99.9	86.2	124	106	113	156	118	104	99.1
	Male	0.75	458	102	181	94.8	102	102	71.2	99.7	95.8	109	129	95.5	109	91.9
	Male	1.0	457	102	158	113	92.5	102	89.5	133	128	103	162	105	129	83.2
	Female	0.0	466	88.2	124	101	56.5	76.6	63.5	50.5	100	100	100	74.9	72.0	78.2
	Female	0.25	389	81.4	88.0	92.4	88.2	68.2	64.9	87.7	93.9	73.8	124	92.3	87.6	60.9
	Female	0.5	402	88.6	88.9	97.4	85.1	77.4	70.2	90.2	93.7	82.7	126	95.1	75.5	70.1
	Female	0.75	463	93.3	120	143	90.2	89.0	86.7	86.0	103	102	129	94.7	95.9	85.8
	Female	1.0	424	87.4	104	89.8	91.9	86.4	84.8	88.7	91.9	87.0	117	101	106	53.5
SEM	S		16.5	2.84	7.17	6.66	3.16	2.19	4.34	7.78	4.21	5.44	3.78	5.11	3.94	5.54
	H		26.0	4.48	11.3	10.5	4.99	3.46	6.86	12.3	6.66	8.61	5.97	8.08	6.23	8.75
	S×H		36.8	6.34	16.0	14.9	7.06	4.89	9.71	17.4	9.42	12.2	8.44	11.4	8.81	12.4
P	S		0.273	0.060	0.000	0.404	0.154	0.000	0.204	0.015	0.246	0.215	0.001	0.088	0.001	0.052
	H		0.697	0.368	0.091	0.311	0.001	0.001	0.586	0.086	0.273	0.224	0.009	0.439	0.016	0.378
	S×H		0.614	0.750	0.595	0.167	0.482	0.646	0.393	0.928	0.132	0.610	0.098	0.809	0.817	0.690

4. CONCLUSION

From the analysis, it is concluded that the live weight and body dimensions of Noi chickens increased with age. The male chickens had relatively higher mean values for most of the measured traits as compared to the females. The addition of herb mixture at levels of 0.75-1.0% significantly improved the wing length, wingspan, thigh and shank length of the chickens.

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EFFECTS OF EMULSIFIERS IN DIETS ON GROWTH PERFORMANCE AND GUT HEALTH OF BROILERS AND THE ADDED VALUE OF A HOLISTIC APPROACH

Nguyen Quang Thieu[1], To Thi Hong Phuong[1], Steven Cools[2], Partha Nukamel[2], Nguyen Kim Ngan[1], Pham Phuc Thinh[1] and Vo Thi Cam Tu[1]*

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ABSTRACT

A trial was conducted to evaluate the effects of emulsifiers in diets on growth performance, the serum biomedical index and gut health of Cobb 500 broiler chickens. Besides, an illustration has been made of the possible added value of a product scoping a more holistic approach of the fat 'housekeeping' process. A total of 924 one-day-old chickens were randomly assigned into seven treatments with 11 replications of 10 chicks per replicate. The seven treatments were POSCON (diets were formulated according to nutrient recommendations of Cobb 500, 2018); NEGCON (used POSCON diets, except for metabolizable energy (ME) which was reduced with 50 kcal/kg); in T1 and T2 treatments POSCON diet was supplemented on top with 500 g/ton feed (ppm) of Emulsifier 1 (Volamel Extra, VE) and Emulsifier 2 (EM2), respectively; in T3, T4 and T5, NEGCON diet was supplemented on top with 500ppm of VE, EM2 and fat navigator Volamel Compass (VC), respectively. Supplementation didn't affect growth performance and feed intake, nor in standard, nor in ME-reduced diets (P>0.05). However, supplementation of fat navigator VC in the ME-reduced diet was improving FCR (P<0.05) when compared to those in broilers fed the NEGCON diet in the final and overall periods. There were no significant differences between the treatments about liver enzymes and total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) (P>0.05). Higher hepatic dry matter and crude fat content were observed when supplementing EM2 in the ME-reduced diet, compared to all other groups (P<0.05). Carcass percentages, abdominal fat, liver ratio, production index (PI) and feed cost per gain were not different between treatments (P>0.05). Supplementation of the nutritional emulsifier Volamel Extra in standard and ME-reduced diets were improving benefits by 2.84 and 4.27%, respectively, while feeding the fat navigator Volamel Compass to ME-reduced diets improved benefits by 5.03%.

Keywords: *Broilers, fat, emulsifiers, fat navigator, liver enzyme, performance*.

[1] Nong Lam University, Ho Chi Minh City
[2] Nukamel Inc., Hoogbuul, Olen, Belgium
* Corresponding Author: Nguyen Quang Thieu, Nong Lam University, Ho Chi Minh City; Tel: (84)913850960; Email: nguyen.quangthieu@hcmuaf.edu.vn;

1. INTRODUCTION

Today, commercial broilers grow at an incredible rate, not only in the first week after hatching but also in subsequent weeks. Therefore, commercial broilers require a high amount of nutrients, especially energy, in their diets. As we know, fat and oil contain the highest level of available energy amongst the feed ingredients, and fat and oil are often used to increase the energy density of the diet of commercial broilers, which leads to improved growth rates and feed efficiency (Blanch *et al*., 1995; Blanch *et al*., 1996; Tavárez *et al*., 2011; Zhang *et al*., 2011). However, supplementation of a high level of fat and oil in broiler diets may impair fat digestibility and performance, especially in young broilers as they have a lower potential to synthesize bile salts and poor emulsification ability, rather than a poor lipase activity (Meng *et al*., 2004; Noy and Sklan 1998; Upadhaya *et al*., 2018). Consequently, the digestion of fat and oil can be improved by an emulsifier, improving dispersion of oil into water (oil-in-water emulsion) by interfacial tension reduction between both phases, fat or oil and water, which are difficult to mix, increasing the penetration of fat and oil into water (Van der Heijden and de Haan, 2010, Aguilar *et al*., 2013). On its turn, this improved emulsification leads to an intensified lipase activity thanks to an increased fat droplet surface (Ravindran *et al*., 2016). Because of these characteristics, emulsifiers are considered by several researchers as a feed additive improving fat digestibility and growth performance of broilers (Huang *et al*., 2007; Zhang *et al*., 2011; Zaefarian *et al*., 2015; Zhao *et al*., 2015). However, trial conclusions are not always that straightforward, by comparing a plenty of studies the supplementation of emulsifiers in the diets of poultry and livestock has different results or even conflicting results on their performance (Aguilar *et al*., 2013; Roy *et al*., 2010; Zhang *et al*., 2011; Zhao *et al*., 2015; Upadhaya *et al*., 2016; Siyal *et al*., 2017; Upadhaya *et al*., 2018). Some studies identified factors, modifying the effects of emulsifiers on performance of livestock and poultry, such as kinds and dosages of emulsifiers and different sources of fat or oil (Roy *et al*., 2010; Zhang *et al*., 2011; Zhao *et al*., 2015; Upadhaya *et al*., 2016; Upadhaya *et al*., 2017; Siyal *et al*., 2017).

To some extent this variation growth performance can also be attributed to a direct effect on fat metabolism by the presence of the emulsifier, specific fat types or an interaction between both (Esteve-Garcia, 2012). Some emulsifiers, some fat/oil types seem to result in more or less intensified fat accumulation. By steering this metabolic activity, one can expect that this variation in growth performance can be leveled off to some extent. An additive focusing on both, the emulsification phase during fat digestion and the fat metabolization after fat absorption (i.e., a more holistic approach), could result in a more predictable outcome. The remnants of the milk fat globule membrane (MFGM) show promising results in orienting fat metabolism in young animals away from lipid accumulation towards beta oxidation, resulting in less adipose tissue formation during growth (i.e., a leaner growth). This has been illustrated in several mammalian species (human neonates, piglets, mice pups) (Bourlieu *et al*., 2015; Baars *et al*., 2016; Bach Korsholm Knudsen *et al*., 2021; Silva *et al*., 2021; Wu *et al*., 2021). But no experiences have been published yet in poultry (as this MFGM is a dairy component, and not present in eggs).

Recently, nutritionists have been dedicated to the use of fat emulsifiers in the lower nutrient density diets to enhance the bioavailability of nutrients and save feed cost on broilers and livestock. Therefore, the objectives of the current study are to evaluate the effects of different fat emulsifiers on growth performance, the serum biomedical index and gut health of Cobb 500 broiler chickens in standard and ME-reduced diets. Next to that, a new additive, with a more holistic approach on 'fat housekeeping' (i.e., fat digestion and metabolism), called as fat navigator, of which MFGM is one of the active components, will be tested in broiler nutrition.

2. MATERIALS AND METHODS

2.1. Experimental design

The trial was conducted at Nong Lam University, Ho Chi Minh City to evaluate the effects of dietary fat emulsifiers on growth performance, the serum biomedical index and gut health of Cobb 500 broiler chickens. Besides, an illustration has been made of the possible added value of a product scoping a more holistic approach of the fat 'housekeeping' process (categorized as a fat navigator). A total of 924 one-day-old chicks (mixed sex) was randomly assigned into seven treatments with 11 replications of 10 chicks per replicate. The seven treatments were POSCON (diet was formulated according to nutrient recommendations of Cobb 500, 2018); NEGCON (used POSCON diet, except for metabolizable energy (ME) which was reduced with 50 kcal/kg); in T1 and T2 treatments, POSCON-diet was fed and supplemented on top with 500 g/ton feed (ppm) of Emulsifier 1 (Volamel Extra, VE) and Emulsifier 2 (EM2), respectively; in T3, T4 and T5, NEGCON-diet was fed and supplemented on top with 500ppm of VE, EM2 and fat navigator (Volamel Compass, VC), respectively. The feeding duration has three phases including phase 1: 1-14 days of age (DOA), phase 2: 15-28 DOA and phase 3: 29-42 DOA. More details about the dietary ingredients and nutrients can by found in table 1 and 2, respectively. Broilers were housed on floor pens with rice husk litter and had free access to water and feed (*ad libitum).*, and feed was provided daily at 08:00am and 16:00pm, while feed residue was recorded at 7:00am next day. Chicks were vaccinated for Newcastle, Bronchitis and Gumboro diseases.

Broilers were weighed on the 1st, 14th, 28th and 42nd day of the trial, feed intake (FI) was recorded daily at replicate level. Mortality of birds was recorded daily (survival rate, SR) by observation during feeding in the early morning and late evening, live weight (LW) of dead birds was recorded together with the date to correct the feed intake and feed conversion ratio (FCR).

Table 1. Ingredient compositions of trial diets (%)

No	Ingradients	1-14d		15-28d		29-42d	
		POSCON	NEGCON	POSCON	NEGCON	POSCON	NEGCON
1	Corn	53.9	55.28	57	58.21	58.97	60.2
2	Soybean meal	37.71	37	34.47	34.27	30.58	30.33
3	Limestone	1.23	1.24	0.72	0.72	0.73	0.73
4	MCP	1.69	1.69				
5	DCP			1.86	1.86	1.91	1.91
6	Salt	0.25	0.22	0.27	0.27	0.2	0.2
7	Soy oil	3.83	2.83				
8	Fish fat			4.51	3.51	6.38	5.38
9	L-Lysin	0.18	0.28	0.15	0.15	0.14	0.16
10	DL-Methionine	0.33	0.52	0.3	0.29	0.28	0.28
11	L-Threonine	0.12	0.13	0.06	0.06	0.05	0.05
12	Premix Vit-Min	0.25	0.25	0.25	0.25	0.25	0.25
13	Additives**	0.46	0.51	0.41	0.41	0.51	0.51
14	Salinomycine	0.05	0.05				
	Total	100	100	100	100	100	100

**The ratio of ingredients in T1, T2 diets are similar to POSCON-diet and the ingredient ratios of T3, T4 and T5 diets are similarly to NEGCON-diet. *Emulsifier: including Volamel Extra, Emulsifier 2 and Volamel Compass, depending on the treatment, an appropriate amount was added to the T1, T2, T3, T4 and T5 treatments, respectively. **Additives: including sodium bicarbonate and choline chloride.*

Table 2. Nutrient composition of trial diets (%) *

No	Ingradients	1-14 days		15-28 days		29-42 days	
		POSCON	NEGCON	POSCON	NEGCON	POSCON	NEGCON
1	Dry Matter	88.8	88.8	88.9	88.8	89.1	89
2	ME (kcal/kg)	2,975	2,925	3,025	2,975	3,150	3,100
3	Crude Protein	22	22	20.7	20.7	19	19
4	Crude Fat	6.09	5.15	6.78	5.86	8.62	7.69
5	Linoleic Acid	3.13	2.65	1.8	1.7	2.04	1.94
6	Choline (mg/kg)	1,700.00	1,700.00	1,500.00	1,500.00	1,500.00	1,500.00
7	Crude Fiber	2.65	2.66	2.61	2.64	2.53	2.55
8	Calcium	0.9	0.9	0.8	0.8	0.8	0.8
9	Total P	0.77	0.77	0.74	0.74	0.72	0.72
10	Available P	0.45	0.45	0.4	0.4	0.4	0.4
11	Arginine	1.358	1.341	1.268	1.266	1.154	1.151
12	Cysteine	0.288	0.286	0.275	0.276	0.257	0.257
13	Isoleucine	0.829	0.819	0.777	0.776	0.71	0.709
14	Leucine	1.638	1.628	1.565	1.569	1.461	1.464
15	Lysin	1.22	1.29	1.12	1.12	1.02	1.031
16	Methionine	0.626	0.812	0.578	0.578	0.546	0.546
17	Met+Cys	0.91	1.095	0.85	0.85	0.8	0.8
18	Threonine	0.83	0.83	0.73	0.73	0.66	0.66
19	Tryptophan	0.234	0.231	0.218	0.218	0.198	0.197
20	Tyrosine	0.657	0.651	0.62	0.621	0.572	0.572
21	Valine	0.902	0.893	0.85	0.85	0.782	0.781

**Amino acids are digestible.*

At 42 days of age, all pens were covered by a clean plastic cloth which was placed on the floor of the pen for half an hour (till 4h at max) in the morning. Next, fresh excreta were collected with minimal litter contamination. The fecal samples of each pen are mixed into one sample per mentioned pen of the treatment and stored at -18°C until analysis of dry matter and fat content by laboratory of department of Animal Nutrition, Nong Lam University, HCMC.

At the final day of the trial, after weighing, 77 birds were chosen (one bird per pen) to evaluate carcass percentages and collect blood samples to measure liver enzyme such as ALT (Alanine Aminotransferase), AST (Aspartate Transaminase), AL(K)P (Alkaline Phosphatase), total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. Blood samples were collected by inserting a sharp needle into the brachial wing vein of broilers, blood samples were stored at 5°C and immediately sent to the laboratory of MEDLATEC hospital in Ho Chi Minh City to measure biomedical blood index. These birds were then sacrificed by severing the jugular vein and carotid artery below the left earlobe by a single incision and allowed to bleed for five minutes. After complete bleeding and cessation of movement, the measurements were carried out to evaluate carcass quality. Besides, the abdominal fat pad and liver were collected and weighed.

2.2. Data analysis

The data were statistically analyzed by Excel 2010 and Minitab 17.0 software using ANOVA, and Tukey and Chi square test to test the factor effects. A significant difference was set at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

The effects on broilers' growth performance of feeding different emulsifiers

(VE and EM2) in the standard and ME-reduced diets and the fat navigator VC in the ME-reduced diet are shown in Table 3. The emulsifiers VE and EM2 and the fat navigator VC did not affect the live weight (LW) and average daily gain (ADG) at any time points and over the whole period (P>0.05). However, LW and ADG at 42 DOA tend to improve in the treatments supplemented with VE (in the standard and ME-reduced diets) and with VC (in the ME-reduced diets). In agreement with the present results, some recent studies found that dietary supplementation of exogenous emulsifiers improved growth performance of broilers (Tan *et al*., 2016; An *et al*., 2020; Ali *et al*., 2022). The improved growth performance, by feeding emulsifiers may come from the higher digestibility of crude fat, protein, apparent metabolizable energy (aME) and from higher nitrogen retention (Boontiam *et al*., 2017; Upadhaya *et al*., 2018; Wenqing Lai *et al*., 2018).

Table 3. Effects of emulsifiers and fat navigator on growth performance of broilers

Items	POSCON	NEGCON	T1	T2	T3	T4	T5	SEM	P
LW1	45.96	45.85	45.91	46.02	45.96	46.17	46.27	0.071	0.714
LW14	488.71	481.98	477.43	479.45	493.4	479.05	478.43	3.36	0.845
LW28	1567.3	1574.9	1565.7	1560.3	1570.3	1545.9	1573.4	5.76	0.27
LW42	2687.3	2690.7	2711.5	2682	2752.6	2658.4	2743.8	11.8	0.304
ADG1-14	31.63	31.15	30.82	30.96	31.96	30.92	30.87	0.24	0.839
ADG15-28	77.02	78.01	77.78	77.19	76.93	76.26	78.14	0.449	0.931
ADG29-42	80.55	79.72	81.85	79.73	84.8	79.53	83.71	0.815	0.379
ADG1-42	63.07	62.96	63.48	62.63	64.56	62.24	64.24	0.296	0.283

In this trial, feed intake (FI) of broilers was not significantly different (P>0.05) in all periods when feeding the emulsifiers in the diets. In contrast to the results of the present study, Tan *et al*. (2016) and An *et al*. (2020) found that supplementing emulsifiers in broiler diets were increasing FI of broilers, but the FCR of these two studies were contradictory. In another study, Ali *et al*. (2022) found that supplementation of emulsifiers in broiler diets was linearly improving (P<0.05) feed efficiency, especially in the low ME-diets. Besides, this study also reveals that better FCR may come from higher ileal digestibility of crude protein, crude fat and aME-values and the response was greater at lower ME-density diets. Similarly, other studies (Abbas *et al*., 2016; Tan *et al*., 2016 and An *et al*., 2020) also found higher digestibility of crude fat, aME, and gross energy when supplementing an emulsifier in broiler diets. The findings in the present study also show FCR was significantly better (P<0.05) in the third phase and overall period compared to those of broilers in the NEGCON-treatment when feeding VE to standard diets (T1) and VC in the ME-reduced diets (T5).

Table 4. Effects of emulsifiers and fat navigator on feed efficiency of broilers

Items	POSCON	NEGCON	T1	T2	T3	T4	T5	SEM	P
FI1-14	32.26	32.71	32.34	31.49	32.16	32.77	32.39	0.206	0.747
FI15-28	108.23	110.19	108.63	108.54	111.03	111.11	108.91	0.508	0.557
FI29-42	148.86	155	147.87	150.17	156.71	149.06	150.14	1.05	0.181
FI1-42	95.34	98.99	95.4	96.34	99.17	97.12	96.37	0.446	0.092
FCR1-14	1.022	1.052	1.050	1.022	1.019	1.061	1.050	0.009	0.777
FCR15-28	1.406	1.416	1.397	1.409	1.447	1.458	1.396	0.008	0.201
FCR29-42	1.86^{ab}	1.95^{a}	1.81^{ab}	1.90^{ab}	1.85^{ab}	1.88^{ab}	1.79^{b}	0.014	0.035
FCR1-42	1.51^{ab}	1.57^{a}	1.50^{b}	1.54^{ab}	1.54^{ab}	1.56^{ab}	1.50^{b}	0.006	0.002

Means in the same row with different superscript letters are significantly different (P<0.05).

The highest survival rate (table 5) of broilers was recorded in the overall period of the NEGCON-treatment, however, there were no significant differences between treatments (P>0.05). Broilers were raised in an open shed. So, temperature and relative humidity depends on the outside climate conditions. Most of the recorded dead birds were autopsied. There was no evidence that the mortalities of broilers were caused by heat stress in the preceding period.

Table 5. Effects of emulsifiers and fat navigator on survival rate of broilers (%)

Items	POSCON	NEGCON	T1	T2	T3	T4	T5	SEM	P
SR1-14	98.48	99.24	97.73	98.48	99.24	96.97	97.73	0.388	0.685
SR15-28	98.35	99.24	97.59	100	98.48	99.24	100	0.361	0.525
SR29-42	95.13	99.24	97.66	97.73	96.9	98.42	94.56	0.548	0.217
SR1-42	92.42	97.73	93.18	96.21	94.7	94.7	92.42	0.78	0.482

Feeding different emulsifiers in standard and ME-reduced diets and fat navigator VC in ME-reduced diet did not affect the liver enzymes, total cholesterol, HDL and LDL-cholesterol (P>0.05; table 6). The findings of the present study are in line to the results of previous studies (Aguilar *et al*., 2013; Rezaeipour *et al*., 2016; Wenqing Lai *et al*., 2018) who found that liver enzyme activities (ALT and AST) and cholesterol (total cholesterol and HDL-cholesterol) were not affected by adding emulsifiers or bovine bile salt powder in broiler diets. In contrast, Saleh *et al*. (2020) found that total cholesterol and HDL-cholesterol were decreased in a ME-reduced diet or a ME-reduced diet supplemented with emulsifiers. A trend of linear reduction (P=0.051) in low-density lipoprotein (LDL) in the serum of broilers fed an emulsifier blend was observed, but excepted for the total cholesterol and HDL-cholesterol (Upadhaya *et al*., 2018). According to the literature serum triglycerides, HDL and LDL-cholesterol concentrations have been regarded as diagnostic markers in lipid metabolism. Fatty acids are synthesized in the liver and transported via LDL or chylomicrons for storage in adipose tissue as triglycerides (Hermier, 1997). The findings of the present study imply that supplemented emulsifiers in diet of broilers were not affecting the transport of cholesterol from peripheral tissues to the liver at the end of the fattening stage.

