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PREFACE

In this edition, Volume 30 No 2, we proudly present articles from animal and veterinary sciences including genetic, reproduction; animal physiology; and veterinary from scientist all over the world. The articles published in this edition are:
"Phenotypic Characterization of Indonesian Jambi Buffalo in the Highland and Lowland Areas using Multivariate Analysis"; "dentification of Protein A and Capsular Structures in Staphylococcus aureus Isolates from Milk of Cows with Subclinical Mastitis"; "Strengthening Breeding Stock Supply: A Study of Community-Based Duck Farming in Magelang under Good Breeding Practice Guidelines"; "The Impact of Adding Egg Yolk In Various Concentrations into The Tyrode Extender on Muscovy Duck (*Cairina moschata*) Sperm Quality"; "Relationship of Growth Performance and Metabolite Profile of Broiler Chickens with the Supplementation of Probiotics *Bacillus coagulans* and *Lactobacillus plantarum*"; and "Effect of Liquid Smoke on the Characteristics of Sensory Quality of Chicken Nugget".

We extent high appreciation to all peer reviewers who make this journal academically high value. Hopefully, these articles would offer any benefit to readers and the end-users of technological innovation, and attract interests from scientists to contribute their papers to the Indonesian Journal of Animal and Veterinary Sciences.

Chief Editor

Bogor, June 2025

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LIST OF CONTENT

Phenotypic Characterization of Indonesian Jambi Buffalo in the Highland and Lowland Areas using Multivariate Analysis

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ABSTRAK

Hendrawan PA, Wijaya SH, Sumantri C, Jakaria. 2025. Karakteristik penotif Kerbau Jambi di dataran tinggi dan dataran rendah menggunakan analisis multivariat. JITV 30(2):68-82. DOI:http://doi.dx.org/10.13443/jitv.v30i2.3515.

Kerbau Jambi dipelihara secara ekstensif dengan sistem tertutup oleh peternak kecil. Studi ini bertujuan menganalisis karakteristik fenotipik kerbau di Provinsi Jambi pada dataran tinggi dan rendah. Sebanyak 160 ekor kerbau digunakan, terdiri dari 53 ekor di dataran tinggi dan 107 ekor di dataran rendah. Analisis deskriptif diterapkan pada data kualitatif, sedangkan analisis varians, Uji Rentang Berganda Duncan, korelasi Pearson, analisis komponen utama (PCA), analisis diskriminan, dan analisis klaster hierarkis (HCA) digunakan untuk data kuantitatif. Hasil menunjukkan keragaman sifat kualitatif pada kedua populasi. Morfometri tubuh kerbau dataran tinggi secara signifikan (P<0,05) lebih besar dibandingkan kerbau dataran rendah. Korelasi Pearson antara morfometri tubuh dan indeks morfometrik berkisar dari rendah hingga tinggi. Kerbau dataran tinggi menunjukkan tiga komponen utama (PC) berdasarkan morfometri tubuh, sedangkan kerbau dataran rendah menunjukkan dua PC. Pada kedua populasi, indeks morfometrik menghasilkan empat komponen utama. Analisis diskriminan mengkarakterisasi sekitar 48,09% kerbau dataran tinggi dan 51,92% dataran rendah berdasarkan morfometri tubuh, serta 57,69% dan 42,31% berdasarkan indeks morfometrik. HCA mengelompokkan kerbau Jambi ke dalam tiga klaster: Merangin (klaster pertama), Kerinci (klaster kedua), dan Tanjung Jabung Barat, Tebo, serta Batanghari (klaster ketiga). Kesimpulan, kerbau Jambi di dataran tinggi dan rendah menunjukkan perbedaan fenotipik signifikan dengan morfometri tubuh lebih besar pada populasi dataran tinggi, serta variasi genetik dan adaptasi lokal yang tercermin dalam pola klasterisasi, memberikan dasar ilmiah penting untuk pengembangan program pemuliaan dan konservasi sesuai kondisi ekosistem.

Kata Kunci: Kerbau, Morfometrik, Multivariat Analisis, Fenotipik

ABSTRACT

Hendrawan PA, Wijaya SH, Sumantri C, Jakaria. 2025. Phenotypic characterization of Indonesian Jambi buffalo in the highland and lowland areas using multivariate analysis. JITV 30(2):68-82. DOI:http://doi.dx.org/10.13443/jitv.v30i2.3515.