Table 6. Effects of emulsifiers and fat navigator on the serum biomedical indices of broilers

Items	POSCON	NEGCON	T1	T2	T3	T4	T5	SEM	P
AST	344.2	338	289.4	298.2	283.5	311.9	323.5	8.83	0.416
ALT	2.486	2.141	2.206	2.025	2.277	1.567	1.941	0.082	0.091
ALP	3769	3241	4336	3458	4359	2943	4342	199	0.289
Total cholesterol	3.245	3.246	3.362	3.055	3.156	3.435	3.177	0.063	0.755
HDL	2.0545	2.03	2.207	2.104	2.121	2.132	2.047	0.035	0.866
LDL	0.93	1.235	0.955	0.745	0.957	0.911	0.856	0.042	0.081

The results in table 7 show that there were no significant treatment effects (P>0.05) in parameters such as carcass, thigh, breast and liver ratios of broilers. However, some recent studies show that supplementation of bile acids or other emulsifiers in broiler diets were improving carcass percentage (Wenqing Lai *et al*., 2018; Saleh *et al*., 2020) and dressing percentages of breast and leg (Boontiam *et al*., 2017). Concerning abdominal fat, the finding of the present study agreed with results of Roy *et al*. (2010) and Boontiam *et al*. (2017) who found that abdominal fat was not affected by emulsifier or lysophospholipid supplementation in broiler diets. However, Wenqing Lai *et al*. (2018) found that abdominal

fat was reduced when broilers were supplemented with bile acids. According to Boontiam *et al*. (2017), the emulsifier might facilitate the conversion of blood lipid and protein fractions into muscular growth rather than towards abdominal fat deposition, which consequently affects fatty acid and amino acid deposits in the meat. In poultry, the abdominal fat pad mass is often used as a reliable parameter for evaluating total body fat content because it is linked directly to total body fat content (Becker *et al*., 1979). There are many factors affecting the deposition of fat in poultry, especially abdominal fat, and nutritional factors such as ME can regulate body fat deposition (Fouad *et al*., 2014).

Table 7. Effects of emulsifiers, fat navigator on broilers carcass, liver weight; abdominal, liver and fecal fat (%)

Item	POSCON	NEGCON	T1	T2	T3	T4	T5	SEM	P
Carcass	72.3	74.62	74.23	72.79	73.21	71.88	73.53	0.411	0.56
Leg	21.61	22.79	22.27	21.50	20.65	21.04	20.81	0.215	0.07
Breast	29.2	28.59	29.13	28.28	29.29	28.15	29.75	0.226	0.462
Abdominal fat	1.56	1.61	1.66	1.75	1.39	1.79	1.56	0.049	0.408
Liver	2.32	2.266	2.11	2.049	2.183	2.143	1.8314	0.053	0.256
Liver DM	24.93^{ab}	23.93^{ab}	23.40^{b}	23.36^{b}	24.66^{ab}	26.31^{a}	24.08^{ab}	0.265	0.035
Liver fat	5.22^{b}	5.06^{b}	4.91^{bc}	4.29^{c}	4.98^{bc}	6.09^{a}	4.96^{bc}	0.083	0.001
Fecal DM	19.95	18.84	19.30	19.38	19.76	21.05	21.5	0.282	0.104
Fecal fat	1.14	1.07	1.12	1.13	1.13	1.25	1.28	0.023	0.172

Table 8. Effects of emulsifiers and fat navigator in diets on production index and economic benefit return

Items	POSCON	NEGCON	T1	T2	T3	T4	T5
PI	383.7	391.56	393.65	392.7	397.08	377.93	395.7
Overall Feed price (VND/kg)	12564.35	12276.28	12633.6	12651.66	12490.57	12510.53	12501.08
FCR (kg feed/kg gain)	1.513	1.572	1.503	1.539	1.538	1.561	1.501
LW42 (kg/bird)	2.687	2.691	2.712	2.682	2.753	2.658	2.744
FCG1[1] (VND/kg of bird)	20799	19780	20468	20286	20347	20696	20402
FCG2[1] (VND/bird)	31473.05	31100.09	30753.17	31222.18	31285.55	32306.46	30617.28
SBR[2] (VND/bird)	83306.3	83411.7	84056.5	83142	85330.6	82410.4	85057.8
NPR1[3] (VNĐ/bird)	51833.25	52311.61	53303.33	51919.82	54045.05	50103.94	54440.52
NPR2[4] (VNĐ/kg)	19288.23	19441.63	19658.24	19358.62	19634.18	18847.41	19841.29
ROI1[5] (VNĐ/bird)							
Compared to POSCON			1470.08	86.56	2211.80	-1729.31	2607.27
Improvement (%)			2.84	0.17	4.27	-3.34	5.03
Compared to NEGCON			991.72	-391.79	1733.45	-2207.66	2128.91
Improvement (%)			1.90	-0.75	3.31	-4.22	4.07
ROI2[6] (VNĐ/kg)							
Compared to POSCON			370.02	70.39	345.96	-440.82	553.06
Improvement (%)			1.92	0.36	1.79	-2.29	2.87
Compared to NEGCON			216.61	-83.02	192.55	-594.23	399.65
Improvement (%)			1.11	-0.43	0.99	-3.06	2.06

The abdominal fat and total body fat were significantly decreased when reducing the energy level from 3,200 to 3,000 kcal/kg in broiler chickens from 21 to 42 DOA without any negative effects on the ADG, FI, or dressing % (Kassim and Suwanpradit, 1996). Besides, feed additives such as conjugated linoleic acid (CLA; one kind of emulsifier) also can reduce the deposition of abdominal fat and lower total body fat in poultry (Fouad *et al*., 2014). However, Zhou (2008) found that there was no difference in abdominal fat when supplementing CLA at 2 and 3% in diets, but the total body fat was reduced. The present study also found highest DM and liver fat in broilers of the T5-treatment (EM2 in the ME-reduced diet; $P<0.05$), while the other emulsifier Volamel Extra, nor the fat navigator Volamel Compass did not affect the hepatic fat level. However, Roy *et al*. (2010) reported that liver fat content decreased linearly with the dietary level of emulsifier. There were no significant treatment effects on the fecal DM and fecal fat content ($P>0.05$).

In this trial, feeding the emulsifier VE in the standard and ME-reduced diets and the fat navigator VC in the ME-reduced diets of broilers induced an improvement on production index (PI) compared to those of the POSCON and NEGCON treatments. The highest PI was found for T3-treatment (ME-reduced diet supplemented with VE emulsifier). This had a positive impact on salable bird return (SBR) and net profits return (NPR) when compared to those of the POSCON and NEGCON treatments. And finally, the return on investment (ROI) of broilers fed the emulsifier VE in standard and ME-reduced diets and the fat navigator VC in ME-reduced diets were higher (2.84-4.27 and 5.03%, respectively), compared to those of the POSCON and NEGCON treatments.

4. CONCLUSIONS

The findings of this study suggest that feeding emulsifier Volamel Extra in the standard and ME-reduced diets and fat navigator Volamel Compass in the ME-reduced diets of broilers improve growth performance and feed efficiency. Moreover, supplementation of the nutritional emulsifier Volamel Extra in standard and ME-reduced diets improved benefits by 2.84 and 4.27%, respectively, while feeding the fat navigator Volamel Compass to ME-reduced diets improved benefits by 5.03%.

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THE CURRENT SITUATION AND FEED UTILIZATION OF BEEF CATTLE RAISING IN CHAU PHU, AN GIANG PROVINCE

Nguyen Thi Thu Hong[1]*

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ABSTRACT

Survey on current status and productivity of cattle production in the Chau Phu District of An Giang province to assess the status of production, feeds, diets and nutritional satisfaction of meat cattle in the Chau Phu district. The study was carried out in three communes Khanh Hoa, My Đuc và O Long Vi, in total 60 households were interviewed to get information on details about the characteristics of the household, cattle production include the number of livestock, rearing systems, animal housing, feeds, breeds, veterinary inputs and sale of animal products. The survey found that cattle production in the Chau Phu is concentrated in provinces of Khanh Hoa, O Long Vy and My Duc with cattle are mainly raised for meat production. There were 93% Crossbred of Brahman x Local cattle and only 7% crossbred Sinddhy. The cattle diets in the Chau Phu were formulated from forages and supplement feeds such as rice bran and concentrate. Dry matter intake was the same in other studies.

Keywords: *Breed, environment, feed, housing, systems.*

1. INTRODUCTION

Livestock is the major source of food and income in rural farm households. Many different factors affect the profitability of cattle keeping including seasonal condition, nutrition and health control... Cattle eat what is available and availability of feed resources is very dependent on season and land utilization. The quality and quantity of available feed resources affects the performance and value of cattle. During periods of abundant feed supply, cattle are in good body condition. Utilization of crop residues and other forages and/or tree leaves is the most appropriate way for production of ruminants (Preston and Leng, 1991).

Cattle taking advantage of abundant feed sources from agriculture and agricultural by-products has contributed to reducing costs, has brought economic benefits to farmers, thereby improve household economy. Besides, fodder is scarce in quantity and poor in quality are disadvantages for cattle production.

Beef cattle husbandry in Vietnam is considered an important livestock industry. To understand current status and productivity of cattle production and the way that cattle production is practiced by rural farmers need to be investigated. The purpose of this study was to the cattle farming methods practiced by rural farmers, the impact of livestock on the cash economy of the farmers, the nature of problems encountered and the solutions farmers preferred. In addition, information about the use of local resources for cattle was also collected.

2. MATERIAL AND METHODS

A total of 60 households of the twelve wards and communes in Khanh Hoa, My Đuc and O Long Vi commune (20 households in each commune) was chosen.

The necessary information for the quantification of the criteria for assessing the current situations of beef cattle husbandry at smallholders is collected through direct interview with smallholders by using a questionnaire.

The information and data collecting

Household information: manpower, activities and sources of income.

[1] An Giang University

* Corresponding Author: Dr. Nguyen Thi Thu Hong, An Giang University, Vietnam National University Ho Chi Minh City (VNU-HCM). Tel: (84)918584419. Email: ntthong@agu.edu.vn.

Interviewees: age, education and gender of main livestock producer; sources of information on new management techniques and experience in livestock production.

Cattle production system: Number of livestock kept and reasons for keeping, cattle breeds.

Livestock production: animal housing; cesspool and use of manure.

Diseases control and prevention: veterinary services and vaccination.

Feeds and feeding systems: availability of feed resources by seasons, chemical composition, availability of technologies, perception and adaptation for cattle production.

Feed amount, feed sampling and chemical analysis

To evaluate dry matter (DM) intake ability of beef cattle, the feed amount was weighed and recorded at smallholders. Feed amount was measured with Nhon Hoa scale when feeding and daily leftovers were weighed in the next morning. In each group of smallholders, each day, the type and amount of feed for cattle was determined at smallholders, continuously for 3 days. The feed offered and refused ere analyzed for DM by drying at 105°C for 24hrs (AOAC, 1990).

Feed samples were taken from the cattle households. The feeds resources used for beef cattle in Chau Phu district were analyzed for DM by drying at 105°C for 24hrs, organic matter (OM) by ashing at 550°C for 4hrs and CP by Kjeldahl technique (AOAC-1990). Neutral detergent fiber (NDF) was be analyzed using the method of Van Soest and Robertson (1991).

Statistical analysis

Summary statistics were calculated to obtain a better understanding of the types of farms, farming methods and economic status of these rural communities. The chemical composition and DM intake was done by descriptive statistical methods to average (Minitab, 2010).

3. RESULTS AND DISCUSSION

3.1. Description of study area

Chau Phu district is one of 11 districts of An Giang province, bordering with Phu Tan, Cho Moi in the East; Tinh Bien and Tri Ton in the West; Chau Thanh in the South; Chau Doc city in the North. The natural land area is 45,100.76km^2, of which agricultural land is 39,774.89ha.

3.2. Characteristics of livestock producers

Average of the interviewees were from 45-50 years old and the level of education of the person mainly responsible for the livestock was still low. People with a low level of education had problems in learning and adopting new technologies and did this only slowly and with difficulty (Vo Tong Anh, 2004).

Table 1. General information about cattle households

Items	O Long Vi	My Duc	Khanh hoa	Chau Phu
Total of surveyed households	20	20	20	60
Average year old of the Interviewees	49.7±9.0	45.6±7.7	47.3±7.0	47.5±8.0
Education of the Interviewees	Secondary	Secondary	Secondary	Secondary
Number of person mainly responsible for cows keeping	2.10±0.4	1.95±0.2	2.05±0.4	2.0±0.4

Table 2. Years of experience of cattle keeping

Years of experience	households	%
<4	7	11.7
5-6	12	20.0
7-8	18	30.0
>8	23	38.3
Total	60	100

The proportion of producers who started keeping livestock less than 4 years ago was lowest (11.7%), followed by those who started from 5-6 years ago (20%), 7-8 years ago (30%), and finally more than 8 years ago (38.3%). This proves that cow raising has brought practical effects to farmers, helping them to improve

their economy, maintain long-term husbandry and it is also a condition to help other livestock households in the area develop together.

Table 3 showed that farmers have paid certain attention to the care and rearing of cattle. Specifically, the number of smallholders vaccinating beef cattle was 97.2. Captive farming accounted for 88.3% of surveyed smallholders. The percentage of smallholders with permanent sheds accounts for 5.0%; Semi-permanent 93.3% and only 1.7% of smallholders have temporary sheds. In general, stables are built permanent or semi-permanent stables ensure the growth and development of cows, avoid rain and drafts, and are convenient for waste collection and barn cleaning. This also contributes to creating a suitable environment for the growth and development of cows. Cattle are almost entirely confined (98.33%), with only 1,67% in combined systems (scavenging and confinement). For water, households mainly use water from rivers and canals (76.67%) and only a few households use tap water (accounting for 23.33%). Manure cattle was used in different ways, only 25.0% farmers also use cattle manure to produce manure for cropping to maintain soil fertility, and (11.7%) biogas production, almost cattle manure was sold (63.3%). Cattle are vaccinated to prevent Food and Mouth Disease and Haemorrhagic Septicaemia. Almost farmers understand the importance of vaccinating their cattle. De-worming is practiced rather in these survey area.

Table 3. Rearing systems and animal housing

	Item	Household	%
Structure of barn	Built permanent	3	5.0
	Semi-permant stables	56	93.3
	Simple barn	1	1.7
Cattle prod system	Entirely confined	59	98.3
	Combined systems	1	1.7
Manure disposal	Produce manure	15	25.0
	Biogas production	7	11.7
	To sold	38	63.3

The common breed found in the study areas is crossbreed of Brahman x Local cattle and constituted 93%, crossbreds are favored because of their good appearance, while Sind crossbred constituted 7% (Fig 1).

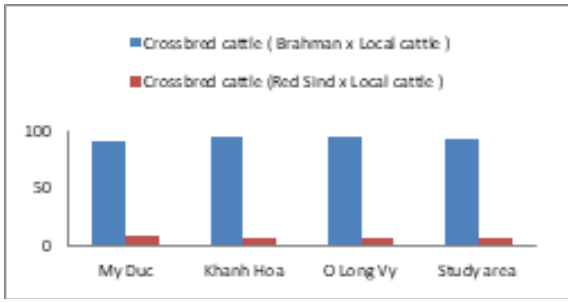

Fig 1. Beef cattle breed of surveyed smallholder

Cattle are kept primarily for meat production and popular is fattening. This also shows that farmers had perceptions about the role of cows in livestock production to keep up with the tastes of the market. Among interviewed farmers 8.3 % keep 1-3 cattle, 61.7% keep 4-5 cattle, 28.3% keep 6-7 cattle and 1.7 % keep more than 10 cattle (Fig 2).

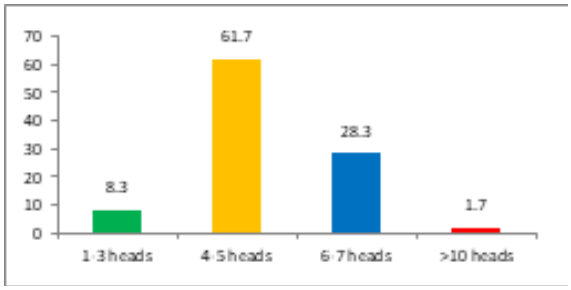

Fig 2. Total number of beef cattle of smallholder

3.3. Feed resources and feed utilization in cattle

Meeting the nutritional needs of livestock is the foundation for creating high-quality livestock products. Feed resources and the methods of using them were found to be similar for the three commune. Chemical composition of some feeds used for beef cattle in Chau Phu district is show in table 4. The type of feed used for cows at smallholders was quite simple.

Feed resource include cultivated grass, rice straw, natural grass and by-products such as baby corn; soybean foliage, *A. hypogaea*. The main forage was still grass and straw.

The concentrated feeds include rice bran and industrial feed. Mineral was supplemented by mineral stone. Rice straw is collected from the paddy field and is made into cakes. Rice straw is used almost year round for all of cattle. Le Duc Ngoan *et al*. (2016) have suggested using forage mixture of elephant grass, rice straw and ruzzi grass or elephant grass, maize foliage and rice straw resulted in increased daily weight gain and reduced enteric methane efficiency compared to using a mixture of elephant grass and rice straw.

Table 4. Chemical composition of feed, %/DM

Feedstuff	DM, %	CP	OM	NDF	ADF
P. purpureum	17.7	10.7	88.9	42.6	33.6
Varisme 06	19.0	9.9	90.0	58.5	46.2
P. maximum	24.3	7.3	86.0	66.7	33.4
H. acutigluma	15.1	10.2	91.1	60.7	32.1
Mix natural grass	22.0	9.4	90.2	64.3	44.5
B. mutica	22.2	6.4	86.8	71.0	31.6
Rice bran	85.0	10.8	88,8	28,4	16,7
Concentrate	88.9	16.0	91.0	33.0	20.7
Rice traw	86.7	5.0	85.5	78.9	49.5

Nutrition plays an important role in livestock production because it directly affects productivity, production costs and product costs. For ruminant cattle, livestock nutrition is converted to dry matter. The dry matter requirement of cows for a day and night was 2.5-3.5% of BW (Nguyen Xuan Trach and Mai Thi Thom, 2004). This research was conducted to investigate of the ingredients of the diets for cows and the DM levels of cows raised in Chau Phu district are shown in Table 5.

Results showed that 100% of households used at least two kind of concentrate and almost households used elephant grass or native grass for cattle. The survey results showed that the percentage of concentrate in the diet of cattle raised in Chau Phu district reached 7.9-10.6%, this shows that farmers have invested in feed resources to ensure the nutritional needs of cows. This result is equivalent to the report of Le Dinh Phung *et al.* (2016) the total amount of concentrate in the diets for cows reached 8% calculated the dry matter basis of the diet. Le Duc Ngoan *et al.* (2016) indicated that increasing dietary concentrate levels of 27-37% resulted in increased DWG from 22 to 49% with reduced enteric methane efficiency of 20-27% compared with the current cattle keeping practice. This is similar to the report by Le Dinh Phung *et al*. (2018) and Le Duc Ngoan *et al*. (2015). Dry matter intake were 4.33, 5.13, 7.42 and 9.33 kg/d with LW of <150, 150-200, 201-300 and> 300kg, respectively DM intake/100kg LW variable from 2.85 to 3.0 of BW. This is sustainable with feeding standard for beef cattle is based on suggestion by NRC (2000) reported that in developing countries, DWG of about 0.5-1.0 kg/d, their DM intake 2.8-3.0 of BW.

Table 5. Ratio of feedstuff in the diet and dry matter intake of beef cattle according to BW (kg)

Items		**<150** n=60 (cages: 17)	**150-200** n=85 (cages: 19)	**201-300** n=90 (cages: 20)	**>300** n=24 (cages: 4)
Ratio of feedstuff in the diet of cattle, %	Concentrate	8.20	10.6	7.90	8.10
	Forage	82.0	77.7	74.0	75.4
	Rice traw	9.80	11.7	18.1	16.5
Dry matter intake of cattle, kg/head/day	Concentrate	0.36	0.54	0.59	0.76
	Forage	3,54	4.00	5.50	7.03
	Rice traw	0.43	0.61	1.33	1.54
	Total DM intake	4.33	5.13	7.42	9.33
	DM/100kg LW	3.00	2.90	2.90	2.85

4. CONCLUSIONS

Based on the survey, it is concluded that cattle production is directly associated with rice cultivation and this relationship is important for profit. Mostly farmers keep small numbers of cattle. Crossbred (Brahman x Local cattle) appear to be the dominant breed. Feeding is mainly based on crop by-

products and natural grasses. In conclusion, feed resources for beef cattle at smallholder were variety, of which included concentrate and roughage feed, however, concentrate ratio in diet was low.

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REPRODUCTIVE PERFORMANCE AND ANIMAL WELFARE ASSESSMENT IN SOWS AND PIGLETS BY FARM SIZE

Dang Thi Ngoc Anh[1], Nguyen Kien Cuong[1], Do Tan Duong[1], Bui Thi Tra Mi[1], Dang Thi Ngoc Anh[1] and Duong Nguyen Khang[1]*

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ABSTRACT

The experiment aimed to assess the effect of three levels of farm size on reproductive performance and animal welfare in sows and piglets. Fourty five farms including small-scale farming (SF), medium-scale farming (MF), and large-scale farming (LF). This study was carried out by using questionnaires to collect data on reproductive performance in sows and to assess the animal welfare status of sows during the period of lactation and sucking piglets at those farms. There were four main principles of animal welfare ranging from physical, psychological, procedural events, and environment, and 16 individual criteria based on the Animal Welfare Assessment Grid (Molly *et al*., 2021*)*. The results showed that farm size had a positive effect on the number of piglets. In particular, the total number of piglets in LF (13.66 piglets) was higher than MF (12.67 piglets) and SF (11.60 piglets) with P<0.001. Similarly, the number of live-born piglets, the number of piglets weaned, and the number of weaned piglets per sow per year tended to be higher in LF (P<0.001). However, there were no differences in the number of pre-weaning piglet mortalities among three treatments (P=0.740). The age of piglets weaned by farm size (SF, MF, and LF) was 26.24, 23.98, and 23.50 days, respectively (P<0.001). The result demonstrated a correlation between the age

[1] Nong Lam University of Ho Chi Minh City, Vietnam

* Corresponding Author: Professor Dr. Duong Nguyen Khang, Nong Lam university of Ho Chi Minh City, Vietnam; Tel: (84)989390179; Email: duongnguyenkhang@gmail.com

of weaning and the time of weaning-to-first-mating interval, with R=0.40. Regarding to animal welfare, the principle of good health had the lowest score in all three farm sizes (P<0.001). The rest of the principles were assessed at an acceptable level and an enhanced level, with a score scale from 2.6 to 7.9.

Keywords: *Animal welfare, farm size, sows, piglets, reproductive performance.*

1. INTRODUCTION

The framework for animal welfare assessment in livestock production consists of five principles that have been proposed and accepted in many countries (UK Farm Animal Welfare Council, 1979). Recently, Molly *et al.* (2021) announced a tool measure an animal's level of welfare using cumulative lifetime experience which called the animal welfare assessment grid (AWAG). Besides, humane treatment of livestock is seen as a strategy for livestock industry development and integration in Vietnam (Livestock law, 2020).

Animal welfare is the physical and mental states of an animal (Duncan, 1993) when it is adapted to habitable environments (Broom, 1986) to maintain mental comfort (Mellor *et al*., 2009) and avoid suffering (Webster, 1995). According to Fraser *et al*., 1997, animal welfare must be concerned with the ability to freely express an important species-specific behaviour, also known as 'naturalness' (Singer, 2013), increased sensory perception positive for livestocks (Grandin, 2020). Today, animal welfare assessment is an area of interest to scientists, as improved feeding conditions and care practices increase animal performance (McInerney, 2004), livestock and poultry will produce better quality products (European Commission, 2002) and increase the economic efficiency of livestock production (McInerney, 2004).

For sustainable development in pig production and increasing higher productivity, it is necessary to change the structure of pig production from household farming to concentrated farming with an orientation toward industrialization. In fact, it has been controversial on animal welfare issues. In commercial farms, lactation sows are kept in small individual cages to facilitate herd management, and limit the spread risk of disease (McGlone, 2013). However, this results in an increased frequency of abnormal behaviour in pigs (Haigh *et al.*, 2019), cardiovascular effects due to reduced exercise (Marchant *et al.*, 1997), and gradually increased stress levels causing reduced fertility in sows (Broom, 1986).

2. MATERIALS AND METHODS

2.1. Data scoure

The data was recorded through direct interview by questionnaire on 45 pig farms in Dong Nai province from October 2022 to April 2023. The farm was based on three scale levels: small-scale farming (SF, 10-30 units), medium-scale farming (MF, 30-300 units), and large-scale farming (LF, over 300 units) (Vietnam's Livestock Law, 2000), which was similar to sows herd identified as approximately 100 sows (SF), 100-500 sows (MF), and above 500 sows (LF). The interviewer will collect the primary figures for reproductive performance through the big data saved on the farm assessed in 2022, and that for animal welfare status will be carried out in one week when observing and contacting sows and nursery pigs in the lactation pen. The principles and criteria assessment have relied on the Animal Welfare Assessment Grid (Molly *et al.*, 2021).

2.2. Assessment protocol for pigs

The assessment of pigs' welfare was studied with the framework of The Animal Welfare Assessment Grid (Molly *et al.*, 2021) in sows and nursery piglets. Through initial interviews with the farming manager will reject the risk of disease on the farm which has a negative effect on the results and randomly choose the number of pens and observation areas. AWAY has 4 main principles attaching physical, procedural, Psychological, and environmental (Table 1).

Table 1. The main principles and the reasons for selection of AWAY method

Principle	Meaning
Physical	This reflects an animal's clinical state of health including factors such as body condition, clinical signs of disease and lameness.
Procedural	This assesses the challenge to the animal arising from experimental events and clinical/ husbandry events, and includes factors such as blood sampling, clinical examination, vaccination and sedation
Behaviour/ Psychological	This reflects an animal's psychological condition, and includes factors such as abnormal/ stereotypic behaviour, expression of natural behaviours and social structure.
Environment	This reflects the animal's housing, including factors such as lighting, flooring, enclosure complexity and enrichment provision.

Each principle was defined by criteria and all criteria ensured distinct differences and avoided double-counting (Table 2).

Table 2. The list of 16 criteria of AWAY method

Principle	Criteria	Measure for sows and piglets
Physical	1. Body condition score	The scale from 1 to 5 points
	2. Lamaness score	No lamaness, or lamaness state (limp, head nod, fully weight bearing, a level of hop, suspected broken leg, impact on standing and moving
	3. Observable clinical signs	No observable of clinical signs level (mild, moderate, severe or chronic clinical signs)
	4. Food intake	Eating normally or lower than normal or not eaten for 1, 2 or 3 days
	5. Presence of injury	Not injuries or appear 1 or some injuries with impact on welfare
Procedural	6. Impact of veterinary procedures	No veterinary procedure; minor, moderate or severe veterinary procedure with short-term or medium-term or prolonged stress/effect on animal
	7. Response to restraint for procedure	No effect or minimal, moderate or change behaviour or show signs of stress or extremely scared
Psychological	8. Abnormal/ Stereotypic behaviour	None or low, moderate, high, higher or very frequency stereotypic behaviours
	9. Respone to human activity	No effect or minimal, moderate, noticeable response and show no or significant stress levels.
	10. Use of enrichment	Regular, rare or no use of enrichment
	11. Natural behaviours	Displaying all, most of or a few of natural behaviours
	12. Social structure	None of, some of these behaviours (submissive behaviour shown within the group, ..)
Environmental	13. Housing	The same wild habitat or lack of factors (space provision, lighting, floor, substrate,…)
	14. Enclosure complexity	All natural behaviours can be expressed with a different level of staff intervention
	15. Enrichment provision	Provision of 1 or 2 optimal or sub-optimal enrichment or no enrichment
	16. Group size	The number of group or individually house can/cannot near others

All criteria will be assessed on a scale from 1 to 10 points, the point based on the specific and exacting standard described in the AWAY method. The mean value of each criterion will be used to calculate the point of the corresponding principle. The result demonstrates animal welfare assessment in sows and piglets with 1 point presenting the best as called the "gold standard" and the higher the score, the worse welfare quality state, and finally 10 points of the worst.

2.3. Measurement of reproductive performance in sows

Reproductive performance in sows, such as total number of piglets, number of live-born piglets, number of stillborn piglets, number of piglets weaned, number of piglets weaned per sow per year, the age of piglets weaned, the percentage of pre-weaning piglets mortality and weaning-to-first-mating interval will be supplied by farm surveyed and the average of each indicator will be represented for the data of the farm meaning that this research will

not access to the impact of swine breeds and different litter regarding on productivity.

2.4. Statistical analysis

Each farm was considered the experimental unit for reproductive performance and animal welfare, whereas individual pig was considered the experimental unit for the other parameters. Data were analyzed as a randomized complete design by ANOVA using the GLM procedure (Minitab 19). When a significant *F* value for treatment means was observed in ANOVA, the treatment means were compared by Tukey's test. The percentage of pre-weaning piglets mortality was compared by Chi-square test. Treatment effects were considered significant at P<0.05.

3. RESULTS

3.1. Effects of farm size on sows reproductive

3.1.1. Effect of farm size on number of piglets born and weaned by litter

Total number born in LF treatment (13.66 piglets) was significantly higher than that in MF (12.67 piglets) and SF (11.60 piglets) with P=0.001 (Table 3). Similarly, NBA, and NW/sow/year tended to be higher for LF treatment compared to the remaining treatments (P=0.001). However, there was no evidence for differences (P>0.05) in number of stillborn (NSB) among farm size treatments (Table 3). The weaning age (WA) by farm size (namely SF, MF, LF) were 26.24, 23.98 and 23.50 days, respectively (P=0.001).

Table 3. Effect of farm size on NB, NBA and WA

Parameters	SF[1]	MF[2]	LF[3]	SEM	P
NB	11.60^{c}	12.67^{b}	13.66^{a}	0.241	0.001
NBA	11.09^{c}	11.98^{b}	13.07^{a}	0.232	0.001
NSB	0.51	0.69	0.59	0.081	0.304
NW	10.23^{c}	11.03^{b}	12.13^{a}	0.189	0.001
NW/sow/year	24.57^{c}	27.11^{b}	30.49^{a}	0.510	0.001
WA, day	26.24^{a}	23.98^{b}	23.50^{b}	0.339	0.001

Means with different superscript letters within a row differ (P<0.05)

3.1.2. Effect of farm size on the percentage of pre-weaning mortality

Although there were no differences (P=0.471) in the percentage of pre-weaning piglet mortality of nursery pigs among the treatments (Figure 1), but the data on the medium-scale farming was the highest in pre-weaning piglet mortality rate with 7.35%, followed by that on large-scale farming and small-scale farming with 7.15 and 6.90%, respectively.

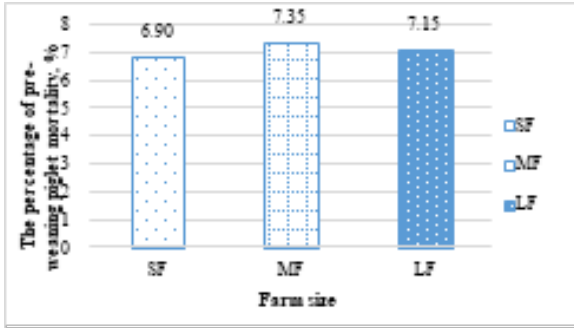

Fig 1. Pre-weaning piglet mortality by farm size

3.1.3. Effect of farm size on weaning-to-first-mating interval

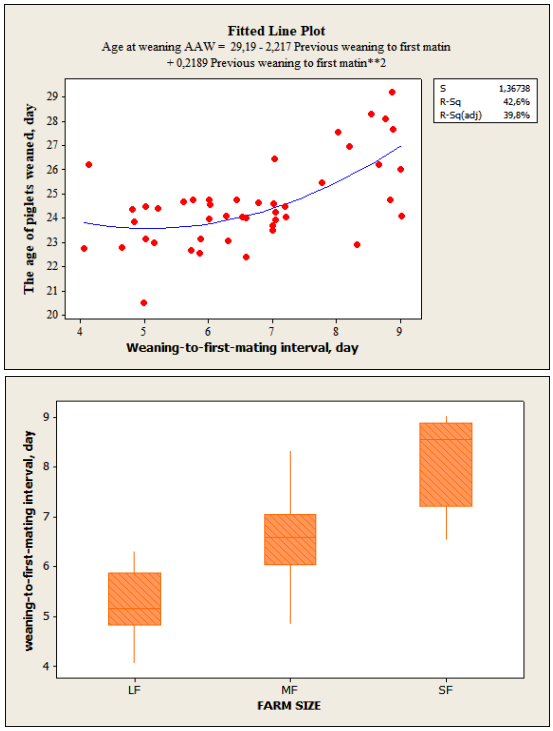

Fig 2. (A) The bar chart of the previous weaning-to-first-mating interval between SF, MF and LF and (B) the correspondence of the age at weaning in piglets and weaning-to-first-mating interval in sows

Farm size has a positive impact on the weaning- to-first- mating days with *P*=0.001. Specifically, the weaning-to-first-mating interval in LF treatment was the lowest with 5.24 days, while that in SF treatment was the highest (8.15 days) compared to the figure for MF (6.61 days) (Fig 2A).

The result demonstrated a slightly correlation between the age of weaning and the time of weaning-to-first-mating interval, with R=0.40 (Fig 2B), meaning that the shorter the period of lactation, the faster the body condition score of swine recovers, which plays an important role in a considerable decrease in non-productive days of swine.

3.2. Animal welfare assessment in sows and piglets

There was no evidence for difference (P>0.05) among treatments for the criterion of respone to human activity (Table 4). Besides, there was a tendency that increasing the scale of farm influenced (P=0.001) criteria belonged to physical principle. In particular, body condition score was greater for LF treatment compared with SF treatment (2, 3 and 4 respectively) while the remaining criteria (lamaness score, observable clinical signs, food intake, presence of injury) illustrated a better physical state of sows and nursery piglets in small-scale farming than that in medium-scale farming and large-scale farming. Procedural principle enclosed 2 criteria such as impact of veterinary procedures and response to restraint for procedure which them score were lower in LT treatment (5.5 and 5.1 respectively) than that in MF treatment (6.4 and 5.5 respectively) and that in SF treatment (7.4 and 6.6 respectively). The score of stereotypic behaviour in SF treatment was significantly greater (2.4) (P=0.001) than that in MF treatment (3.1) and that in LF treatment (3.5). Farm size demonstrated its important effect on the score of natural behaviour and social structure, meaning that the small-scale farming was better insurance for sows and piglets than other farms.

Table 4. Criterion scores in sows and piglets from SF, MF and LF

Welfare principle	Welfare criteria scores	SF[1]	MF[2]	LF[3]	SEM	P
Physical	Body condition score	4.0^{a}	2.7^{b}	2.3^{b}	0.194	0.001
	Lamaness score	2.1^{b}	2.5^{ab}	3.2^{a}	0.219	0.005
	Observable clinical signs	2.1^{c}	3.5^{b}	6.4^{a}	0.394	0.001
	Food intake	3.4^{b}	4.8^{b}	6.6^{a}	0.511	0.001
	Presence of injury	1.6^{b}	2.8^{a}	3.7^{a}	0.273	0.001
Procedural	Impact of veterinary procedures	7.4^{a}	6.4^{b}	5.5^{c}	0.253	0.001
	Response to restraint for procedure	6.6^{a}	5.5^{b}	5.1^{b}	0.226	0.001
Psychological	Abnormal/Stereotypic behaviour	2.4^{b}	3.1^{a}	3.5^{a}	0.191	0.001
	Respone to human activity	1.8	2.3	2.4	0.212	0.119
	Use of enrichment	4.6^{b}	5.7^{ab}	6.2^{a}	0.355	0.011
	Natural behaviours	2.0^{c}	3.8^{b}	5.8^{a}	0.267	0.001
	Social structure	1.4^{b}	2.5^{a}	2.2^{a}	0.155	0.001
Environmental	Housing	7.1^{b}	8.0^{a}	7.9^{a}	0.170	0.001
	Enclosure complexity	5.2^{b}	5.8^{b}	6.7^{a}	0.205	0.001
	Enrichment provision	4.8^{c}	6.4^{b}	7.9^{a}	0.235	0.002
	Group size	10.0	9.0	9.0	0.000	*

The score of housing by farm size (namely SF, MF, LF) were 7.1, 8 and 7.9 points, respectively (P=0.001). There was considerably difference (P=0.001) in the score of enclosure complexity among treatments (5.2, 5.8 and 6.7 points). This is the same trend in the data on enrichment provision, the score of this criteria among treatments were 4.8, 6.4 and 7.9 points, respectively. The group size point by farm size was very high, about 9-10 point and with

P=0.001 which the difference was significantly analysis.

The score of physical principle was lower (2.6 points) in small-scale farming than that in medium-scale farming (3.3 points) and that in large-scale farming (4.4 points) (P=0.001) (Figure 3) whereas the trend of procedural principle point, this principle was a tendency that the score of SF treatment was the highest (7.0 points), followed by 6.0 points of that of MF treatment and 5.3 points of LF treatment (P=0.001). Both the score of psychological principle and the score of environmental principle showed that SF treatment was the lowest point (2.5 and 6.8 points, respectively) compared with the data on MF treatment (3.5 and 7.3 points, respectively) and that on LF treatment (4.0 and 7.9 points).

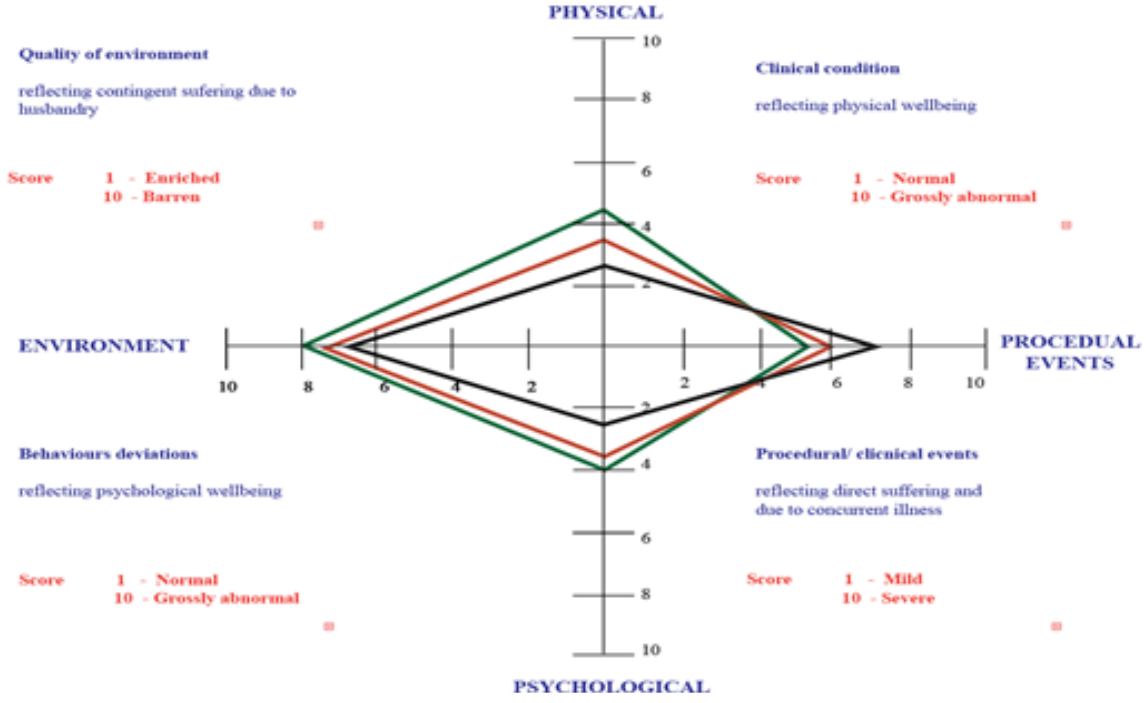

Fig 3. Principle scores in sows and piglets from SF, MF and LF

4. DISCUSSION

Reproductive performance has an essential effect on economic profitability in the pig industry (Ran *et al.*, 2022) through 2 factors: fertility (reproductive cycle) and prolificacy (NBA) (Koketsu *et al.*, 2017). The sow fertility traits, such as the NB, and NW/sow/year, are indicators with low heritability (h^2<0.2) (Popovac *et al.*, 2012), meaning that they are susceptible affected by farm size, farming environment, and management (Popovac *et al.*, 2012). The increased herd scale means more investment in livestock equipment, care processes, quality of human resources for the farm, and application of information technology. Using big data management will help to assess the potential and risks earlier which can lead to better stable performance in sows (Zhang *et al.*, 2021). The large-scale herd has quality in better breeding pigs (Guan *et al.*, 2021) due to the interest in genetic advancement (Yang *et al.*, 2018) to select desirable traits in order to continuously improve the reproductive performance of sows. Kotetsu *et al.* (2020) comparing reproductive performance in small-scale farming (180 sows) and large-scale farming (1,300 sows) showed that the number of live-born piglets per litter increased by 0.3

pigs in the former compared with the latter. Sows farrow an average total number of piglets from 13 to 14 will stimulate better the development of mammary (46) the amount of milk produced in sows will increase which leads to improve the number of weaned piglets per year (PIC, 2021). In addition, using teaser boar to accurately test the time of estrus in sows (Gerritsen *et al*., 2005) is widely applied in medium-scale and large-scale farming, late insemination time has a great influence on the number of newborn piglets (Bai *et al*., 2021), applying post-cervical artificial insemination in sows (Roberts and Bilkei, 2005) reducing the distance and time of sperm movement (Sbardella *et al*., 2014) increasing the number of embryos (Roberts and Bilkei, 2005), increasing the number of piglets born leading to increase the number of weaned piglets. On the other hand, the biggest difference between small-scale and large-scale farming is the climate control inside cages (Ignatkin *et al*., 2023) by the combination of cooling pads and ventilators, large-scale farming often builds a indoor farm and ensures temperature, humidity, and air speed in pen (Forcada and Abecia, 2019) while the small-scale farm does not have enough funds for initial investment so that it is mainly an open farm; the hotter outside environment is, the more length the weaning-to-first-mating interval is (Koketsu and Dial, 1997) due to decreased GnRH production and impaired follicle development (De Rensis *et al*., 2012); the total number of piglets born decreased by 0.6 pigs when the ambient temperature increased by 5^{o}C (from 25^{o}C to 30^{o}C) (Iida *et al*., 2013).

Changing farm size has a negative argument on animal welfare (Friedrich *et al*., 2020) pigs which lived in large-scale farming illustrated poorer welfare than those in small-scale farming (Tonson *et al*., 2009). The pig farrowing crate is about 0.8x2.2m in the commercial farm, with the small cage's acreage (Dumniem *et al*., 2023) and combine with individual pens for lifetime production (Ignatkin *et al*., 2023) lameness state (Heinonen *et al*., 2013) and clinical signs (Jensen *et al*., 2010) witnessed a significant increase. Narrow environmental habitat led to slightly growing in stress levels which as well as caused climbing the frequency of appearing abnormal in sows and nursery piglets (Zhang *et al*., 2020); natural behaviours were distinctly presented when they feel free and relaxed in their living environment (Kõlves *et al*., 2013). The suitable feeding process, to feed based on the assessment of the body condition score (Esbenshade *et al*., 1986) and to feed relied on automatic feeding systems (Ignatkin *et al*., 2023) which improve effective feed intake of sows in the period of lactation and length the time of production, above matters very limited in small-scale farming. However, the feeding process was handicrafts by labour which was easier to add enrichments to the pigs' diet such as vitamins, probiotics, or prebiotics. Other procedures were always done in nursery piglets within a minimum of 24hrs after their birth, such as teeth clipping and tail docking (Fu *et al*., 2019). Almost farm sizes have made both these tasks without anaesthesia for piglets (Zhou *et al*., 2013) they are noticeably stressed or scared (Fu *et al*., 2019) and even the pains cause decreasing the amount of milk intake in nursery piglets after teeth clipping by cutting pliers (Fu *et al*., 2019).

5. CONCLUSIONS

According to our results, farm size has a positive impact on reproductive performance in sows, the number of live-born piglets and number of piglets weaned per sow per year was higher and the weaning-to-first-mating interval was demonstrated shorter in large-scale and medium-scale farming than small-scale farming. Although animal welfare assessment in large-scale farming was poor quality than small-scale farming, the average score of principles in 3 scales were all acceptable level and enhanced level.

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EFFECT OF BREWERS YEAST ON GROWTH PERFOMANCE OF SINDHI CROSSBRED CATTLE FED WHEAT DUST BY-PRODUCT

*Le Phap Luat[1], Dang Hoang Dao[2], Le Viet Bao[3], Howard Huang[4], Pradeep Padmaja Jayaprasad[4] and Duong Nguyen Khang[1]**

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ABSTRACT

Thirty cross Sindhi female cattle of 158±24kg live weight and 7-8 months of age were ramdomly allocated to three treaments in a ramdomized complete design (RCD) in the experiment (1) LBY_0: Fed *ad libitum* the basal diet of wheat dust by-product without supplementation of Leiber brewers' yeast-BT®; (2) LBY_{100}: Fed *ad libitum* the basal diet of wheat dust by-product with supplementation of 100g dose of Leiber brewers' yeast-BT® per cattle; and (3) LBY_{150}: Fed *ad libitum* the basal diet of wheat dust by-product with supplementation of 150g dose of Leiber brewers' yeast-BT® per

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

cattle. In treameants, the total of crude protein will be the same value. Growth rates were 1022, 989 and 914 g/day when the diet of supplementation with 150, 100 and 0g of Leiber brewers' Yeast-BT®. Live weight gains were 10% higher (989 and 1022 g/day) when the protein was in the form of brewers' yeast, compared with the ingredients were not mixed Leiber brewers' yeast-BT® (914 g/day). Feed conversion rates were 6.85 and 7.02 for the supplementation of 150 and 100g dose of Leiber brewers' yeast-BT® compared with 7.53 for the control. The results confirmed the beneficial effects supplementary Leiber brewers' Yeast-BT®, especially source of by-pass protein. Growth rate was increased; feed conversion was improved in treament which had Leiber brewers' Yeast-BT® in a basal diet of wheat dust by-product.