Jambi buffaloes are extensively reared under a closed system by smallholder farmers. This study aimed to analyze the phenotypic characteristics of buffaloes in Jambi Province, Indonesia, across the Highland and lowland areas. A total of 160 buffaloes were sampled, comprising 53 from the highlands and 107 from the lowlands. Descriptive analysis was applied to qualitative data, while analysis of variance, Duncan's Multiple Range Test, Pearson correlation, principal component analysis (PCA), discriminant analysis, and hierarchical cluster analysis (HCA) were used for quantitative data. Results revealed considerable diversity in qualitative traits across both populations. Highland buffaloes exhibited significantly larger body morphometrics (P<0.05) than their lowland counterparts. Pearson correlations between body morphometrics and morphometric indices ranged from low to high. PCA identified three principal components (PCs) for highland buffaloes and two PCs for lowland buffaloes based on body morphometrics, while four PCs were extracted from morphometric indices in both groups. Discriminant analysis characterized approximately 48.09% of Highland and 51.92% of lowland buffaloes based on body morphometrics, and 57.69% and 42.31%, respectively, based on morphometric indices. HCA classified Jambi buffaloes into three clusters: Merangin (first cluster), Kerinci (second cluster), and Tanjung Jabung Barat, Tebo, and Batanghari (third cluster). In conclusion, buffaloes from Highland and lowland areas exhibit significant phenotypic differences, characterized by larger body size in highland populations, alongside genetic variation and local adaptations reflected in clustering patterns. These findings provide a scientific foundation for breeding and conservation programs tailored to the specific conditions of each ecosystem.

Key Words: Buffalo; Body Morphometric; Multivariate Analysis; Phenotypic

INTRODUCTION

Indonesia has mega biodiversity, including the genetic resources of livestock. Buffalo is one of Indonesia's livestock species. Buffaloes play a significant role in contributing to meat and milk (Prihandini et al. 2023), are integral to traditional and religious activities (Kurniadi & Putri 2021), and exhibit

excellent adaptability (Saputraa et al. 2020). Based on their phenotype and genotype, we can categorize buffaloes into two types: river buffaloes and swamp buffaloes. River buffaloes are characterized by black body color and curved horns, while swamp buffaloes are dark gray with one to two chevron lines (Curaudeau et al. 2021). Based on the number of chromosomes, river buffaloes have 50 pairs of chromosomes, while mud buffaloes have 48 pairs (Barker et al. 1997).

Differences in geographical conditions or the isolation of areas by natural features such as rivers, mountains, and forests can result in livestock species with distinct characteristics. The information provided by characterization studies is essential for planning the management of (Animal genetic resources) AnGR at local, national, regional, and global levels (FAO 2012). Characterization of livestock phenotypes and body biometry measures, particularly for lesser-known livestock groups, is essential to provide information on their genetic diversity (Vohra et al. 2015).

Jambi Province has a large buffalo population of 46,106 (Badan Pusat Statistik Provinsi Jambi 2024), which is spread across several districts. Smallholder farmers typically keep buffaloes in extensive systems. Compared to other livestock, including those in Jambi Province, buffalo characterization activities in Indonesia remain limited. There is no report comparing the phenotypic diversity of qualitative and quantitative traits based on multivariate analysis of buffaloes from the Highland and lowland areas in Jambi province. Phenotypic data, like morphometric data, can be analyzed using multivariate analysis. Multivariate analysis is a standard statistical approach used in analyzing compound data with multiple variables (Rezende et al. 2017). Multivariate analyses included PCA, Pearson correlation, discriminant analysis, and hierarchical cluster analysis (HCA). In addition to assessing genetic diversity, morphometric analysis can also be used to estimate genetic distance within and between livestock populations (Yunusa et al. 2013).

Based on these reasons, this study aims to analyze the phenotypic diversity of Jambi buffaloes in the highlands and lowlands. The study's results can inform the conservation and breeding strategies of Jambi buffalo.

MATERIALS AND METHODS

Ethical approval

The methods in this study were approved by the Animal Ethics Commission of the Faculty of Medicine, Andalas University, Indonesia, with permit number 588/UN.16.2/KEP-FK/2023.