Keywords: *Leiber brewers' Yeast-BT®, wheat dust by-product, weight gain, feed conversion rate*

1. INTRODUCTION

Feeding cereal grain to cattle in the SE Asia area is neither politically or socially desirable nor economically feasible (Preston and Leng, 1987). However, there are byproducts which could substitute for the cereal grains and which presently are often major sources of environmental pollution. The barley dust by-product, a presently under-utilized and often polluting by-product from manufacture from beer production, is such as example. The wheat dust by-product contains low amounts of crude protein, essential amino acids and minerals; therefore, was recommended to use with the protein higher diet by supplementation.

One of the challenges for extending the application of this fattening system has been to identify a local replacement for the brewers' grains, which was the initial source of the by-pass protein that plays a key role in supplementing the microbial protein derived from urea. Fresh brewers' grains are known to be an excellent source of by-pass protein (Promkot and Wanapat, 2003). Brewers' grains contain about 26% crude protein; but their protein quality, essential amino acids and minerals are poor (Arosemena *et al.*, 1995). In pig, it was recommended to limit the level of inclusion to approximately 5% in diets (Boucqué and Fiems, 1988). In dairy cow, feeding up to 30% of brewers' grain was not found to reduce milk production in dairy cattle (West *et al*., 1994; Murugan *et al*., 2014). It was shown that the brewers' grains apparently assisted in the detoxification of the HCN precursors in cassava foliage, as reflected in reduced excretion of thiocyanate in the urine of cattle fed the bitter cassava foliage (Phanthavong *et al*., 2016; Binh *et al*., 2017; Phanthavong *et al*., 2018).

The aim of this study to facilitate and carry out the effect of Leiber brewers' Yeast-bt® on cross-bred Sindhi cattle fed wheat dust by-product. The effects expected from Leiber brewers' Yeast-bt® such as the treatments to be compared, the parameters to be measured, the analysis to be performed as well as the statistical needs are presented.

2. MATERIALS AND METHODS

2.1. Location

The experiment was conducted in the cattle farm of the Research and Technology Transfer Center of Nong Lam University from July to December 2022.

2.2. Treatments and experimental design

Thirty cross Sindhi female cattle were allocated to three pens according to live weight and fed a basal diet of wheat dust by-product and sulphur-rich minerals (2% of diet DM). Each pen received one of the following treatments according to a completely randomized design:

LBY_0: No supplementation of Leiber brewers' Yeast-BT®.

LBY_{100}: Supplementation with 100g dose of Leiber brewers' Yeast-BT®.

LBY_{150}: Supplementation with 150g dose of Leiber brewers' Yeast-BT®.

Product profile on ingredients, effectiveness and use

Ingredients: 40% brewers' yeast (*Saccharomyces cerevisiae*) bound to 60% spent grains.

Effectiveness: Leiber brewers' Yeast-BT® product contains the *Saccharomyces cerevisiae* yeast for animal diets. It also includes an additional benefit of spent grain with high content of rumen resistant protein up to 46%.

Usage: Breeding sows: 3-5% in the ration or 100-200g per animal/day with gestating and lactating sows. Piglets: 3-5% in pig starter feed. Dairy cattle/cattle: 100-300g per day. Calves: 50-100g/day.

2.3. Animals and housing

The cross Sindhi female cattle had an initial weight in range of 158±24kg and were allocated to 3 pens so that mean live weights within each pen were similar. Vaccination was done against epidemic diseases and the cattle were drenched against internal parasites before the commencement of the experiment. The cattle were weighed before morning feeding at the beginning of the trial and every 30 days.

2.4. Feeding and management

Cross Sindhi female cattle were adapted gradually to experimental feeds for two weeks prior to starting experiment. The wheat dust by-product was bought from Intermalt Vietnam Co, LTD factory in Ba ria Vung Tau province and fed *ad libitum* for each treatment. The amount of Leiber brewers' Yeast-BT® was mixed into 5kg the wheat dust by-product, transferred and provided for each treatment. Feeds were offered two times a day, at 7.30am and 2.30pm. Feeds offered and refused were recorded daily. Water was supplied all day.

2.5. Data collection and measureaments

Cattle were weighed at beginning and monthly individual record, using an electronic balance. Feeds offers were weighed before giving them to cattle. Feed refusals were collected each morning prior to offering fresh feed and weighed to measurea feed intake. Samples of feeds offered and refused were collected every 14 days to determine DM and crude protein according to AOAC (1990) methods. The morbidity rate was recorded using the diagnosis of treatment condition ie, displaced abomasum, pneumonia, bloat, lameness, fever, diarrhea for each animal determined to be sick. Provide the information on all medications given including vaccines, de-wormers, implants, metaphylatic and therapeutic antimicrobial treatments. Specific medications and dosages administered to each animal.

2.6. Chemical analysis

DM and crude protein in feeds were analysed using the methods of AOAC (1990). Protein solubility was measuread by weighing 3 g of sample (DM basis), followed by shaking in 100 ml of M NaCl for 3h (Whitelaw *et al*., 1961). The suspension was then filtered through Whatman No. 4 filter paper and washed 3 times with distilled water. All the filtrate was then transferred to a kjeldahl flask for digestion, distillation and titration according to AOAC (1990). Protein solubility was calculated as the N content of the filtrate as a percentage of the N in the original sample.

2.7. Statistical analysis

Response curves were fitted to the data using linear and quadratic equations in Microsoft Office Excel software, with level of Liber brewers' yeast as the independent variable (X) and the response component (eg: feed intake, weight gain...) as dependent variable (Y).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of feeds

There were major differences in the solubility of protein with higher values for Leiber brewers' Yeast-bT® than for wheat dust by-product (Table 1).

Table 1. Composition of diet ingredients

Parameters		Wheat dust by-product	Leiber brewers' Yeast-bT®: 40% brewers' yeast bonded to 60% brewers' spent grains
DM, %		88.2	91.6
% in DM	Ash	4.7	6.5
	Crude protein	9.7	31.0
	Crude fibre	7.9	9.5
	Crude fat	2.7	7.0
	Lysin	-	1.8
	Methionine	-	0.6
	N solubility#	12.2	33.9

 # % N soluble in M NaCl

3.2. Dry matter intake, feed conversion rate and live weight

Table 2. Mean values for changes in live weight, DM intake, feed conversion rate in diets

Treatments		LBY_0	LBY_{100}	LBY_{150}	SEM	P
Live weight, kg	Initial	158.6	156.9	158.6	24.78	0.98
	Final	310.4	321.1	328.2	37.56	0.57
	Total weight gain, kg	151.8	164.2	169.6	17.79	0.09
	Daily weight gain, kg/day	0.914	0.989	1.022	-	-
DM intake, kg/cattle/day	Total feed intake, kg in dry matter	6.89	6.94	7.00	0.37	0.78
	Wheat dust by-product	6.89	6.84	6.85	0.35	0.81
	Leiber brewers' Yeast-BT®	0	0.1	0.15	-	-
	Percent of Leiber brewers' Yeast-BT®	0.00	1.44	2.14	-	-
Feed conversion rate	Total	7.53	7.02	6.85	0.42	0.74
	Wheat dust by-product	7.53	6.91	6.70	0.48	0.72
	Leiber brewers' Yeast-BT®	0	0.00061	0.00088	-	-

DM intake decreased with a curvilinear trend as supplementation with Leiber brewers' Yeast-bT® in treament when Leiber brewers' Yeast-bT® was fed at 150g per cattle per day (Table 2, Figure 1). Feed conversion was improved by supplementation with Leiber brewers' Yeast-bT®, reflecting the high rates of live weight gain with 1.02kg per day (Table 2, Figure 2 and 3). Growth rate increased with the treatment supplementation of Leiber brewers' Yeast-bT®. The increasing rate of response in live weight gain when using Leiber brewers' Yeast-bT® supplementation is in accordance with similar studies in which protein-rich supplements were fed in increasing quantities in diets rich in carbohydrates (eg: fish meal and molasses-urea, Preston and Leng, 1987; cotton seed cake and ammoniated wheat straw, Weixian *et al*., 1994).

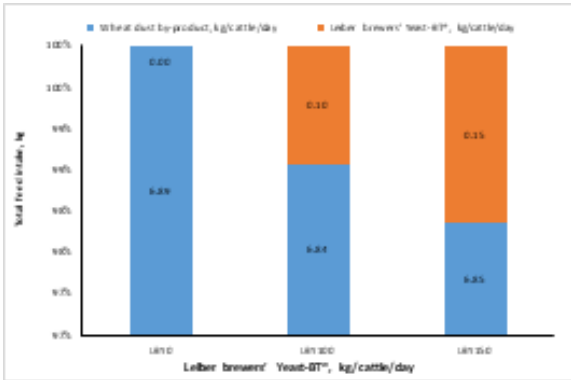

Fig 1. Proportion of dietary intake as wheat dust by-product and Leiber brewers' Yeast-bT® according to dietary treaments

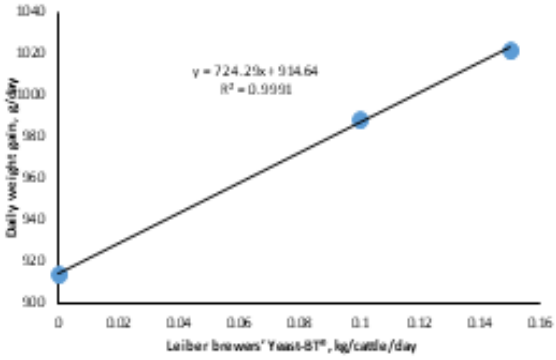

Fig 2. Effect of Leiber brewers' Yeast-bT® on ADG of cattle fed wheat dust by-product as basal diet

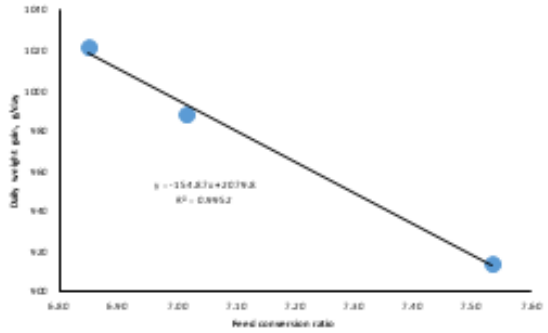

Fig 3. Effect of Leiber brewers' Yeast-bT® on FCR of cattle fed wheat dust by-product as basal diet

4. CONCLUSIONS

Growth rate was increased and feed conversion was improved in treament which had Leiber brewers' Yeast-bT® supplementation in a basal diet of wheat dust by-product.

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CURRENT ANTIBIOTIC USE IN CHICKENS AND PIGS FARMED IN NORTHERN VIETNAM

*Chu Thi Thanh Huong[1], Dong Van Hieu[1], Vu Thi Thu Tra[1] and Truong Ha Thai[1]**

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ASTRACT

A total of 30 antibiotics of different groups have been widely used in pig and chicken farms with different purposes. There were 18 antibiotics were used for the therapeutic purpose in both pigs and chickens. Of which, Tylosin, Gentamicin, Colistin and Enrofloxacin were the most widespreadly used. Antibiotic selections were mainly depended on the farmer's experiences (43.8%) and drug-sellers (26.7%). Only 20.3% of farms selected antibiotics after getting the diagnosis results. In the farms, animals were injected antibiotics by animal caretaker (92.4%). 72.5% of the farmers used antibiotics following the instructions, while 25.5% used antibiotic with overdoses for animals when prevention or therapeutic. In addition, 47% the farmers did not stop using antibiotics before slaughter. This finding suggested that the inappropriate use of antibiotics in animal farming in Vietnam could led to the risk of appearance of antibiotic resistant bacteria and transmit to humans.

Keywords: *Antibiotics, poultry, pigs, Vietnam*.

1. INTRODUCTION

Antimicrobials are essential to treat microbial infections and preserve the health of both humans and animals. However, imprudent antimicrobial use (AMU) accelerates the development of antimicrobial resistance, diminishing treatment efficiency and endangering the future of human and animal medical interventions (WHO, 2011; WHO, 2015). Therefore, the increasing antibiotics resistance seen today is a global public health concern (EclinicalMedicine, 2012). Globally, animal production accounts for about three quarters of total antimicrobial use (Tiseo *et al*., 2020). A recent study estimated that, in Vietnam total annual antimicrobial use amounts to 3,838 tons, of which 71.7% correspond to animal use (Carrique-Mas *et al*., 2020; Le et al., 2021).

In animal production, antibiotics are used for disease treatment, disease prevention and growth promotion. The permitted uses vary among countries and regions, antibiotics are no longer be used as animal growth promoters in European Union but these practices are permitted in many other countries (Gyles, 2008). Furthermore, the amount of antibiotic usage per animal differs between countries as it also depends on the effectiveness of regulation enforcement, and the knowledge of the community about antibiotic use and resistance. Besides, the incidence of resistance to antibiotics of bacteria originating from food animals or retail meat is high in developing countries (Van *et al*., 2007; Fashae *et al*., 2010; Yang *et al*., 2010), possibly as the result of the inappropriate or uncontrolled use of antibiotic in farming practices.

In Viet Nam, exact data on quantities of antimicrobial consumption in animal production are unavailable, and information is limited to point surveys in defined geographical areas (Nhung *et al*., 2016). Previous researches suggested that prophylactic antimicrobial use practices were and are still widespread in chicken and pork production (Choisy *et al*., 2019; Pham *et al*., 2019). For several decades, all veterinary medicine products had to be registered with the Department of Animal Health, Ministry of Agriculture and Rural

[1] Faculty of Veterinary Medicine, Vietnam National University of Agriculture

* Corresponding Author: Dr. Truong Ha Thai, Faculty of Veterinary Medicine, Vietnam National University of Agriculture; Tel: (84)967861168; Email: ththai@vnua.edu.vn

Development. Currently, there are more than 15,000 registed-veterinary products, of which about seventy percent antimicrobial-containing products are authorized in the country. Antimicrobials intended for animal production have a dedicated supply chain and can be legally purchased without prescription by anyone from any of the approximately 12,000 veterinary drug stores across the country (Carrique-Mas *et al*., 2019).

Currently, there is no active nationwide antibiotic usage surveillance program in Vietnam. However, it was estimated that a large number of antibiotic products are used in animal husbandry. The aims of this study were to show the antibiotic usage in animal production in the North of Vietnam.

2. MATERIALS AND METHODS

2.1. Materials

A total of 172 chicken farms and 79 pig farms were selected in 6 provinces (Ha Noi, Hoa Binh, Vinh Phuc, Bac Ninh, Thai Nguyen, Hai Phong) for the investigation of antibiotic usage. In this study, the numbers of chickens and pigs at each farm ranged from 200 to 5,000 and 50 to 2,000, respectively.

2.2. Methods

Information collection: a cross-sectional investigation of antibiotic usage in pig and chicken farms for understanding on veterinary drug usage of farmers and owner in the northern Vietnam was designed and conducted from July 2020 to June 2022. Questionnaires, contents of which were compiled after test survey and adjustment, were used for direct interviews of owners, technical cadres or veterinarians of the farms. The information of veterinary drugs, antibiotic components and active elements which weren't noted in the farm were tracked down and collected through labels on remedy packs or jars left around animal housing or at local veterinary medicine pharmacy. In order to ensure the objectivity of full remedy use information exploitation, all householders' names and addresses were kept in security through encoding addresses just at the survey time.

Table 1. Sample size and localities of the study

		Ha Noi	Hoa Binh	Vinh Phuc	Bac Ninh	Thai Nguyen	Hai Phong	Total
Pig	Semi-industrial	17	-	-	10	8	11	46
	Industrial	19	-	3	11	-	-	33
	Total	35	-	3	21	8	11	79
Chicken	Semi-industrial	15	-	7	25	13	7	67
	Industrial	14	11	5	10	24	41	105
	Total	29	11	12	35	37	48	172

Data and statistical analyses: statistical comparisons of antibiotic resistance rates between source isolations, serovars will be analyzed using the Chi-square test (Microsoft Excel 2010).

3. RESULTS

At least 30 antibiotics of more than 10 groups have been used in pig and chicken farms with difference purposes (Table 2). There were 18 antibiotics were used in both pig and chicken farms, including Aminoglycosides (6 types of antibiotics), Beta-lactams (3), Fluoroquinolones (4), Macrolides (7), Sulfamides (2), Tetracyclines (3), Phenicols (3), and other antibiotics (3). Of which, Colistin, Amoxicillin, Tilmicosin, Ampicillin, Enrofloxacin, Norfloxacin, Tylosin and Oxytetracycline were commonly used for prevention and disease treatment purposes (Table 2).

Table 2. Purposes of antibiotic used in animal farm

Groups	Antibiotic	Chicken (n=172)		Pig (n=79)	
		Prev	Treat	Prev	Treat
Aminoglycosides	Gentamycin	22	48	8	14
	Kanamycin	-	5	4	11
	Neomycin	11	5	3	7
	Spectinomycin	-	-	-	15
	Streptomycin	-	5	-	8
	Tobramycin	-	-	-	12
Beta lactams	Amoxicillin	33	118	25	48
	Ampicillin	10	27	5	16
	Penicillin	-	-	-	5
Fluoroquinolones	Enrofloxacin	8	81	18	37
	Norfloxacin	-	17	5	19
	Flumequine	-	-	-	11
	Ciprofloxacin	-	5	-	2
Macrolides	Erythromycin	-	-	-	2
	Tilmicosi	25	121	-	23
	Tylosin	78	145	11	47
	Tulathromycin	-	-	-	8
	Spiramycin	-	-	-	3
	Tiamulin Tyvalosin	-	-	-	5
Sulfamides	Sulfachlorpyrazin	-	5	-	-
	Sulfamethoxazole	-	31	-	4
Tetracyclines	Doxycylin	-	-	-	5
	Oxytetracyclin	40	22	15	23
	Tetracycline	-	5	2	7
Phenicols	Chloramphenicol	-	2	-	5
	Flophenicol	78	115	4	21
	Thiamphenicol	-	-	-	3
Others	Lincomycin	-	-	3	10
	Colistin	126	155	9	33
	Trimethoprim	5	22	-	14

The data in Table 3 showed that antibiotic selections were mainly depended on the farmer's experiences (43.8%), followed by advice of drug-sellers (26.7%). There were only 20.3% of farms selected antibiotics after getting the diagnosis results. However, 31.9% of farmers in the industrial farms chose antibiotic after getting the diagnosis results.

In the chicken and pig farms, antibiotics were mainly given to animals by farmers or workers, 100% in the industrial farms and 83.2% in the semi-industrial farms (Table 4). Our data showed that 98.8% farmers gave antibiotic to animals by themselves in the chicken farms, while 78.5% in pig farms.

The data in Table 5 showed that 72.5% farms used antibiotic following the instructions and 25.5% the farms used the over dosage for animal. This situation became more serious in semi-industrial farms, where 27.3% pig farms and 40.3% chicken farms used the higher dosage for animal. In addition, 47% the farms did not stop using antibiotic before slaughter. Especially, it happened in 67.4% of the pig semi-industrial farms and 45.9% of the chicken semi-industrial farms.

Table 3. Antibiotic selection at different systems of animal production

Systems animals production		Experience n (%)	Supplier n (%)	Local vet. n (%)	Diagnosis results n (%)	Others n (%)
Pigs	Semi-industry (n=46)	17 (37.0)	12 (38.3)	8 (17.4)	2 (4.3)	7 (15.2)
	Industry (n=33)	14 (42.4)	11 (33.3)	0 (0.0)	8 (24.2)	0 (0.0)
	Total (n=79)	31 (39.2)	23 (29.1)	8 (10.1)	10 (12.7)	7 (8.9)
Chickens	Semi-industry (n=67)	31 (46.2)	23 (34.3)	2 (3.0)	5 (7.5)	6 (9.0)
	Industry (n=105)	48 (45.7)	21 (20.0)	0 (0.0)	36 (34.3)	0 (0.0)
	Total (n=172)	79 (45.9)	44 (25.6)	2 (1.2)	41 (23.8)	6 (3.5)
Total	Semi-industry (n=113)	48 (42.5)	35 (31.0)	10 (8.8)	7 (6.2)	13 (11.5)
	Industry (n=138)	62 (44.9)	32 (23.2)	0 (0.0)	44 (31.9)	0 (0.0)
	Total (n=251)	110 (43.8)	67 (26.7)	10 (4.0)	51 (20.3)	13 (5.2)

Table 4. Drugs injection for animals at different systems production

Systems animals production		Breeders/workers (n, %)	Local veterinarian (n, %)	Both of them (n, %)
Pigs	Semi-Industry (n=46)	29 (63.0)	8 (17.4)	9 (19.6)
	Industry (n=33)	33 (100)	0 (0.0)	0 (0.0)
	Total (n=79)	62 (78.5)	8 (10.1)	9 (11.4)
Chickens	Semi-Industry (n=67)	65 (97.0)	2 (3.0)	0 (0.0)
	Industry (n=105)	105 (100)	0 (0.0)	0 (0.0)
	Total (n=172)	170 (98.8)	2 (1.2)	0 (0.0)
Total	Semi-industry (n=113)	94 (83.2)	10 (8.8)	9 (8.0)
	Industry (n=138)	138 (138)	0 (0.0)	0 (0.0)
	Total (n=251)	232 (92.4)	10 (4.0)	9 (3.6)

Table 5. The drugs dosage used for animals at different systems production

Systems animals production		Drugs dosage			Stop drugs before slaughter	
		Instruction n (%)	Higher n (%)	Double n (%)	Yes n (%)	No n (%)
Pigs	Semi-industry (n=46)	33 (77.7)	10 (21.7)	3 (6.5)	15 (32.6)	31 (67.4)
	Industry (n=33)	24 (72.7)	9 (27.3)	0 (0.0)	25 (75.8)	8 (24.2)
	Total (n=79)	57 (72.2)	19 (24.0)	3 (3.8)	37 (46.8)	42 (53.2)
Chickens	Semi-industry (n=67)	38 (56.7)	27 (40.3)	2 (3.0)	28 (41.8)	39 (58.2)
	Industry (n=105)	87 (82.9)	18 (17.1)	0 (0.0)	65 (61.9)	40 (38.1)
	Total (n=172)	125 (76.7)	45 (26.2)	2 (1.2)	93 (54.1)	79 (45.9)
Total	Semi-industry (n=113)	71 (62.8)	37 (32.8)	5 (4.4)	43 (38.1)	70 (61.9)
	Industry (n=138)	111 (80.4)	27 (19.6)	0 (0.0)	90 (65.2)	48 (34.8)
	Total (n=251)	182 (72.5)	64 (25.5)	5 (2.0)	133 (53.0)	118 (47.0)

4. DISCUSSION

In Vietnam, more than 14,000 veterinary products are using for animals, including antibiotics, vitamins and anti-parasitic. Of which antibiotics (about 70% of all products) were the most common registered by many veterinary medicine companies (MARD, 2016). The pharmaceutical products were sold commonly by the veterinary medicine representation in 64 provinces and cities, where antibiotics are available "over the counter" and the control of antibiotic use is insufficient and the understanding in the community of prudent use of antibiotics is limited (Larsson *et al*., 2000; Larsson, 2003; Duong *et al*., 2006). This study observed that antibiotics were widely used in animal husbandry for therapy and prevention of bacterial infections in pig and chicken farms. At least 30 antibiotics belong to more than 10 groups were found including Aminoglycosides, Sulfamides, Tetracyclines, Phenicols, Beta-lactams and (Fluoro)-quinolones which were commonly used for disease prevention and treatment purpose. Interestingly, antibiotics are used in all breeding, even though such use is not supported by the Ministry of Agriculture and Rural Development such as Ciprofloxacin, Chloramphenicol, etc. was found in several farms. In addition, almost of the antibiotics used for animals are also used to treat human infection diseases in Vietnam such as Neomycin, Streptomycin, Penicillin, Norfloxacin, Trimethoprim, Erythromycin, etc. Several lines of evidence indicate that antibiotic resistance in animal husbandry results from the use of antibiotics in food animals (Rosenfren *et al*., 2007; Thakur *et al*., 2007; Harada *et al*., 2008; Miranda *et al*., 2008). It may suggest the high prevalence of antibiotic resistance in the bacteria isolates from both human and veterinary in Vietnam recently (Van *et al*., 2007; Ogasawara *et al*., 2008;

Vo *et al*., 2010) may link to the widespread of antibiotic use in animal husbandry.

In the pig farms, the majority of antibiotics use is for treatment or prophylaxis of respiratory and enteric disease, while in the poultry farms, antimicrobials are primarily used for mycoplasmosis and intestinal infections, namely colibacillosis salmonellosis, and necrotic enteritis. The method of administration and the volume of antimicrobial used will vary depending on the animal species, stage of production, and risk of disease. In these farms, antibiotics were selected by farmer's experiences (43.8%), by suppliers (26.7%) and follow the prescription of veterinarians after getting the diagnosis results (20.3%). Interestingly, more than one fourth of the farms used antibiotics with over dosage for animals. The concerns became more serious in the semi-industrial farms, where 32.8% of the farms abused antibiotic. In addition, farmers often do not comply with regulations that require them to stop using antibiotics before slaughter. These may link to the high prevalence of antibiotic residue above acceptable limits in foodstuffs in Vietnam (Vo *et al*., 2010; Dang *et al*., 2011). These findings observed that antibiotics were mainly given to animals by farmers or workers. Of which, 92.4% of them undertake themselves the therapy for animals and only 4% need the local veterinarian's assistance. Previous study also showed that farmers normally use antibiotics to treat sick animals with higher dose and using their own experience without veterinary prescription, supervision and laboratory diagnosis (Duong *et al*., 2006). In addition, recent reports in Vietnam (Dang *et al*., 2011) and other countries (da Costa *et al*., 2008) confirmed that the volume of antibiotics used in food animal production has led to concerns in the public, regulatory, and scientific arenas that antibiotic use in food animals is contributing to the antibiotic resistance problem by creating a reservoir of resistant bacteria.

There is a high prevalence of antibiotic resistant bacteria in Vietnam (Van *et al*., 2007; Ogasawara *et al*., 2008; Vo *et al*., 2010). This is almost certainly a consequence of the high levels of antibiotic use, much of which is inappropriate. Whilst antibiotic use in animals is widespread, the role of these in resistance levels in human pathogens remains unclear in Vietnam and other countries. As a consequence of the high rates of resistance, many antibiotic regimens advised in current treatment guidelines are unlikely to be effective. Although very hard to quantify, the profile of antibiotic resistance observed in Vietnam undoubtedly causes negative health and economic impacts.

In present study, data were observed the antibiotic overuse and illegal use in pig and chicken production in the North of Vietnam. It may create difficulties for the control and management on animal diseases in Vietnam. Prudent use principles are a guide for optimal use of antibiotics. Such experience was performed in Denmark, where reductions in the use of growth promoting antimicrobials have been followed by reduction in the prevalence of resistance to these agents in bacterial species sampled from food animals. The farmers and veterinarians should not be interpreted so restrictively as to replace professional judgment of practitioners or to compromise animal health or welfare. In all cases, animals should receive prompt and effective treatment as deemed necessary by the prescribing or supervising veterinarians.

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DETECTION OF *RHABDITIS* (*RHABDITELLA) AXEI* IN DIARRHEA FECES OF A DOMESTIC PIGEONS (*COLUMBA LIVIA*)

Nguyen Thi Hoang Yen[1] and Nguyen Thi Lan Anh[1]*

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ABSTRACT

Rhabditis (Rhabditella) axei is usually a free-living inhabitant in the soil on decaying organic materials, but it has been documented associated in urine and stool of humans as well as some other animals such as dogs, cattle, monkeys, porcupine, and chickens. Just some of them showed symptoms such as diarrhoea, respiratory problem. We herein reported a case of a domestic pigeon association with diarrhoea. Diarrhea fecal was collected and examined by sedimentation and floatation techniques. The result showed that one kind of nematode with a typical rahditiform esophagus was discovered. Next, PCR and sequencing assays were performed based on 18S rDNA gene. In result, it was identified to be *Rhabditis axei.* This is the first report for the identification of *R. axei* in pigeons. Though there was not enough evidence of the presence of *R. axei* in the pigeon intestine and transmission route of this worm to the pigeon in the farm, however the discovery of *R. axei* gives the warning about the risk of transmission of *R. axei* to humans, especially for the barn cleaning-related people in this farm.

Keywords: *Rhabditis axei, Diarrhoea feces, pigeon.*

1. INTRODUCTION

Rhabitida is a large order of Aphasmidia comprising free-living and parasitic nematode worms with a typical rhabditiform esophagus which is more or less clearly divided into three regions including corpus, isthmus, and bulb. Only three genera in this order Rhabditida parasitize domestic animals: *Rhabtidis* (syn., *Pelodera*), *Halicephalobus* (syn., *Micronema*), and *Strongyloides.*

Rhabditis (Rhabditella) axei is usually a free-livng inhabitant in the soil on decaying organic materials, but it has been documented associated in human urine and stool (Meamar *et al*., 2007; Yang *et al*., 2018; Yu *et al*., 2019), in feces of dogs (Stachurska-Hagen *et al*., 2016); monkeys (Kreis and Faust, 1993), chickens (El-Azazy *et al*., 1988), a porcupine (Rakhshanpour *et al*., 2012), cattle (Duarte *et al*., 2001), and piglets (Stachurska-Hagen *et al*., 2016). The cases of infected human patients were due to be exposed to polluted sewage or through drinking or contacting wastewater. The migration of nematodes in human caused true signs of hematuria, diarrhea, and high eosinophilia (Yang *et al*., 2018; Campos *et al*., 2022). Besides, ear infection (Teschner *et al*., 2014), in ADIS patient (Meamar *et al*., 2007) association with *R. axei* in feces were reported. The cases of animals were almost found *R. axei* among the hairs of dogs and monkeys (Kreis and Faust, 1993), or soiled feet or feathers of chickens (El-Azazy *et al*., 1988). Almost infected animals didn't show any symptom, except for the abdominal respiratory in dogs (Kreis and Faust, 1993) or diarrhoea in piglets or external otitis in cattle (Duarte *et al*., 2001; Stachurska-Hagen *et al*., 2016).

We herein reported a case of identification *R. axei* in the diarrhoea feces of a domestic pigeon by PCR and sequencing techniques.

2. MATERIALS AND METHODS

2.1. Fecal samples

A diarrhoea fecal sample of the pigeon was transferred to the laboratory of veterinary

[1] Vietnam National University of Agriculture

* Corresponding Author: Dr. Nguyen Thi Hoang Yen, Department of Parasitology, Faculty of Veterinary medicine, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Ha Noi, Vietnam; Tel: (84)982595128; Email: nthyen@vnua.edu.vn.

parasitology, faculty of Veterinary Medicine, Vietnam National University of Agriculture to be examined for parasite infection on July, 2019. The pigeon was raised in a pigeon farm which was located at Luc Nam district, Bac Giang province. This sample was collected on the fecal stray by 5ml syringe immediately after the pigeon excreted. The sample was then stored at 4^{o}C and examined on next day.

2.2. Fecal examination method

Wisconsin Sugar centrifugal-floatation technique with some small modifications was applied by using Sheather's sugar (specific gravity 1.27) for fecal examination. Briefly, the fecal sample was mixed thoroughly, and 5g of feces was placed in a 100mm mesh sieve, and all put all into porcelain mortar with adding of 50ml tap water. Pestle was used to wash the feces. Fecal solution was then filtered through the mesh sieve, the remaining debris was removed. Next, 20ml of the fecal solution was taken and equally divided into two 15ml test tubes (10 ml/tube) which were then centrifuged at 1500rpm (650xg) for 10 minutes. After centrifuging, the supernatant was removed, floatation medium was added in each tube, filling the tube halfway. A wooden applicator stick was used to thoroughly break up the feces and mix them with the medium in each tube to ensure that the parasite eggs were exposed to the medium. Then, floatation medium was added again until the 15ml line of each test tube. The second centrifugation step was repeated as above. The tubes were then filled with floatation solution until a slight positive meniscus formed, and a 22x22-mm glass coverslip was placed on top of each tube for 15min. Finally, the coverslip was taking out and placed onto the glass slides to be examined under a compound microscope at a viewing magnification of 10x, 40x.

After checking the possible parasites on the coverslip, the sugar solution supernatant was removed, the sediment was kept, diluted into tap water, settled down for 15min. Finally, the supernatant was removed, the sediment was poured into a petri dish in order to find possible parasites.

The size of parasites was measured by Olympus CX3 EP50 microscope. The parameters included the length of eosophagus, the length and width of adult male and female. The average length and width were calculated based on 10 individuals for each.

2.3. DNA extraction

Parasite DNA was extracted by Alkaline -lysis method (Nguyen *et al*., 2016). Briefly, 180ml of 50mM NaOH was added into each 1.5ml tube containing suspected parasites. The tubes were then incubated at 95^{o}C for 10min before adding 20ml of 1M Tris-HCl (pH 8.0). Next, the mixture was vortexed thoroughly and centrifuged at 14,000x*g* for 10min. Finally, the supernatant was separated and stored at -20^{o}C until using.

2.4. Polymerase chain reaction (PCR) assay

A segment (935bp) of the 18S rDNA gene was amplified using a pair of primers 988F (5'-CTCAAAGATTAAGCCATGC-3') and 1912R (5'-TTTACGGTCAGAACTAGGG-3') (Nagayasu *et al*., 2017). PCR was performed in a 50µl reaction mixture containing 25µl of Mastermix 2X_100_Tracking dye (Phusa Biochem LTD. Company, Can Tho, Viet Nam), 0.4mM of each primer and 1µl of 10x diluted parasite lysate. The amplification condition was conducted as follows: 94°C for 2min; followed by 40 cycles of 94°C for 10sec, 55°C for 30sec, and 68°C for 1min; and finally 68°C for 7min (Phoo *et al*., 2019). After that, 10µl of PCR product was separated on 1% agarose gel electrophoresis.

2.4. DNA sequencing and Phylogenetic tree analysis

PCR products were sent to Suran Medical and Scientific Solutions Join Stock Company (Ha Noi, Viet Nam) in order to be purified and sequenced in both directions using the same primers as employed in the PCR. DNA sequences were then aligned using Geneious Prime (Biomatters Company) and compared

with sequences from the GenBank database via the BLAST search tool. Phylogenetic tree was constructed by the maximum likelihood algorithm using the best substitution model, implemented in MEGA XI.

3. RESULTS

3.1. The result of fecal examination

By fecal examination, just one kind of rhabditida roundworm was determined, without any other parasites (Fig. 1). Both adult and larvae - stage worms were recognized with the speciality of rhabditiform eosophagus and remarkble long tails, neither adult nor larva with filariform eosophagus was discovered. The average length of female and male parasites was 1300-1500 and 1000-1200mm, respectively. The average width of body in female and male was 90 and 65mm, respectively. Besides, the length of rhabditiform eosophagus was measured in 130-147mm. Embryonated eggs could be observed in the female uteri. The number of worm was around 20 ones including larvae and adults per g feces.

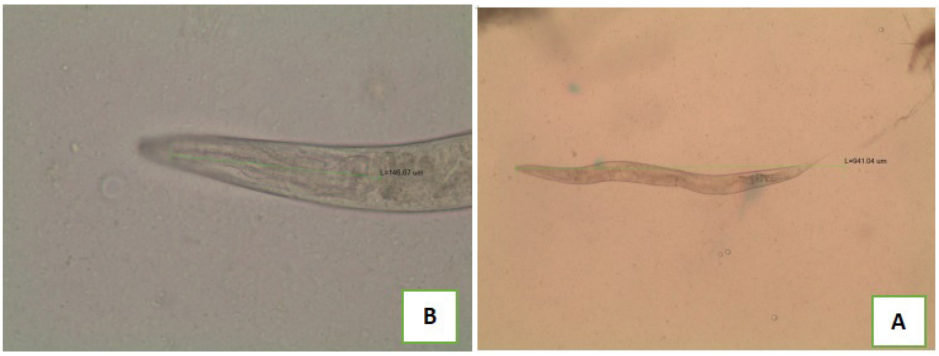

Fig 1. The male of *Rhabditis axei* (A), and Rhabditiform esophagus (B)

3.2. The result of DNA and sequencing analysis

The rhabditida worm - suspected samples were analyzed by the PCR assay, and the result showed that an around 1,000bp region of 18S rDNA was successfully amplified (Fig 2). Sequencing analysis of PCR product revealed a high degree of similarity (99.77-99.86%) with reference sequences of *Rhabitella axei* (Fig 3).

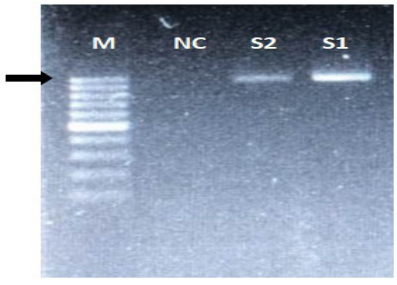

Fig 2. The photo of electrophoresis of *Rhabditida worm*

M: 100bp ladder, NC: negative control, S1: DNA template, S2: 10xdilution of DNA template. Black arrow shows the position of 1,000bp band

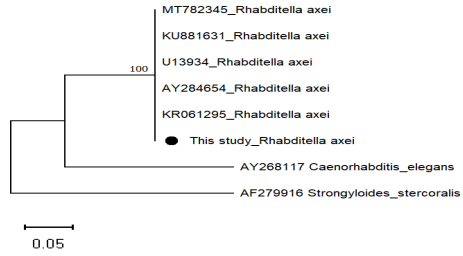

Fig 3. Maximum-likelihood tree based on the 18S rDNA gene haplotype data

MEGA XI software was used to build a phylogenetic tree using a dataset 10 sequences (obtained from the GenBank database). The numbers at the nodes indicate bootstrap values. Black button represented for the sequence obtained in this study.

4. DISCUSSION

Pigeons belonging to the order Columbiformes are ubiquitous birds and

distributed over the world. Along with raising pigeons as ornamental, homing as well as companion animals; pigeons are also raised to provide the food for humans. The farm in this study has about 50,000 pigeons in number, hence the farm owner is very concerned about the diseases in his flock of pigeons. The diarrhoea feces of the pigeon was sent to our laboratory to examine parasites, and there was the only rhabditiform worm detected. Our initial identification was *Strongyloides* worm under microscope with the typical rhabditiform esophagus. Morphologically, *Strongyloides* and *Rhabditis (Rhabditella)* genus have similar characterisitics with the typical rhabditiform esophagus comprising of three regions: corpus, isthmus, and bulb (Bowman, 2014). Thus, misidentification between two genus is possible, particularly in the areas where *Strongyloides* is prevalent as Vietnam (Nguyen *et al*., 2019). However, by PCR and sequencing techniques, we confirmed this worm to be *R. axei*.

As above mentioned, *R. axei* is one of the free-living roundworms which live in the soil, on decaying organic materials. However, it was found on hair or feather of dogs and chickens (El-Azazy *et al*., 1988; Kreis and Faust, 1993), and this roundworm did not cause serious health problems for those animals, except for the symptoms of abdominal respiratory in dogs or diarrhoea in piglets or external otitis in cattle (Kreis and Faust, 1993; Duarte *et al*., 2001; Stachurska-Hagen *et al*., 2016). However, the serious symptoms were observed in humans who were infected with *R. axei*, such as hematuria, diarrhea, and high eosinophilia (Yang *et al*., 2018; Campos *et al*., 2022), and ear infection (Teschner *et al*., 2014).

Up to now, there has not been reported for the presence *Rhabditis* genus in pigeons. Some experimental study of *R. axei* infection in dogs and chickens showed that this nematode worm did not develop in these animals. Unfortunately, we had no chance to necropsy this pigeon to look for this roundworm in the intestine of the pigeon. Thus, there is inadequate proof of establisment of *R. axei* in the intestinal in the pigeon in order to consider it as true parasites.

5. CONCLUSION

This is the first report for identification of *R. axei* in the pigeon diarrhoea feces. Even though there was not enough evidence of the presence of *R. axei* in the pigeon intestine and transmission route of this worm to the pigeon in the farm, however the discovery of *R. axei* gives the warning about the risk of transmission to humans, especially the barn cleaning related-people in this farm.

ACKNOWLEDGEMENT

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IDENTIFICATION OF CO-INFECTION OF NEWCASTLE DISEASE VIRUS AND INFECTIOUS LARYNGOTRACHEITIS VIRUS IN CHICKENS IN NORTHERN VIETNAM BY MULTIPLEX POLYMERASE CHAIN REACTION

Tran Thi Huong Giang[1], Nguyen Thi Sinh[2], Huynh Thi My Le[1] and Dong Van Hieu[1]*

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ABSTRACT

This research was conducted to evaluate the co-infection of Newcastle disease virus (NDV) and Infectious laryngotracheitis virus (ILTV) in diseased chicken flocks and utilize multiplex polymerase chain reaction (mPCR) in diagnosis. A total of 50 pooled tissue samples were collected from clinically suspected flocks and diseased chickens farmed in northern Vietnam from December, 2021 to May 2022. The results indicated that the positive rates for the NDV genome according to individual sample and farm levels were 14% and 30.76%, respectively; for the ILTV genome were 12% and 30.76%, respectively; and for co-infection of the two viral genomes were 6% and 23.07%. mPCR was utilized to detect both the NDV and ILTV genomes in samples with high sensitivity (100%) and specificity (100%), compared to single conventional PCR. The finding of this study gains a better understanding of NDV and ILTV, leading to the development of diagnostic methods and disease prevention strategies in the chicken industry.

Keywords: *Chickens, Coinfection, ILTV, mPCR, NDV.*

1. INTRODUCTION

Newcastle disease (ND) and Infectious Laryngotracheitis (ILT) are highly contagious respiratory diseases in the poultry industry. The ND is caused by ND virus (NDV), or avian paramyxovirus type 1, a species belonging to the genus *Avian orthoavulavirus 1* of the family *Paramyxoviridae* (Dimitrov *et al*., 2019). Regarding ILT, the disease is caused by a herpes virus (*Gallid alphaherpesvirus 1* of the family *Alphaherpesvirinae*) (Maekawa *et al*., 2019).

[1] Vietnam National University of Agriculture
[2] Hanvet Pharmaceutical Company
[*] Corresponding author: Dr. Dong Van Hieu, Faculty of Veterinary Medicine, Vietnam National University of Agriculture; Tel: +84-914977587; Email: dvhieuvet@vnua.edu.vn.

The etiology of the respiratory disease is complex, suggesting that symptoms involve more than one pathogen. Chickens of all ages can be infected with NDV, while ILTV has been recognized in broiler chickens at three weeks of age (Dormitorio *et al*., 2013). These viruses can cause disease not only independently but also in association with each other (Ali and Reynolds, 2000). It is needed to diagnose and differentiate the pathogen agent in respiratory disease conditions in domestic chickens.

A multiplex polymerase chain reaction (mPCR) assay can simultaneously detect and distinguish pathogens in a single reaction. It is recommended to be a highly advantageous screening assay in both the clinical and the research laboratory. It would greatly aid in the diagnosis and control of outbreaks rapidly.

We herein reported cases of identification of the NDV and ILTV genome in diseased chickens in some provinces in northern Vietnam by mPCR technique.

2. MATERIALS AND METHODS

2.1. Materials

Sample collections: a total of 50 pooled samples including larynx, lung, and brain were collected from diseased chickens in Phu Tho (n=10), Bac Ninh (n=10), Thai Nguyen (n=15), and Hai Phong (n=15) during December 2021 to May 2022 . In the study, all chickens were collected from ND-vaccinated chicken farms, however, they did not vaccinate with ILTV vaccine. Samples were transferred to and processed at the Faculty of Veterinary Medicine, Vietnam National University of Agriculture.

Preparation of positive controls: the attenuated La Sota strain (TW-XI-16, Vetvaco, Vietnam) and the Medivac ILT vaccine (Medion-Bandung-West Jave, Indonesia) were used as positive controls.

2.2. Methods

RNA/DNA extraction, cDNA synthesis: total RNA and DNA were extracted from homogenated samples using the Viral Gene-spin™ Viral DNA/RNA Extraction Kit (iNtRON Biotechnology, Seoul, Korea) according to the manufacturer's instructions. cDNA was synthesized using the Maxime™ RT PreMix Kit (iNtRON Biotechnology, Seoul, Korea). The reaction was performed following conditions: 45^{o}C for 60min, 95^{o}C for 5min.

Conventinal polymerase chain reaction (PCR) assay: positive and negative samples for NDV and/or ILTV by single PCR were used for the initial setup and application of mPCR using two set of primers as previous reported (Table 1) (Stäuber *et al*., 1995; Pang *et al*., 2002).