Research area and animals

This study was conducted in highland areas (>500-1000 m above sea level), including the Merangin and Kerinci districts, and in lowland areas (0-100 m above sea level), including the Batanghari, Tebo, and Tanjung Jabung Barat districts (Figure 1). Climatic conditions in the research areas are presented in Table 1. Jambi Province is located at a latitude of 0°45′–2°45′ S and a longitude of 101°10′–104°55′ E. A total of 52 buffaloes were collected from highland areas, with a proportion of (male, 21 and female, 31), and 107 buffaloes were collected from lowland areas, with a proportion of (male, 36 and female, 71). To avoid sex effects, only adult buffaloes (3 to 6 years old) were observed; age determination was based on birth records and also on changes in fixed incisors (Badan Standarisasi Nasional, Bibit kerbau - Bagian 1: Lumpur 2011).

Livestock rearing management

The observed livestock were reared using semi-intensive methods, where they were released in the morning and penned in the afternoon without being provided with forage or concentrates. The housing system was colony-based, and the mating system was natural.

Data collection

The data collected include qualitative traits such as 1) skin color, 2) hair color, 3) horn shape, 4) chevron, 5) stocking, 6) back line, 7) hoof shape, 8) whorls on the head, 9) whorls on the back, and 10) whorls on the waist (Suhardi et al. 2020; Abdullah et al. 2024).

The quantitative trait of body measurements is measured based on (Vohra et al. 2015; Maleo et al. 2018). Quantitative properties of body measurements were measured based on Vohra et al. (2015) and Maleo et al. (2018) were 1) Shoulder height (SH), the highest distance of the shoulder through the back of the shoulder blade perpendicular to the ground (cm); 2) Hip height (HH), the highest distance of the hip perpendicular to the ground (cm); 3) Hip width (HW), the vast distance between the two hip joints (cm); 4) Body length (BL), the straight-line distance from the edge of the processus spinosus bone to the protrusion of the tapis bone (Os ischium) (cm); 5) Chest circumference (CC), measured right around the chest cavity, behind the shoulder joint (os scapula), using a tape measure (cm); 6) Deep chest (DC), the distance between the highest point of the shoulder and the base of the sternum (cm); 7) Chest width (CW), the distance between the left and right shoulder joints (between the humeral tuberosity and the dextral tuberosity) (cm); 8) Buttock length (RL), measured from the groin to the hip (cm); 9) Rump width

Table 1. Cinematic condition of the research area

Description	Highland		Lowland		
	Kerinci	Merangin	Batanghari	Tebo	Tanjung Jabung Barat
Temperature (^{0}C)	23.24	23.24	27.58	27.58	27.27
Humidity (%)	80.10	80.10	82.68	82.68	87.62
Rainfall (mm/year)	1,996	1,996.50	2,091.20	2,091.20	-
Regional Height (asl)	1000	501	36	43	37

Source: Badan Pusat Statistik Provinsi Jambi (2024)

Table 2. Calculations for body index

No.	Variables	Formula
1.	Height index (HI)	Shoulder Height/Body length
2.	Rump length index (RLI)	Rump Length/Body Length*100
3.	Over increase index (OI)	Hip Height/Shoulder Height*100
4.	Height slope (HS)	Hip Height-Shoulder Height
5.	Length index (1) (LI1)	Body Length/Shoulder Height
6.	Width slope (WS)	Hip Width/Chest Width
7.	Body weight index (BWI)	Body weight/Hip Height
8.	Length index (2) LI2)	Body Length/Chest Deep
9.	Balance: (Ba)	(Rump Width*Rump Length)/(Chest deep*Chest Circumference)
10.	Depth index: (DI)	Chest deep/Hip Height
11.	Foreleg length: (FL)	Hip Height-Chest deep
12.	Body index[a] (Bia)	Body Length*100/Chest Width
13.	Body ratio[c] (Bre)	Hip Height/Shoulder Height
14.	Transverse pelvic (Tp)	Rump Width*100/Shoulder Height