Multiplex PCR (mPCR): The mPCR technique was performed using two pairs of primers (Table 1). A total of 25µl volumes, in which the reaction mixture included 12,5µl of 2X Gotag® Green Master Mix (Promega, USA), 1µl of forward and reverse primers with final concentration 0,25 pmol/µl of each (Table 1), 1µl of cDNA, and 1µl of DNA, and 6,5µl of distilled water, was added in a PCR tube. The amplification condition was conducted as follows: 95°C for 5min; followed by 40 cycles of 95°C for 10 sec, 55°C for 30sec, and 72°C for 1min; and finally 72°C for 5min. After that, 10µl of PCR product was separated on 1.2% agarose gel electrophoresis.

Table 1. Primers were used in the study

Name	Nucleotide sequencee (5′-3′)	PCR product (bp)	References
NDV-F	GGAGGATGTTGGCAGCATT	310	(Stäuber *et al*., 1995)
NDV-R	GTCAACATATACACCTCATC		
ILTV-F1	ACGATGACTCCGACTTTC	647	(Pang *et al*., 2002)
ILTV-R1	CGTTGGAGGTAGGTGGTA		

2.3. Data analysis

Data were analyzed by Excel software. Sensitivity, specificity, and Kappa coefficient of the mPCR method compared with PCR were evaluated according to the previous studies (Altman and Bland, 1994; Feuerman and Miller, 2008).

3. RESULTS

3.1. Clinical and Postmortem Findings

The common clinical signs of chickens suspected of ND and ILT recorded in the present study were twisting of the head, depression, and difficulty breathing (Fig 1A). At necropsy, some diseased chickens showed mucous on the trachea (Fig 1B), point hemorrhage in the larynx (Fig 1C), and pin-point hemorrhages in the proventriculus (Fig 1D).

3.2. Detection NDV and ILTV genomes in the field samples

The NDV and/or ILTV genomes detected in the diseased chickens by mPCR were summarized in Table 2. In detail, 7/50 (14%) samples were determined positive with the NDV genome, while 6/50 (12%) samples were positive with the ILTV genome. In addition, the coinfection-positive rate for the NDV and ILTV genomes was 6% (3/50) (Fig. 2).

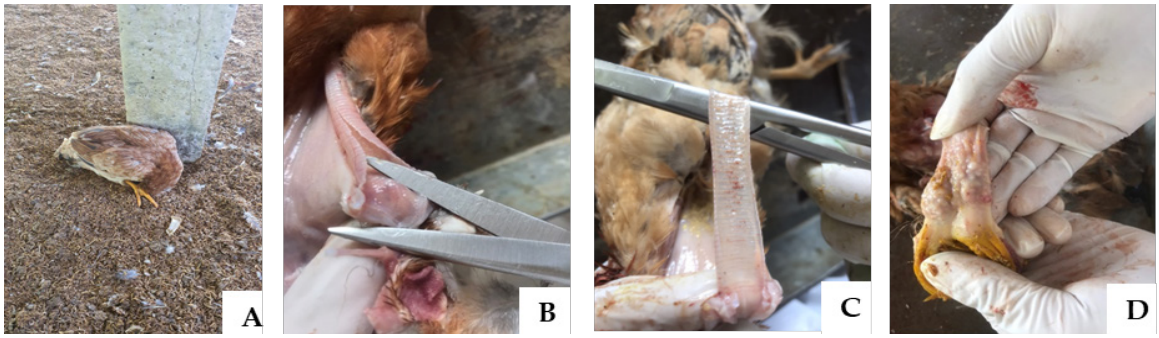

Fig 1. Clinical and postmortem findings in diseased chickens

(A) twisting of the head, difficulty breathing; (B) mucous on the trachea; (C) point hemorrhage in the larynx, (D) pin-point hemorrhages in the proventriculus

Table 2. Detection of NDV and/or ILTV genome in domestic chickens in some northern Vietnam

City/ Provinces	No. of samples	NDV		ILTV		NDV + ILTV	
		No. of positive samples	Positive rate (%)	No. of positive samples	Positive rate (%)	No. of positive samples	Positive rate (%)
Phu Tho	10	2	20	1	10	1	10
Bac Ninh	10	0	0	2	20	0	0
Thai Nguyen	15	2	13.33	1	6.67	1	6.67
Hai Phong	15	3	20	2	13.33	1	6.67
Total	50	7	14	6	12	3	6

Table 3. Detection of NDV and/or ILTV genome in domestic chicken flocks in northern Vietnam

City/ Provinces	No. of flocks	NDV		ILTV		NDV + ILTV	
		No. of positive flocks	Positive rate (%)	No. of positive flocks	Positive rate (%)	No. of positive flocks	Positive rate (%)
Phu Tho	3	1	33.3	1	33.3	1	33.3
Bac Ninh	2	0	0	1	50	0	0
Thai Nguyen	4	1	25	1	25	1	25
Hai Phong	4	2	50	1	25	1	25
Total	13	4	30.76	4	30.76	3	23.07

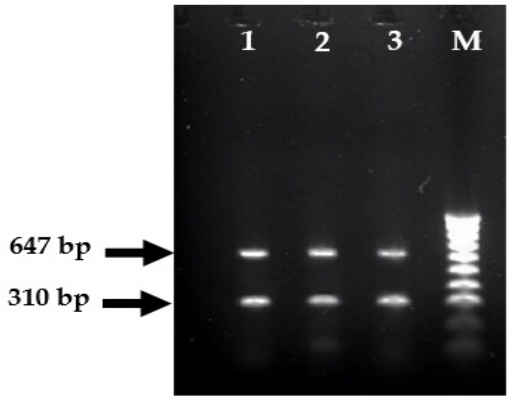

Fig 2. PCR product NDV (310bp), ILTV (647bp) genome by mPCR (M: marker 100bp; 1, 2, 3: field samples)

Of the 13 farms with suspected infections, 4 (30.76%) flocks were positive for the NDV and ILTV genome individually (Table 3). Regarding the co-infection of NDV and ILTV, 3 flocks were evaluated to be positive (23.07%).

Among 50 samples, the samples were determined to be positive for NDV (7), ILTV (6), and coinfection of NDV and ILTV (3) (Table 4). The results indicated that mPCR showed sensitive (100%) and specificity (100%) when compared with single PCR. In addition, the Kappa index was 1 when mPCR and single PCR were applied to detect NDV and ILTV genomes in the field samples (Table 4).

Table 4. Sensitivity and specificity of mPCR compared with single PCR

mPCR	NDV		ILTV		NDV + ILTV		
	No. of samples +	No. of samples -	No. of samples +	No. of samples -	No. of samples +	No. of samples -	
No. of samples +	7	0	6	0	3	0	Positive predictive value: 100%
No. of samples -	0	43	0	44	0	47	Negative predictive value: 100%
	Sensitive 100%	Specificity 100%	Sensitive 100%	Specificity 100%	Sensitive 100%	Specificity 100%	

Kappa = 1, "+": positive; "-": negative

4. DISCUSSION

NDV is still a serious pathogen threat to the poultry industry, even though a vaccination program has been applied. Some studies reported ND outbreaks among vaccinated commercial flocks (Xiao *et al*., 2012; Mariappan *et al*., 2018). ILT is also a contagious disease of poultry and is spread all over the world, especially it is present in regions with developed poultry farming. ILTV outbreaks were reported in commercial layer flocks in some Asian countries (Yan *et al*., 2016; Yang *et al*., 2020).

ND outbreaks were also reported among vaccinated commercial flocks in Vietnam (Choi *et al*., 2014; Le *et al*., 2018). Although ILTV has been reported in commercial flocks in some countries, the information on ILTV still limits in Vietnam (Dormitorio *et al*., 2013; Maekawa *et al*., 2019). Recently, the genome of ILTV was reported in chickens farmed in Hanoi, Vietnam in 2022 (Dong *et al*., 2023). In the present study, the samples were collected from the diseased chickens showing some clinical and postmortem signs such as twisting of the head, depression, difficulty breathing, point hemorrhage in the larynx, and pin-point hemorrhages in the proventriculus. In addition, the number of samples collected might influence the positive rate of NDV and/or ILTV in the current study. Therefore, increasing the number and geography of sample collection is necessary

for understanding well the coinfection of NDV and ILTV.

mPCR is described to be able to detect RNA from some viral pathogens (Infectious bronchitis virus, avian influenza virus, and NDV), as well as NDV from ILTV and some bacteries (Pang *et al*., 2002). Here, the mPCR was applied to detect NDV and ILTV genomes in domestic chickens. In preliminary detection of NDV and/or ILTV in the field samples, mPCR showed high sensitivity and specificity (100%) with the Kappa value equal to 1. mPCR with rapid, specific detection would greatly aid diagnosis and control of outbreaks.

5. CONCLUSION

The positive rate for NDV (14%), ILTV (12%), and coinfection of NDV and ILTV (6%) in domestic chickens were reported in the present study. Regarding the chicken flocks, the positive rate for NDV (30.76%), ILTV (30.76%), and coinfection of NDV and ILTV (23,07%). Furthermore, mPCR was applied to detect the NDV and/or ILTV genome with high sensitivity and specificity compared with a single PCR.

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EFFECT OF ULTRAVIOLET-C (UV-C) LIGHT ON THE REDUCTION OF *VIBRIO* SPP.

Vu Thi Thu Tra[1,2,], Thomas Alter[2] and Stephan Huehn[3]*

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ABSTRACT

Vibrio spp. are ubiquitous and abundant in aquatic environments, including estuaries, marine coastal waters and sediments, as well as aquacultures worldwide. Human infections caused by *Vibrio* are usually associated with the consumption of raw or undercooked seafood. To minimize the risk of *Vibrio* infections, decontamination methods involving thermal and non-thermal processes have been used to eliminate these pathogenic bacteria in the food chain. This study was conducted to determine the effects of UV-C irradiation on the reduction of *Vibrio* spp. in pure culture. The obtained data showed that UV-C light could effectively reduce the number of *Vibrio* spp. Among four *Vibrio* spp. tested, *V. alginolyticus*, *V. cholerae* and *V. vulnificus* were more susceptible to UV-C light than *V. parahaemolyticus*.

Keywords: *UV-C, Vibrio spp., pure cultures.*

1. INTRODUCTION

Vibrio (*V.*) spp. are ubiquitous and abundant in aquatic environments, including estuaries, marine coastal waters and sediments, as well as aquacultures worldwide (Thompson *et al*., 2004). Among the family *Vibrionaceae*, some species such as *V. alginolyticus*, *V. cholerae*, *V. damselae*, *V. fluvialis*, *V. furnissii*, *V. harveyi*, *V. hollisae*, *V. metschnikovii*, *V. mimicus*, *V. parahaemolyticus* and *V. vulnificus* can cause diseases in both aquatic animals and humans (Austin, 2010). Human infections caused by *Vibrio* are usually associated with the consumption of raw or undercooked seafood (e.g. *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*), or septicaemia and wound infections via contact with contaminated seawater or seafood (e.g. *V. alginolyticus*, *V. vulnificus*) (Igbinosa and Okoh, 2008). Due to the global warming with rising seawater temperatures, Vibrio-associated diseases in humans and marine animals have been highlighted in recent reports (Thompson *et al*., 2004; Le *et al*., 2015).

To minimize the risk of *Vibrio* infections, decontamination methods involving thermal and non-thermal processes have been used to eliminate these pathogenic bacteria in the food chain. UV-C light irradiation has been reported as an effective non-thermal treatment for the inactivation of pathogenic microorganisms in water and wastewater (Hijnen *et al*., 2006, Turtoi, 2013) as well as on food products (Chun *et al*., 2009; Birmpa *et al*., 2013; Crook *et al*., 2015; Ha *et al*., 2016; Lee *et al*., 2016). The UV-C light with wavelengths between 200-280nm effectively inactivates most types of microorganisms and a wavelength of 254nm is commonly used because of the highest germicidal effect (Turtoi, 2013; Ha *et al*., 2016). The inactivation mechanism of UV light is based on the damage of nucleic acid of the cells by the formation of photoproducts especially pyrimidine dimers in the DNA, resulting in interruption of DNA transcription and replication (Hijnen *et al*., 2006, Koutchma *et al*., 2009, Turtoi, 2013).

However, to date there is little information on the application of UV-C light

[1] Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi, Vietnam

[2] Institute of Food Safety and Food Hygiene, Freie Universitaet Berlin, 14163 Berlin, Germany

[3] Life Sciences and Technology, Beuth University of Applied Sciences, 13353 Berlin, Germany

[*] Corresponding author: Dr. Vu Thi Thu Tra, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Ha Noi, Vietnam; Tel: (84)866173680; Email: vutra@vnua.edu.vn.

to inactivate *Vibrio* spp. and no comparable data on reduction levels of different *Vibrio* species under UV-C treatments. Therefore, the purpose of this study was to investigate the effects of UV-C on the reduction of *Vibrio* spp. in pure cultures.

2. MATERIALS AND METHODS

2.1. Materials

Four different *Vibrio* species, *V. alginolyticus*, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* were used in the study (details are given in Table 1).

Table 1. Characteristics and sources of *Vibrio* strains used in this study

Vibrio strains	Characteristics and sources
V. alginolyticus (ATCC 17749)	Serotype XII Spoiled horse mackerel which caused food poisoning in Japan
V. cholerae (NCTC 4711)	Serotype O:2 (non-O1, non-O139) Isolated from a case of cholera in Nanking, China
V. parahaemolyticus (RIMD 2210633)	Serotype O3:K6, *tdh* (+) Clinical strain isolated in Osaka, Japan
V. vulnificus (V57/10)	Biotype 1 Human isolate from wound infection in Germany

2.2. Methods

*Bacterial culture preparation: p*rior use, all strains were stored in cryovials at -80°C (Cryobank; Mast Diagnostica, Bootle, England). Initially, bacterial cells were resuscitated in alkaline peptone water (APW, 1l contains 10g peptone from casein, 20g NaCl, and 3g yeast extract (Merck, Darmstadt, Germany)) at 37°C for 24h. Enrichments were streaked on thiosulfate citrate bile salt sucrose (TCBS) agar (Oxoid, Basingstoke, Hampshire, England) followed by incubating at 37°C overnight. A single colony on TCBS agar was transferred into 10 ml of APW (2% NaCl) and incubated under constant shaking (200rpm) at 37°C for 4h. Then 100µl of 4h cultures were diluted into 150 ml APW (2% NaCl) and grown at 37°C overnight.

*UV-C light treatments: t*he UV light treatments were performed with the BioLink DNA Crosslinker. The internal chamber (height: 14.5cm, depth: 33cm, width: 26cm) contained five UV tubes (8W, 254nm), the UV irradiation energy range was between 0-99.99 J/cm^2. Overnight bacterial culture plates were placed in the middle of the treatment chamber and exposed to four different doses of 0.5, 1, 2 and 3 J/cm^2. Five repetitions for each *Vibrio* spp. culture were conducted. To ensure that bacteria were not killed by temperature, for all trials the temperatures of the internal chamber before treatment was set at 21°C and temperatures of the bacterial cultures before treatment were adjusted to room temperature (21.2±0.4°C). After treatments, temperatures of the bacterial culture as well as temperatures of the internal chamber were recorded. ΔT_{Sample} and $\Delta T_{Chamber}$ were calculated as follows:

ΔT_{Sample} (°C) = temperatures of bacterial cultures after treatment - temperatures of bacterial cultures before treatment;

$\Delta T_{Chamber}$ (°C) = temperatures of internal chamber after treatment - temperatures of internal chamber before treatment.

Vibrio enumeration: Vibrio counts were determined using the drop plating method on tryptic soy agar (TSA; Merck) containing 1% NaCl for determination of initial counts and on TSA agar for determination of UV-treated cell counts. Briefly, samples were 10-fold serially diluted in APW (2% NaCl) and 0.05 ml of diluent was dropped onto agar plates. All plates were incubated at 37°C for 24h and colonies were counted. Initial counts of *Vibrio* spp. in pure cultures were between 8.1-8.8log$_{10}$ (CFU/ml).

*Data analysis: l*og reductions of *Vibrio* spp. were calculated by $\log_{10}$ (N_0/N) with N_0 as the initial counts of *Vibrio* spp., and N as the

numbers of cells surviving from the UV-treated samples. Statistical analysis was done using SPSS software (IBM SPSS Statistics Version 25). One-way analysis of variance (ANOVA) and the PostHoc Turkey test were used for comparison of different treatments; the significance level was set at P-value of <0.05.

3. RESULTS AND DISCUSSION

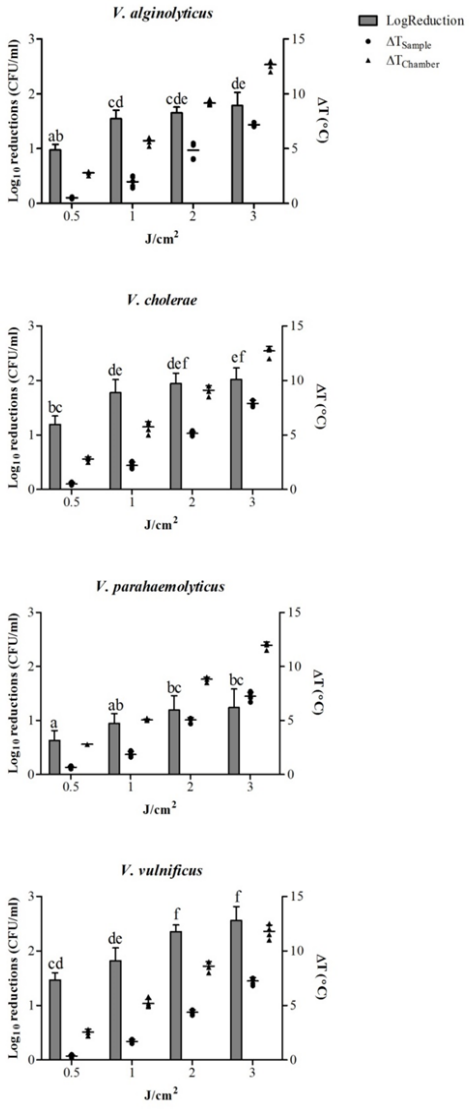

Fig 1. Effect of UV-C light on inactivation of *Vibrio* spp. in pure cultures.

Groups with different letters are significantly different (P<0.05). Data are Mean±SD.

The effects of UV-C irradiation on the reduction of *Vibrio* spp. are showed in Figure 1. Treatment at 0.5, 1, 2 and 3 J/cm^2 obtained 0.63, 0.95, 1.19 and 1.24log reductions of *V. parahaemolyticus*, respectively. In addition, the reduction levels of *V. parahaemolyticus* at each treatment dosage were significantly lower (P<0.05) compared to the other three *Vibrio* spp. Treatments at the same conditions (0.5, 1, 2 and 3 J/cm^2) decreased the numbers of *V. alginolyticus* by 0.97, 1.55, 1.65 and 1.78 log CFU/ml; of *V. cholerae* by 1.19, 1.78, 1.94 and 2.02 log CFU/ml and 1.47, 1.82, 2.35 and 2.56log CFU/ml for *V. vulnificus*, respectively.

A previous study of Mori *et al*. (2006) showed that under exposure of UV-A LED (365nm) for 2.5, 5 and 10min, the germicidal effects of 30, 70 and 100% for *V. parahaemolyticus* (O3:K6) were observed. In our study, *V. parahaemolyticus* was found to be the most resistant species to UV-C irradiation. The findings of Hamamoto *et al*. (2010) highlighted the role of the SOS response (a global regulatory network for repairing DNA damage in bacteria induced by various environmental stresses) to UV-C resistance in *V. parahaemolyticus*, this response is stronger with UV-C irradiation than with UV-A irradiation.

CONCLUSION

The obtained results indicate that UV-C irradiation could effectively reduce the number of *Vibrio* spp. in pure cultures. *V. alginolyticus*, *V. cholerae* and *V. vulnificus* were more susceptible to UV-C light than *V. parahaemolyticus*.

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STUDY ON GROWTH PERFORMANCE OF BLACK SOLDIER FLY LARVAE FED HOUSEHOLD FOOD WASTES

Nguyen Huu Hoa[1], Van Hoai Thinh[2], Dang Hoang Dao[3], Le Huynh Quang Thong[1] and Duong Nguyen Khang[1]*

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ABSTRACT

The study was carried out from February to April 2023 at households of Nhon trach district, Dong Nai province to determine the growth rate of black soldier fly larvae fed different residues and wastes. The study was surveyed the food input and wastes of 150 households, recorded the growth rate of black soldier fly larvae fed these different residues and wastes of 30 households selected randomly. Before raising the black soldier fly larvae, organic waste is treated by collection service, unsorted and un-reused. Thirty households classified and divided into 5 groups of wastes for the study including soybean residues, household leftovers, spoiled fish from market, fruit waste and spoiled vegetables. Each waste for larvae rearing was randomly assigned to 6 farmers, three trays for each household. During larvae rearing, 100 individuals were randomly selected from each tray of each household. All 30 trays were randomly selected from 30 households out of 30 surveyed households to record the growth of larvae on 5 these different wastes. On the first day of experiment, each tray was added 10 grams of eggs with the same laying date as 200 grams of chicken

[1] Nong Lam University of Ho Chi Minh City
[2] Regional Animal Health Office No. 6
[3] HUTECH university
[*] Corresponding Author: Professor Dr. Duong Nguyen Khang, Nong Lam University of Ho Chi Minh City; Tel: (84)989390179; Email: duongnguyenkhang@gmail.com

concentrate feed. After 5 days, the experimental trays received the amount of food according to the arranged ration formula. Parameter criteria included length, width and weight through days 7, 14 and 21 after hatching. The results showed that soybean waste, household leftovers, spoiled fish, fruit wastes and spoiled vegetables effected on the length of black soldier fly larvae at day 7 of 7.54, 7.17, 7.03, 6.80 and 7.74 mm, respectively (P<0.001); on the day 14 were 13.23, 13.10, 13.93, 13.73 and 11.18 mm, respectively (P<0.001); on the day 21 were 19.84, 20.91, 21.70, 20.94 and 19.20 mm, respectively (P<0.001). The width of the black soldier fly larvae at day 7 were 2.05, 2.13, 2.24, 2.27 and 1.75 mm, respectively (P<0.001); at day 14 were 3.43, 3.29, 3.84, 3.66 and 2.71 mm, respectively (P<0.001); at day 21 were 3.96, 3.88, 4.56, 4.17 and 3.18 mm, respectively (P<0.001). The weight of black soldier fly larvae at day 7 were 1.70, 1.65, 2.15, 1.86 and 1.66 g, respectively; at day 14 were 7.65, 7.30, 9.99, 8.73 and 6.95 g, respectively; at day 21 were 17.36, 17.10, 19.05, 18.63 and 16.98 g, respectively. Conclusion, black soldier fly larvae fed the death fish had the best growth rate.

Keywords: *Black soldier fly larvae, soybean waste, death fish, fruit wastes, food leftovers, vegetable wastes, growth.*

1. INTRODUCTION

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 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). The quality of BSF larvae is determined by the nutrition of the feed consumed. Katayane *et al.* (2014) found that different feed would produce different nutritional content of BSF larvae, particularly the protein content. BSF larvae cultivation can be done using waste-based organic matter and agricultural and livestock by-products (Herlinae *et al.*, 2021). BSF larvae can decompose organic waste as manure (Wardhana, 2016). They are capable of converting large amounts of waste biomass into stored protein (ranges 35-57%) and fat ranges 15-49% (Barragan-Fonseca *et al.*, 2017; Shumo *et al.*, 2019; Chia *et al.*, 2020; Smets *et al.*, 2020). 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 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, methane, and nitrogen dioxide, as well as less products responsible for soil eutrophication notably ammonia (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 23-30% 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. The aim of this study was to determine the growth performance of black soldier fly larvae fed by different residues and wastes available household residues. The effects growth performance from difference household residues and wastes to be compared, the parameters to be measured, the analysis to be performed as well as the statistical needs are presented.

2. MATERIALS AND METHODS

2.1. Location, treatments and experimental design

The study was carried out from Feb to Apr 2023 at households of Nhon Trach district, Dong Nai province to determine the growth rate of BSFL fed different residues and wastes. The study was surveyed the food input and wastes of 150 households, recorded the growth rate of BSFL fed these different residues and wastes of 30 households selected randomly. In 150 households with an average number of 4 people in household showed that the average food consumption was 310g of starch, 176g of protein, and 152g of other foods/person/day. Organic waste is leftover food and vegetables, accounting for 497 g/household/day.

Thirty household in this study classified and divided into 5 groups of wastes. Each household received one of the following treatments according to a completely randomized design:

- Soybean residues.
- Household leftovers.
- Spoiled fish.
- Fruit waste.
- Spoiled vegetables.

2.2. Micro-organism fermented food wastes, feeding and management

Micro-organism fermented household food wastes: Dissolve 1l of Digest One product containing the *Latobacillus spp., Bacillus spp.* and *Saccharomyces cerevisiae* bacterial strains in 50l of clean water and 1.0kg molasses into a container, make up to 52l. Spray uniformly on surface of the household food wastes according to the recommendations. Dosage and dilution may vary depending on the intended application and sprayer capacity. It should follow technical recommendations before use.

Feeding and management: On the first day of experiment, each tray was added 10g of eggs with the same laying date as 200g of chicken concentrate feed. After 5 days, the experimental trays received the amount of food according to the arranged ration formula. BSFL were fed gradually to household food wastes into three weeks of experimental period. Soybean residues (SR), spoiled fish (SF), fruit waste (FW) and spoiled vegetables (SV) were collected and carried from market. The food leftovers were collected and used by households. All food wastes were offered the larvae one time at 7.30am into one place of the tray.

2.3. Data collection and measureaments

Feed were weighed to measure the feed intake for everytime feeding. Samples of feeds were collected at the first days of experiment to determine DM, Ash, CP, CF according to AOAC methods (2005). Parameter criteria included length and width of larvae were recorded through days 7, 14 and 21 after hatching. The live weight larvae (LWL) will be weighed at the day of 21 in the end of experiment.

2.4. Chemical analysis

All samples were analyzed for dry matter (DM), ash, crude protein (CP), crude fiber (CF), lipid (EE), NDF and ADF according to AOAC (2005). While NDF and ADF analysis was followed the Van Soest *et al*. (1991); GE, DE, ME, NEM, NEG, TDN, NEL and NFC was calculated following the formula suggested by Sauvant *et al*. (2004).

2.5. Statistical analysis

The data were subjected to analysis of variance using the General Linear Model procedure of Minitab software version 17.01. Tukey's pairwise comparisons ($P<0.05$) were

applied to determine the differences between dietary treatments for BSFL. Response curves were fitted to the data using linear and quadratic equations in Microsoft Office Excel software, with different total mixed rations as the independent variable (X) and the response component feed intake (FI), LW, FCR... as dependent variable (Y).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of feeds

There were major differences in CP, CF and EE contents with higher values for SF, SR and HL than for FW and SV (Table 1).

3.2. Feed intake, feed conversion rate and live weight

DM intake, LW, FCR in SR, HL, SF, FW and SV diets were statistical difference (Table 2). Relationship of FCR and LW in different food wastes shown in Figure 1. Effect of dry matter intake on FLW in different FW shown in fig 2.

Table 1. Composition of diet ingredients
(% in DM)

	SR	HL	SF	FW	SV
DM, %	19.85	31.24	21.45	19.25	18.25
CP, %	18.17	15.7	18.09	8.29	2.17
CF, %	13.32	11.32	1.13	0.82	15.75
Lipid, %	11.77	6.72	27.74	1.97	0.5
Ash, %	4.01	6.20	3.13	1.08	2.22
TDN	84.90	79.01	113.16	86.64	69.67
NDF, %	34.16	52.35	32.46	4.22	55.9
ADF, %	24.7	35.92	22.74	2.78	43.3
Lignin, %	3.43	10.7	2.93	0.34	6.9
NFC, %	31.89	19.03	18.58	84.44	39.21
Starch, %	2.79	20.24	32.24	1.12	2.34
GE, Mcal/kg	4.22	3.82	3.24	4.62	2.14
DE, Mcal/kg	3.74	3.48	4.98	3.81	3.07
ME, Mcal/kg	3.06	2.85	4.08	3.13	2.51
NEM, Mcal/kg	2.08	1.91	2.89	2.13	1.62
NEG, Mcal/kg	1.67	1.49	2.53	1.73	1.18
NEL, Mcal/kg	1.96	1.82	2.65	2.00	1.59

Table 2. Changes in parameter criteria on growth of larvae, DM intake and FCR in different food wastes

Trait	Item	SR	HL	SF	FW	SV	SEM	P
Growth rate of larvae	Length at 7 days, mm	7.54^{a}	7.17^{b}	7.03^{bc}	6.80^{c}	7.74^{a}	0.028	0.001
	Length at 14 days, mm	13.23^{b}	13.10^{b}	13.93^{a}	13.73^{a}	11.18^{c}	0.040	0.001
	Length at 21 days, mm	19.84^{c}	20.91^{b}	21.70^{a}	20.94^{b}	19.20^{d}	0.032	0.001
	Width at 7 days, mm	2.05^{c}	2.13^{b}	2.24^{a}	2.27^{a}	1.75^{d}	0.010	0.001
	Width at 14 days, mm	3.43^{c}	3.29^{d}	3.84^{a}	3.66^{b}	2.71^{e}	0.130	0.001
	Width at 21 days, mm	3.96^{c}	3.88^{c}	4.56^{a}	4.17^{b}	3.18^{d}	0.013	0.001
LW	Weight of 100 larvae at 7 days, g	1.70^{c}	1.65^{d}	2.15^{a}	1.86^{b}	1.66^{d}	0.036	0.001
	Weight of 100 larvae at 14 days, g	7.65^{c}	7.30^{d}	9.99^{a}	8.73^{b}	6.95^{e}	0.207	0.001
	Weight of 100 larvae at 21 days, g	17.36^{b}	17.10^{b}	19.05^{a}	18.63^{a}	16.98^{b}	0.165	0.001
	Total weight in fresh, kg	8.73^{cd}	10.10^{c}	21.15^{a}	14.33^{b}	7.22^{d}	0.954	0.001
	Total weight in DM, kg	2.74	3.17	6.64	4.50	2.27	0.300	-
FI	Total FI, kg in fresh	45.5^{d}	28.7^{e}	63.0^{c}	123.2^{a}	99.6^{b}	6.280	0.001
	Total FI, kg in DM	9.0^{d}	9.0^{d}	13.5^{c}	23.7^{a}	18.2^{b}	1.030	-
	Fresh FI on kg fresh larvae	5.21^{c}	2.85^{d}	2.98^{d}	8.60^{b}	13.80^{a}	0.727	0.001
	DM FI on kg fresh larvae	1.03^{c}	0.89^{c}	0.64^{d}	1.66^{b}	2.52^{a}	0.119	0.001
	DM FI on kg dry larvae	3.29	2.83	2.03	5.27	8.02	0.378	-

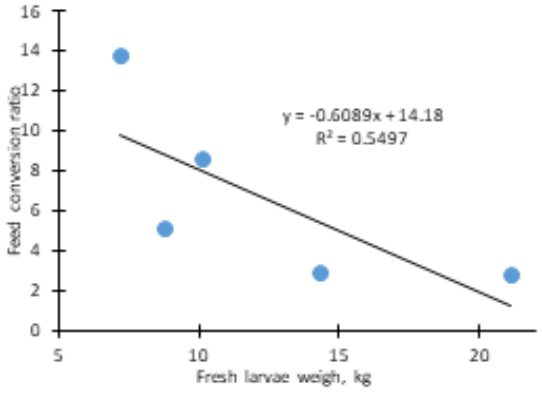

Fig 1. Effect of FCR on LW in different food waste

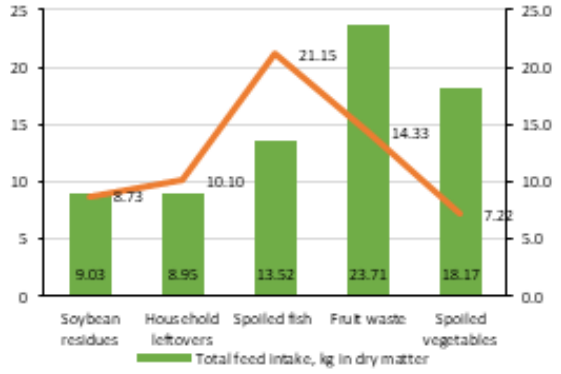

Fig 2. Effect of DMI on FLW in different food waste

3.3. Cost and profit

Cost and profit of the trial were recorded and calculated based on the experiental results (Table 3).

Table 3. Cost and profit of the trial

Item	SR	HL	SF	FW	SV
FLW	8.73	10.10	21.15	14.33	7.22
DLW	2.74	3.17	6.64	4.50	2.27
TFI, kg in fresh	45.48	28.65	63.02	123.18	99.57
TFI, kg in DM	9.03	8.95	13.52	23.71	18.17
FCR	5.21	2.85	2.98	8.60	13.80
DM on kg FL	1.03	0.89	0.64	1.66	2.52
DM on kg DL	3.29	2.83	2.03	5.27	8.02
Cost, VNĐ					
Price for 1kg feed	100	0	1500	100	100
Feed	4,548	0	94,525	12,318	9,957
Labor per day	1,000	1,000	1,000	1,000	1,000
Price 10g BSF egg	40,000	40,000	40,000	40,000	40,000
Total	154,645	141,000	424,575	177,955	170,870
Price 1kg FL, VNĐ	15,000	15,000	15,000	15,000	15,000
Price all WG, VNĐ	393,000	454,500	951,750	645,000	324,750
Benefit, VNĐ	238,355	313,500	527,175	467,045	153,880

Note: Price of fresh larvae in average is 15,000 VND/kg.

4. CONCLUSIONS

The usage of SR, HF, SF from market, FW and SV has no negative impact on larvae growth. Growth rate and FCR are different in FW from SR, HF, SF from market, FW and SV. It is concluded that the BSFL fed the SF had the best growth rate.

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EFFECT OF SOYA BEAN WASTE AND COOKED BROKEN RICE IN DIETS ON GROWTH PERFORMANCE OF BLACK SOLDIER FLY LARVAE

Vu Thu Giang[1], Phan Thi Lieu[1], Le Huynh Quang Thong[2], Do Duc Luc[1] and Duong Nguyen Khang[2]*

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ABSTRACT

The study was carried out from Feb to Apr 2023 at Dairy farm, Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City to determine the appropriate proportion of rice energy in diets on growth rate of black soldier fly larvae (BSFL). The experiment was setup in a one factor completely randomized block design with three replications for each treatment. For each replication, 100 individuals were randomly selected from an experimental tray. All total of 30 trays were used to evaluate the effect of replacing the soya bean waste with cooked broken rice on the growth performance of BSFL between 5 treatments: T_1 (100% soya bean waste + 0% cooked broken rice), T_2 (75% soya bean waste + 25% cooked broken rice), T_3 (50% soya bean waste + 50% cooked broken rice), T_4 (25% soya bean waste + 75% cooked broken rice), T_5 (0% soya bean waste + 100% cooked broken rice). On the first day of experiment, each treatment was added 10g of eggs of BSFL with the same laying date and 200 grams of concentrate feed. After 5 days, in the experimental trays, the amount of food was added according to the treatment ration formula. The studied parameter were length, width and weight of black soldier fly larvae in 7, 14 and 21 days after hatching. The experiment was repeated once time. The results showed that replication 0, 25, 50, 75 and 100% of cooked broken rice affected length of BSFL. These values at day 7 were 7.58, 7.62, 7.88, 7.61 and 7.54mm, respectively (P=0.155); at day 14 were 13.29, 13.33, 13.37, 13.33 and 13.25mm, respectively (P=969); at day 21 were 17.79, 18.83 19.27, 18.21 and 17.62mm, respectively (P=0.001). The width of BSFL at day 7 were 2.09, 2.12, 2.22, 2.00 and 2.05mm, respectively (P=0.001); at day 14 were 3.13, 3.26, 3.39, 3.11 and 3.03mm, respectively (P=0.001); at day 21 were 3.83, 3.91, 4.14, 3.88 and 3.62mm, respectively (P=0.001). The weights of BSFL at day 7 were 1.69, 1.72, 1.73, 1.70 and 1.69gr (P=0.089), respectively; at day 14 were 7.36, 7.78, 7.86, 7.70 and 7.34gr (P=0.001), respectively; at day 21 were 16.01, 16.45, 17.60, 16.22 and 15.38g (P=0.001), respectively. Conclusion, BSFL fed a diet with 50% of cooked broken rice had the best growth rate.

Keywords: *Black soldier fly larvae, soya bean waste, cooked broken rice, growth, feed intake.*

[1] Vietnam National University of Agriculture
[2] Nong Lam University of Ho Chi Minh City
[*] Corresponding author: Professor Dr. Duong Nguyen Khang, Nong Lam University of Ho Chi Minh City; Tel: (84)989390179; Email: duongnguyenkhang@gmail.com

1. INTRODUCTION

Black soldier fly larvae (BSFL) could consume many kinds of organic material, and could use and treat for the waste management purposes in the small-scale by using the media substrates such as food waste (Green *et al*., 2012), manure (Sheppard *et al*., 1983; Yu *et al*., 2009), distillers' grains (Webster *et al*., 2016), animal offal, kitchen waste (Nguyen *et al*., 2015), fecal sludge (Lalander *et al*., 2013; Banks *et al*., 2014), rice straw (Zheng *et al*., 2012). The diversity of substrates BSFL could eat, process, effect and efficiency with which they have done these ways may be the highest among the flies (Kim *et al*., 2011). It is very surprise that their feed conversion ratios (FCR) are known to be superior to both crickets and mealworms. In compared with the crickets and mealworms, BSFL survival rate and nitrogen and phosphorus compositions have done not vary as highly with diet (Oonincx *et al*., 2010). BSFL accumulated lipids from their diet for use as energy by the adult (Li *et al*., 2011; Wang *et al*., 2017; Nguyen *et al*., 2017; Mohd-Noor *et al*., 2017). The refused feed that BSFL did not consume, combined with their rich nitrogen manure, could be used as fertilizer (Green *et al*., 2012; Lalander *et al*., 2015). One other important bioactivity, the larvae development time of over three weeks was longer than that of flies such as house fly; it means a single larva consumed a larger amount of substrate and produce larger pupae (Čičková *et al*., 2015). Additionally, in the pre-pupa stage, they leave the substrate and move to a high, clean place, and their behavior called "self-harvesting" that reduces the labor cost in farming system (Sheppard *et al*., 1994; Diener *et al*., 2011). All these benefits make BSFL practical to rear and a suitable tool to use waste, and possibly a sustainable animal feed source.

As mentioned above, BSFL can consume a wide range of organic materials, including animal manure, kitchen scraps, and agricultural waste. Some of these wastes are difficult to use, such as rice straws, which are high in lignocellulosic matter and thus are low quality as livestock feed (Zheng *et al*., 2012; Manurung *et al*., 2016). Some of these wastes are potentially pollutants and/or attract disease-vectoring pest flies, and all represent reduced revenue both in terms of lost nutrients as well as the cost of waste treatment. Wild black soldier fly larvae are already used to manage manure successfully (Sheppard *et al*., 2002), reducing odor and pest fly populations (Sheppard *et al*., 1983). The proposed solution is to supply BSFL as a sustainable source of protein to livestock/and aquaculture farmers to raise rural farmers' income and improve farming practices.

The aim of this study was to determine the growth performance of BSFL fed by different the ratio of soya bean waste (SBW) and cooked broken rice (CBR) in diet. The effects of growth and benefit from black soldier fly will be compared, the parameters to be measured, the analysis to be performed as well as the statistical needs are presented.

2. MATERIALS AND METHODS

2.1. Materials and location

The SBW and CBR was used for the experiment conducting at the Ruminant Research and Technology Transfer Farm, Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City, Vietnam from Feb to Apr 2023.

2.2. Experimental design and treatments

Thirty trays (0.24m^2 per tray) were divided into 5 treatments. Each tray received one of the following treatments according to a one factor completely randomized design:

T_1: 100% SBW + 0% CBR

T_2: 75% SBW + 25% CBR

T_3: 50% SBW + 50% CBR

T_4: 25% SBW + 75% CBR

T_5: 0% SBW + 100% CBR

2.3. Feeding and management

Egg incubation period: In the first day of experiment, each tray was put 10g of eggs and 200g of concentrate feed. After 5 days, the larvae in this tray were randomly divided into 3 trays for feeding the experimental diets.

Feeding experimental period: The young larvae trays received an amount of feed according to the arranged ration formula. BSFL were fed gradually to different ratio of soya bean waste and cooked broken rice into three weeks of experiment. Soya bean waste was collected and carried from the market and cooked broken rice were prepared at the Research farm. Feed was supplied to the larvae one time at 7.30AM into one place of the tray.

2.4. Data collection and measurement

Feed was weighed to measure the feed intake for everytime feeding. Samples of feeds were collected at the first days of experiment to determine dry matter (DM), Ash, crude protein (CP), crude fiber (CF) according to AOAC methods (2005). The studied parameters were length and width of larvae were recorded through days 7, 14 and 21 after hatching. The live weight of larvae was weighed at the day of 21 in the end of experiment.

2.5. Chemical analysis

All samples were analyzed for DM, ash, CP, CF, lipid (EE), NDF and ADF according to AOAC (2005). While NDF and ADF analysis was followed the Van Soest *et al.* (1991); GE, DE, ME, NEM, NEG, TDN, NEL and NFC was calculated following the formula suggested by Sauvant *et al.* (2004).

2.6. Statistical analysis

The data were subjected to analysis of variance using the General Linear Model procedure by Minitab software version 17.01. Tukey's pairwise comparisons were applied to determine the differences between dietary treatments for BSFL at significant level at $P<0.05$. Response curves were fitted to the data using linear and quadratic equations in Microsoft Office Excel software, with different total mixed rations as the independent variable (X) and the response component such as feed intake (FI), live weight (LW), feed conversion rate (FCR) as dependent variable (Y).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of feeds

The major differences of feed chemical composition were the higher in CP and EE values contents but and the lower in starch value in the SBW compared with the CBR (Table 1). The nutritional contents of each diet were presented in Table 2.

Table 1. Composition of diet ingredients (% DM)

Item	SBW	CBR
DM (%)	19.85	24.31
CP (%)	18.17	8.36
CF (%)	13.32	16.64
Lipid (%)	11.77	4.01
Ash (%)	4.01	9.96
TDN ()	81.19	86.79
NDF (%)	34.16	35.02
ADF (%)	24.7	20.54
Lignin (%)	3.43	6.36
NFC (%)	31.89	42.64
Starch (%)	2.79	29.63
GE (Mcal/kg)	4.22	3.86
DE (Mcal/kg)	3.57	3.82
ME (Mcal/kg)	2.93	3.13
NEM (Mcal/kg)	1.97	2.14
NEG (Mcal/kg)	1.55	1.73
NEL (Mcal/kg)	1.87	2.01

Table 2. Nutrients in diets

Item	T_1	T_2	T_3	T_4	T_5
DM (%)	19.85	20.97	22.08	23.20	24.31
ME (Kcal/kg)	2930	2980	3030	3080	3130
CP (%)	18.17	15.72	13.27	10.81	8.36
CF (%)	13.32	14.15	14.98	15.81	16.64
Lipid (%)	11.77	9.83	7.89	5.95	4.01
Starch (%)	2.79	9.51	16.23	22.95	29.67

3.2. Feed intake, FCR and live weight

DM intake, larvae weight, FCR were statistical difference between treatments (Table 3). Correlation between treatment and FCR are presented in Figure 1. The relationship of FCR and DM intake in different treatments is shown in Figure 2.