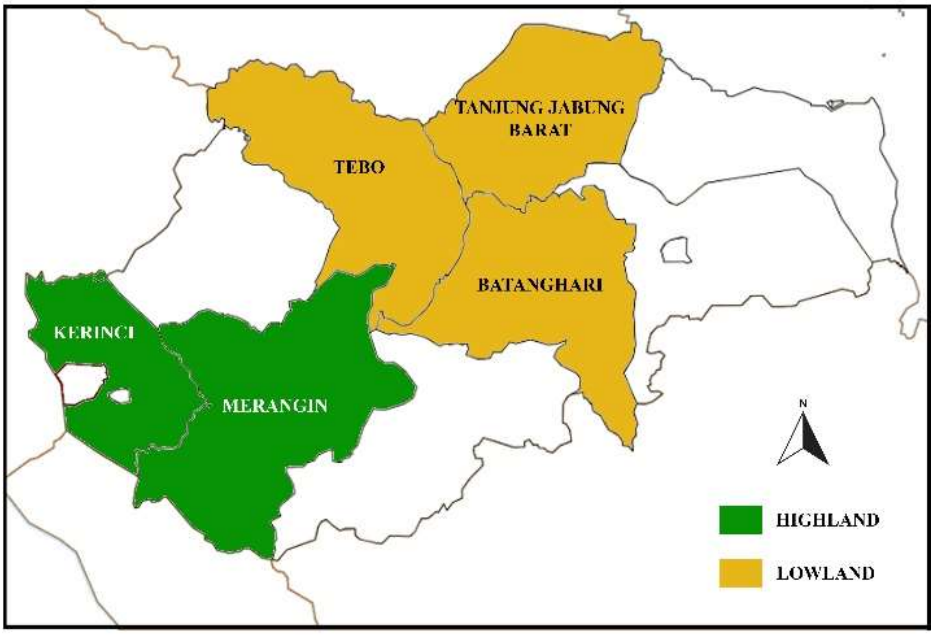

Figure 1. Research location in Jambi Province, Sumatra Island

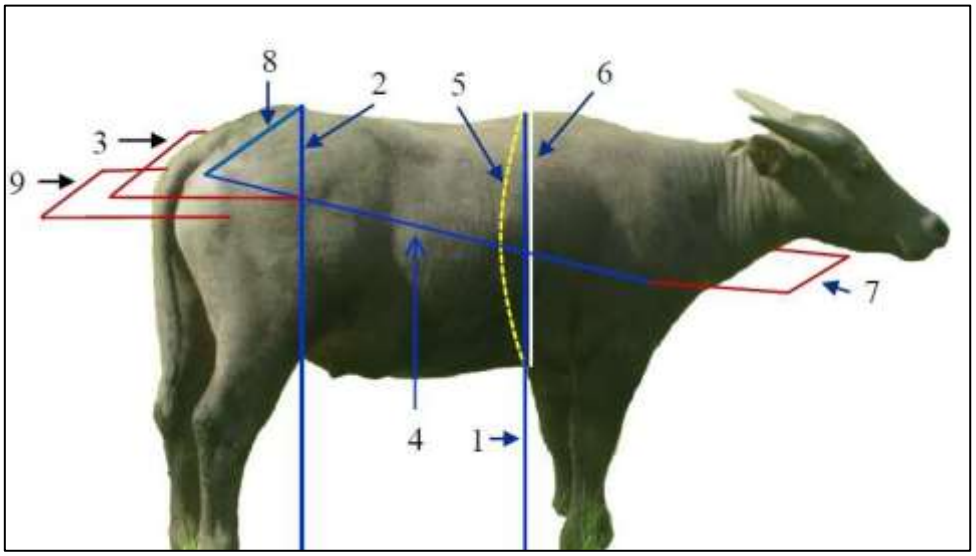

Figure 2. Illustration of buffalo body measurements. 1= Shoulder height, 2= Hip height, 3= Hip width, 4= Body length. 5= Chest circumference, 6= Deep , 7= Chest width , 8= Buttock length, 9= Rump width