Table 3. Mean values for changes in parameter criteria on growth of larvae, DM intake and feed conversion rate in different treatments

Item	Time/Item	T_1	T_2	T_3	T_4	T_5	SEM	P
Length larvae (mm)	7 days	7.58	7.62	7.88	7.61	7.54	0.048	0.155
	14 days	13.29	13.33	13.37	13.33	13.25	0.053	0.969
	21 days	17.79^{bc}	18.83^{a}	19.27^{a}	18.21^{b}	17.62^{c}	0.054	0.001
Width larvae (mm)	7 days	2.09^{b}	2.12^{ab}	2.22^{a}	2.00^{b}	2.05^{b}	0.015	0.001
	14 days	3.13^{b}	3.26^{a}	3.39^{a}	3.11^{b}	3.03^{b}	0.016	0.001
	21 days	3.83^{b}	3.91^{b}	4.14^{a}	3.88^{b}	3.62^{c}	0.014	0.001
W100 larvae (g)	7 days	1.69	1.72	1.73	1.70	1.69	0.007	0.089
	14 days	7.36^{b}	7.78^{a}	7.86^{a}	7.70^{a}	7.34^{b}	0.059	0.001
	21 days	16.01^{b}	16.45^{b}	17.60^{a}	16.22^{b}	15.38^{c}	0.199	0.001
TLW (kg)	In fresh	2.50^{c}	2.90^{b}	3.30^{a}	3.00^{b}	2.80^{b}	0.073	0.001
	In DM	0.79	0.91	1.04	0.94	0.88	0.023	-
TFI (kg)	In fresh	14.00	14.00	14.00	14.00	14.00	0.000	-
	In DM	2.78	2.94	3.09	3.25	3.40	0.059	-
FCR	Fresh feed/kg fresh larvae	5.61^{a}	4.83^{b}	4.25^{c}	4.67^{bc}	5.00^{b}	0.125	0.001
	DM feed/kg fresh larvae	1.11^{b}	1.01^{bc}	0.94^{c}	1.08^{b}	1.22^{a}	0.027	0.001
	DM feed/kg dry larvae	3.54	3.22	2.98	3.45	3.87	0.084	-

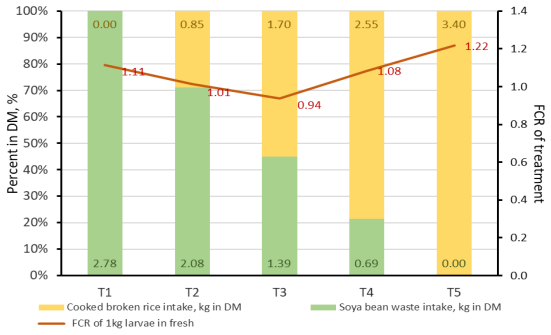

Fig 1. Correlation between treatment with FCR

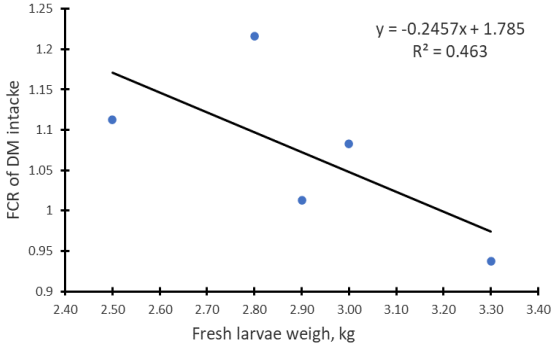

Fig 2. Effect of FCR on FLLW

For the first two weeks, lengths of larvae were not significantly different between treatments (P>0.05). However, these values were observed difference between diets (P<0.05). Lengths of larvae were highest in T2 (18.83mm) and T3 (19.27). Widths of larvae at 3 moments (7, 14 and 21 days) were always different between diets (P<0.05). BSFL in T3 had the highest widths at 3 moments (Table 3). At 21 days, weight of 100 larvae and total larvae weight were also observed highest at T3 (Table 3). These values were 17.60g and 3.30kg in fresh for weight of 100 larvae and total larvae weight (TLW) respectively. Inversely, FCR were lowest at T3 for fresh feed (4.25kg) and DM feed (0.94kg) for one kg of fresh larvae.

3.3. Cost and profit

Cost and profit according to treatments were recorded, calculated based on the experimental results and are presented in Table 4.

Table 4. Cost and profit according to treatments

Item	T_1	T_2	T_3	T_4	T_5
FLW (kg)	2.50	2.90	3.30	3.00	2.80
DLW (kg)	0.79	0.91	1.04	0.94	0.88
FI in fresh (kg)	14.0	14.0	14.0	14.0	14.0
FI in DM (kg)	2.78	2.94	3.09	3.25	3.40
SBW in fresh (kg)	14	10.5	7	3.5	0
CBR in fresh (kg)	0	3.5	7	10.5	14
FCR (kg)	5.61	4.83	4.25	4.67	5.00
DM feed/kg FL (kg)	1.11	1.01	0.94	1.08	1.22
DM feed/kg DL (kg)	3.54	3.22	2.98	3.45	3.87
Expenses (1,000VND)					
Feeding	1.40	1.75	2.10	2.45	2.80
Labor/day	0.5	0.5	0.5	0.5	0.5
Price 10g BSFeggs	30	30	30	30	30
Total expenses	78.9	81.0	83.1	85.2	87.3
Total revenues	225	261	297	270	252
Benefit	146.1	180.0	213.9	184.8	164.7

Note: Price of SBW is 100 VND/kg, CBR is 200 VND/kg and FL is 15,000 VND/kg.

The profit was lowest in T1 (146,100VND) and highest in T3 (213,900VND). This result suggests that diet with 50% of SBW and 50% of CBR was the best for BSFL.

4. CONCLUSIONS

The combined use of SBW and CBR in diets had a positive impact on larvae growth. Growth rate was improved and FCR were reduced in treatments combined between SBW and CBR. The black soldier fly larvae fed a diet with 50% of SBW and 50% of CBR had the best growth and performance.

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TOP 10 LIVESTOCK MANAGEMENT TRENDS IN 2023

Dr. Pham Kim Cuong

Technological advances in livestock management improve global food security and sustainability as well as animal welfare. For example, farm automation solutions use sensors and robotics to automate livestock management tasks and reduce labor costs. Genome sequencing and health monitoring, on the other hand, enhance animal efficiency and productivity while semen analyzers and breeding programs improve breeding potential. This research provides you with the top 10 livestock management trends, ranging from artificial intelligence (AI) and drones to blockchain and cloud computing. Discover the latest developments in the livestock industry in this data-driven report.

1. LIVESTOCK HEALTH MANAGEMENT

Disease management errors result in livestock productivity loss and high-risk health scenarios. Therefore, farmers continuously monitor livestock health to detect diseases early on. This enables them to increase the likelihood of successful treatment. To aid this, startups and scaleups are developing technologies that simplify disease diagnosing and treatment. Such solutions assist farmers in managing nutrients and milking their herds. Further, detection solutions, such as estrus, lameness, and facial detection, improve disease diagnostics efficiency. Portable polymerase chain reaction (PCR) kits also ensure the well-being of livestock by enabling on-demand testing.

CattleEye enables Autonomous Livestock Health Monitoring

UK-based startup **CattleEye** develops a hardware-independent autonomous livestock monitoring platform. It collects data from security cameras and displays them in a proprietary dashboard, CattleEye Insights. This enables a hands-free method to monitor and recognize the patterns and behaviors of the cow as they exit the milking parlor. Consequently, farmers are able to eliminate collars or pedometers. Additionally, the platform detects early signs of lameness in cows. The startup's dashboard insights enable farmers to take required actions, improve livestock welfare, and maintain the overall farm welfare standards.

Victory Genomics aids Horse DNA Genome Sequencing

US-based startup **Victory Genomics** decodes horse DNA using whole genome sequencing (WGS). The startup sequences, digitizes, and analyzes horse DNA to identify the gene color of mare and stallion for farmers to predict the foal's coat. Additionally, it identifies genetic diseases and diagnoses illnesses. Farmers further receive personalized recommendations on optimal feeding, training regimens, and sleep cycles to ensure the horses' wellness.

2. FARM AUTOMATION

Automated farming addresses major challenges such as the growing food demand and worker shortages as well as improves livestock productivity and welfare. Startups are developing solutions such as automated dairy installations, computerized feeders, cleaning systems, and incubators. Additionally, farms control the barn environment through automated ventilation and lighting adjustment systems. By leveraging robotics, animal farms are able to further bring automation to livestock

management. For instance, milking and pusher robots speed up farm processes while eliminating frequent manual interventions.

PharmRobotics delivers Robotic Herd Health Management

US-based startup PharmRobotics makes Sureshot, a robotic health center to automate livestock pharmaceutical administration. It utilizes radio frequency identification (RFID) readers and cameras to detect cows requiring inoculation. Upon identifying illness, they are separated from the herd and weighed on a scale, where they are scanned again to determine medication needs. Sureshot's robotic arm then administers the medication in the neck region while sensors monitor precise dosage. This extends livestock lifespan and results in more efficient herd management.

Corvitac offers an Automated Pig Counter System

German startup **Corvitac** develops an automated pig counter system. Its camera system counts the pigs automatically when rehousing. Farmers turn on the system during rehousing and receive accurate count reports. The startup also videotapes the data for evidence and automatically generates reports. This allows farmers to eliminate manual counting and reduce counting errors.

3. ARTIFICIAL INTELLIGENCE

AI offers better insights into livestock behavior as well as disease control and prevention. Additionally, AI-powered digital twins enable the prediction of breeding heat cycles and discourage negative livestock behavior. It also allows farms to build energy-efficient housing structures. Farmers use translation algorithms to better understand the grunts and squeals of animals for ensuring their well-being. Startups are improving big data and analytics workflows with AI and machine learning (ML) for livestock management. Such solutions collect animal and farm-level data for enhancing product quality, animal health, and management practices.

myAnIML simplifies Cattle Disease Prediction & Monitoring

US-based startup myAnIML analyzes cattle faces and muzzles using computer vision for disease prediction and monitoring. For this, the startup deploys cameras on feeder trucks, milking booths, water ponds, and semi-trailers. They track the change in the cow's muzzle and compare it with the disease database. myAnIML's solution also identifies conjunctivitis in infected cows and alerts farmers. This allows them to isolate infected cows and prevent disease spread. The startup's AI-powered disease prediction and tracking solution thus reduces treatment costs and increases revenue.

Animals.ai offers AI-based Behavioral Animal Care

Swedish startup Animals.ai simplifies livestock management through behavioral monitoring. The startup's solution leverages computer vision and deep learning to track herds in real time. It monitors behavioral patterns such as increased activity, chin resting, sniffing, and mounting in cows. This provides accurate insights into heat detection and reproduction at an individual level. Additionally, the solution monitors non-cycling and irregular cycling cows to improve herd management. These features enable farmers to improve reproduction results and increase labor efficiency.

4. ANIMAL BREEDING TECHNOLOGIES

Farmers use animal breeding technologies to identify the healthiest and most productive animals for maintaining a quality herd.

Several technologies such as sex selection, embryo culture, parthenogenesis, and gene transfer enable them to better use their resources. DNA marker technology increases the frequency of desirable characteristics in future generations of farm animals. Further, DNA profiling facilitates parentage testing. Embryo transfer and in vitro fertilization (IVF) technology also speed up the genetic progress in one generation that otherwise takes five generations through traditional breeding.

Ongo Vettech offers a Portable Semen Analyzer

Hungarian startup Ongo Vettech makes Ongo CASA, a portable computer-assisted semen analyzer. It measures sperm quality and simplifies sperm analysis. The sperm sample is inserted in a slide and placed within Ongo CASA. It conducts concentration and motility assessments as well as shows results on the display screen. With this, farmers ensure that the cell count and motility are of the right quality for mating. Getting accurate sperm analysis on animals eliminates the need to send sperm samples to the laboratory. This, in turn, saves significant costs and avoids delays.

Danish Genetics specializes in Pig Breeding

Denmark-based startup Danish Genetics drives pig breeding programs for farmers. The startup's system, Danish Genetics Evaluation System (DGENES), collects the breeding data and stores it in the database. It combines numerous farm records and genomic data in genomic best linear unbiased prediction (BLUP) to generate the Danish Genetics Breeding Index(DGI). This further assists farmers in better pig production. Additionally, the farmers get to utilize their animal's breeding potential optimally.

5. PRECISION LIVESTOCK FARMING

Improving animal well-being while reducing environmental impacts is the primary benefit of precision livestock farming. Considering each animal and its different reactions to the environment, they are labeled as complex, individual, and time-variant (CIT) systems. PLF monitors the livestock in real-time to help farmers understand their condition and intervene accordingly. Startups are developing technologies to measure and record livestock behavior, weight, food and water intake, temperature, and respiration rate. Alongside this, it allows for flagging and addressing deviations from normal processes.

Pastoral advances Precision Regenerative Livestock Farming

UK-based startup Pastoral develops a precision regenerative livestock farming platform. It provides dairy farm data using satellite and animal sensors to the farmers. The platform also delivers weather-based precision feeding information and fine-scale animal management strategies. This enables farmers to improve their livestock capacity and efficiency while reducing operational costs.

FarmSee develops a Pig Production Support System

FarmSee is an Israeli startup that creates a pig production support system. The startup's camera-based weighing sensor collects, stores, and processes information on the cloud. Its web application then provides real-time data and insights into operations like slaughter planning and avoiding overweight penalties. It also shares the optimal time for switching food compositions to minimize the feed conversion ratio (FCR). Farmers use the startup's system to increase the quality and volume of pig meat while ensuring their welfare.

6. FEED SUPPLEMENTS

Livestock health decides the animals' overall value and is dependent on their diet

and nutrient intake. This encourages farmers to use feed supplements. Farmers provide animals with proper nutrition, which is very important for their growth and development. The feed supplements and additives are added to the basal feed to improve the gain rate and feed efficiency. It also assists in preventing and controlling diseases. Further, feeding the supplements in the right quantity and right time boosts fertility. Some supplements also reduce gut emissions in cattle.

Rumin8 produces Climate-friendly Livestock Feed Supplements

Australian startup **Rumin8** manufactures feed supplements that reduce livestock methane emissions. The startup's technology stabilizes compounds in plants that target methanogenic pathways in livestock rumen. With the reduction of ruminant methane, livestock converts otherwise lost energy into compounds for increased productivity. Using Rumin8's feed supplements, farmers contribute to sustainability and improve their livestock performance.

UniFAHS provides a Sustainable Animal Antibiotics & Chemicals Alternatives

UniFAHS is a Thai startup that offers sustainable alternatives to animal antibiotics and chemicals. The startup works with farmers to identify the potential sources of pathogens such as Salmonella, E. coli, Listeria, and Vibrio on their farms. Using its phage development platform, UniFAHS then provides customized solutions. For example, the startup's SalmoGuard is a phage-based bio-feed additive for poultry to combat Salmonella serovars.

7. BLOCKCHAIN

Blockchain enables immutable data storage for farm-related transactions, enabling traceability and promoting sustainable food production. As products and animals go through their production cycle, blockchain-based solutions track the entire supply chain. This improves feed safety and animal welfare significantly. The distributed ledger technology (DLT) system also connects farmers, traders, and consumers to improve end-to-end supply chain visibility and simplifies product tracking.

Breedr creates a Livestock Supply Chain Platform

UK-based startup **Breedr** makes a blockchain-based livestock supply chain platform to analyze individual animal data such as daily weight gain, medicine intake, and movement. It shares this information with farmers to optimize the yield, quality, and profitability of the herd. The startup also provides animal management tools that enable farm managers to produce meat more efficiently. Additionally, the startup provides an app, Grass Fed, that connects farmers to build a sustainable supply chain and deliver grass-fed beef and lamb with improved traceability.

Aqgromalin simplifies Farm Diversification

Indian startup Aqgromalin develops a blockchain-enabled supply chain platform. The startup allows farmers to order certified input materials from its app, AQAI. Additionally, Aqgromalin offers farm-gate delivery in remote locations and assistance in setting up micro-farms. Its buy-back option enables farmers to sell the farm produce back to them. Else, the startup connects farming and trading communities and provides consistent deliveries at transparent prices through a blockchain ecosystem. With the startup's farm diversification platform, farmers gain access to information, machinery, and materials to make their farms profitable.

8. BIOSAFETY SURVEILLANCE

Biosafety surveillance prevents the entry of infectious agents into the farm and mitigates their spread. Farmers use this technology for preserving the sanitary status of their farm animals through bioexclusion and biocontainment. Additionally, biosensors detect and identify infectious diseases in livestock as well as identify contaminants and toxins in feed. This benefits the farmers by improving animal welfare and productivity. Further, biosafety surveillance decreases the economic losses due to diseases that are not treatable through vaccines.

Livet aids Infectious Disease Testing

Swiss startup **livet** enables on-site rapid testing for infectious diseases in horses. The startup allows veterinarians to analyze multiple samples and diagnose infectious diseases within thirty minutes. For this, they use livet's isothermal amplification technology that offers accurate and reliable results in less time. Further, it is portable and enables disease diagnosis closer to patients, increasing the quality of health care. Additionally, the startup's system allows horse owners to make informed decisions and take adequate measures for minimizing disease spread.

Micron Agritech simplifies Animal Disease Diagnostics

Irish startup Micron Agritech allows farmers and veterinarians to test animals on-site for parasites. The testing process is simple and includes collecting, preparing, and analyzing the sample using the startup's application. With this data, farmers are able to make real-time decisions on whether their animals require medication or not. This, in turn, enables farmers to save costs on medications while increasing animal yield and performance.

9. DRONES

Managing massive livestock herds is expensive, tedious, and time-consuming. Drones solve these problems by enabling remote livestock monitoring. It is fitted with cameras and thermal imaging sensors that track livestock movement, performs counts, and find stray cattle and sheep. By providing real-time information, it also enables precision farming. Livestock theft is another major issue that farmers tackle using connected sensors and drones. This saves time significantly and reduces the frequent trips to the farm. Additionally, it provides farmers with a vast pool of information to manage their livestock.

Herd-itt offers Remote Cattle Monitoring

Israeli company Herd-itt develops drones to provide remote cattle monitoring. The drones hover near cows and offer farmers live video feeds. Herd-itt's system combines an IoT collar and integrated drone services that send signals to its tower. This data includes the cattle's location, hostility, count, and health status. Additionally, it tracks cattle behaviors and sends real-time alerts in case of illness suspicion. Farm managers and owners use the startup's drone to increase their cattle yield and reduce operational costs.

BeeFree Agro enables Autonomous Herd Tracking

Israeli startup BeeFree Agro offers an autonomous drone-based herding system that monitors pastures, infrastructure, and livestock. It scans pastures and tracks livestock count and location automatically. Additionally, the system provides an overview of the status of the facilities including gates, water troughs, fences, and more. As a result, farmers save time and resources by implementing the startup's solution.

10. CLOUD COMPUTING

Cloud computing enables data aggregation and analytical capabilities while removing IT overhead from farmers. Therefore, startups are developing cloud computing platforms that allow farmers to better understand the farm environment and manage operations cost-effectively. Such platforms also display information such as livestock's nutritional needs, health, and breeding conditions. This enables them to realize the maximum livestock production potential.

ADIS makes an Animal Digital Information System

Indian startup ADIS builds a digital information system for farm animal management. The startup's app provides unique biometric identities for each animal and stores the data in the cloud. The centralized cloud enables farmers to access animal details such as pedigree and health records more flexibly. Additionally, the startup's system provides breeding assistance and insights into animal performance to make informed and assisted decisions. This allows farmers to reduce expenses and, most importantly, maximize profits sustainably.

BellCloud provides a Cloud-based Farm Management Solution

Mexican startup BellCloud develops a comprehensive farm management tool to help farmers easily manage livestock. This tool collects and analyzes data in real time, allowing farmers to quickly access records and generate reports on their animals. It also offers heat, illness, and treatment alerts that can be sent to farmers or veterinarians with recommended actions and instructions. By using this farm management system, farmers increase livestock production, reduce veterinary costs, and maximize profits.

THE INTERNATIONAL FOOD ANIMAL CONFERENCE (IFAC2023) WAS SUCCESSFULLY ORGANIZED BY CAN THO UNIVERSITY, VIETNAM

Prof. Dr. Tran Trung Tinh - Vice Rector of Can Tho University delivered the opening speech of the IFAC2023

Nowadays, meat, milk, eggs, and other animal products play an important role in achieving food security. The production of animals contributes to economic growth by generating income and employment opportunities for those involved in livestock keeping and other related activities. The livestock value chain comprises every facet, spanning from the initial production through processing, all the way to the final delivery of animal products to consumers. However, while working towards enhancing animal production and product quality, it is also essential to consider the potential environmental impacts of livestock. Therefore, the International Food Animal Conference 2023 (IFAC2023) organized by Can Tho University, was taken place from August 30th

to September 1st, 2023, under the theme of "***Animals, food, and environment: a value chain approach***".

The objectives of the International Food Animal Conference 2023 were to facilitate the exchange and dissemination of the latest advancements in scientific knowledge and technologies related to animal production, animal health management, animal-based food technology, and environmental factors, all geared towards enhancing the productivity, quality, and safety of livestock products. It also aimed to establish extensive research, publication, and technology transfer cooperation between institutes, universities, management agencies, and enterprises raising animals. Moreover, it could help to develop focus research and strategies for continued advances in the livestock industry that will enable livestock production to bring more income for farmers and produce safe and nutritious animal products for human consumption.

The content of the conference included 8 overview reports at the plenary session by Vietnamese and foreign speakers, 21 oral reports at 3 scientific subcommittees, and 34 poster reports. Out of the 72 papers submitted to the conference, 30 have been chosen for publication in

The invited Speakers and Chairpersons of the IFAC2023

a Scopus Q3 journal, while an additional 15 have been selected for publication in the Journal of Animal Husbandry Sciences and Technics (JAHST) in its English version. The remaining papers have been included in the conference proceedings. The IFAC2023 was also honored to receive sponsorship from many different companies: 1 diamond sponsor, 3 bronze sponsors, and 8 contributing sponsors. The conference also attracted 9 businesses to display and introduce products related to livestock and veterinary medicine.

Participating delegations and IFAC2023 Organizing Committee

It is undeniable that the 3-day event of IFAC2023 at Can Tho University took place enthusiastically and effectively thanks to the high-level and comprehensive programs offered by the IFAC2023. The series of scientific-sharing activities, including speeches of keynote speakers, plenary speakers, and invited speakers, the oral and poster sessions, brought participants together to discuss and network in the field of animal science. At the plenary session, there were reports from 8 keynote speakers, including 6 foreign professors from the USA, Canada, Japan, and Thailand; 1 Vietnamese professor and 1 expert from De Heus Vietnam Corporation.

As expected, the total number of delegates attending both in person and online was 1,039 people, including 29 foreigners from 10 different countries; 160 Vietnamese attendees came from institutions, state units, and businesses operating in the fields of livestock, veterinary medicine, chemicals, and equipment; with the participation of 850 students of Can Tho University.

The IFAC2023 Organizing Committee and Can Tho University wish to convey our heartfelt appreciation to the National and International Advisory Committee for their invaluable

guidance. We also extend our gratitude to the esteemed invited Speakers and Chairpersons for generously dedicating their time and participation to this event. We would like to extend a special note of thanks to all the attendees who joined us at this gathering. This conference served as an exceptional platform for the exchange of scientific ideas, the kindling of fresh research endeavors, and the cultivation of new connections for more profound collaborative efforts.

Last but not least, we are pleased to announce to scientists, institutes, and universities that the next IFAC will be hosted by Kasetsart University, Thailand, on 17-19 February 2025.

Looking forward to seeing you again soon!

Keynote speakers received flowers from IFAC2023 organizers

Scientific report activities in sessions

Students found great value in exploring the companies actively participating in IFAC2023

Kasetsart University, the host of the second IFAC in Thailand, has officially inaugurated the conference program