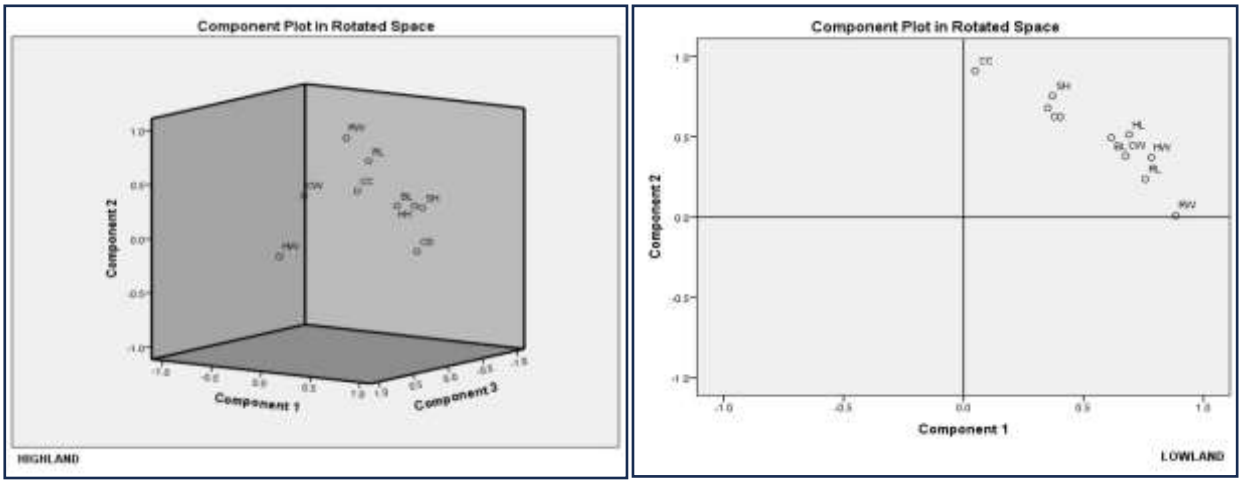

Figure 3. Component plot in rotated space for body morphometric

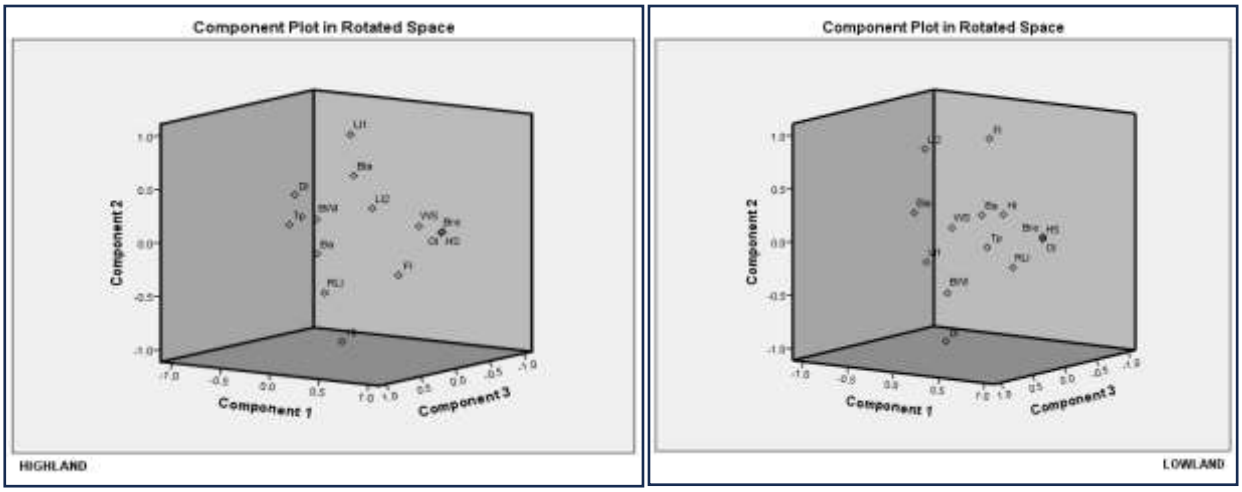

Figure 4. Component plot in rotated space for morphometric index

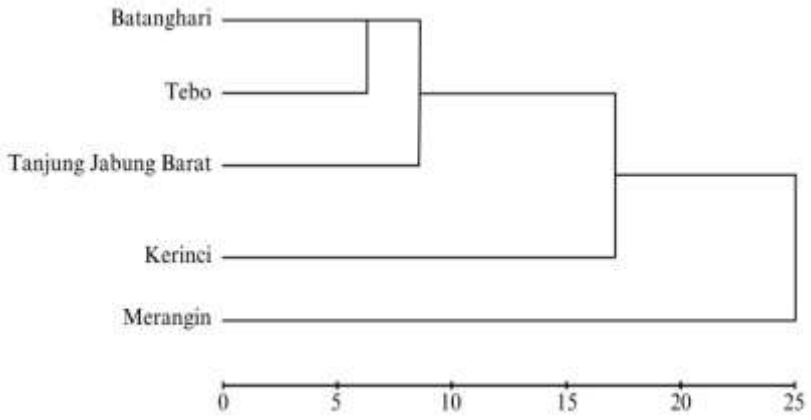

Figure 5. Dendrogram of genetic distances between five populations of Jambi buffalo

(RW), the distance between the left and right tuber femoris (cm) (Figure 2). SH, HH, HW, BL, and DC were measured using a yardstick. CW, RL, and RW were measured using callipers, and CC was measured using a tape measure. The body index in this study was analyzed based on the equation in Table 2, according to (Alderson 1999; Benerjee et al. 2014).

Statistical analysis

Qualitative trait data were analyzed by percentage and discussed descriptively using Microsoft Excel 21 version software. Body size and body index data were calculated as means and standard deviations. Pearson correlation, principal component analysis (PCA), discriminant analysis, and hierarchical cluster analysis (HCA) were analysed using SPSS 25 version software (IBM Corp, 2017). The Kaiser-Meyer-Olkin (KMO) sample adequacy test and Bartlett (1950) test were used in PCA to see if the data set was valid at a 1% significance level. This number, known as the Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO-MSA), indicates the extent to which variation in certain biometric traits can be attributed to underlying factors (Kaiser 1958). Rotation of principal components: We used Varimax rotation through component transformation to simplify the interpretation of factor analysis. HCA is used to group buffalo from different populations. We performed HCA using combined data (body size and body index), the nearest neighbor method, the Euclidean distance measure, and the Z-score transformation value.

RESULTS AND DISCUSSION

Qualitative and quantitative traits

Table 3 presents observations of qualitative traits of highland and lowland buffaloes. The skin color of buffaloes (both male and female) in both populations is generally dark gray to black. Observations of coat color also showed a similar trend, although in the lowland population, the black coat color was more dominant. The results revealed that the skin color and coat color in this study were in agreement with the Paraguas et al. (2018) on swamp buffalo in Calayan Island, Philippines; Suhardi et al. (2020) on Kalimantan, Indonesia, and Thailand buffaloes; and buffaloes from East Java, Indonesia (Putri et al. 2022). Others reported buffaloes in Bali, Indonesia (Adebowale et al. 2022), India (Vandre et al. 2022), and in Bangladesh (Yeasmin et al., 2016), which are predominantly black. In general, swamp buffalo have a characteristic dark gray to black coat color (Yusnizar et al. 2015; Liang et al. 2021). Under extreme environmental conditions, differences in phenotypes, such as coat color, can be observed, as reported by Khan et al. (2013), who found that the majority of Azikheli buffaloes in the mountainous areas of Pakistan have a brown coat color. This variation is strongly suspected to be related to adaptation to the environment, as the area lacks swamps for bathing, making it difficult for buffalo to reduce heat stress.

The Tranga horn shape dominates the horn shape in both populations; however, in the lowland male population, the Sikki horn shape is more prevalent. Maulana et al. (2023). The Taranga horn shape (horns grow upwards to form a half circle) was also found in Toraya buffalo populations, Musi Rawas Utara Setiawan (2022), Bangladesh Rahman et al. (2015), and buffalo in Konawe (Nafiu et al. 2023), while in the Indian state, buffalo have a backward curved horn shape (Khatke et al. 2023).

Double strip chevrons dominate the number of chevrons in both populations. One of the characteristics of the swamp buffalo is the presence of chevrons on the neck, typically one or two in white color (Zhang et al. 2020). The Chevron lines of swamp buffaloes in Jepara are predominantly single-line Chevron (Nur et al. 2018). The results of the study, Suhardi et al. (2020), report that

double strip chevrons dominated buffaloes from East Kalimantan and South Kalimantan, while single chevrons dominated Thale Noi Buffaloes from Thailand. Meanwhile, light gray and white dominate the stocking colors of both populations. Nur et al. (2018) reported that white stockings were found on the majority of buffaloes in Jepara, Indonesia, and the Nagpuri buffalo of the Purnathadi strain in India (Thalkar et al. 2018). In contrast, buffaloes in Konawe, Indonesia, have light gray legs (Karabu et al. 2021).

The backline shape of highland buffaloes is predominantly curved, while in lowland populations, the more common shape is flat. Buffaloes have a dorsal line that can be used to characterize meat proportions; a straight dorsal line indicates a high proportion of fat in the body (Rusdin et al. 2022). Krisnandi et al (2013) reported that buffaloes in Garut, Indonesia, have curved (56.67%) and flat (43.33%) backlines, while Suhardi et al. (2020) reported that buffalo populations in Kalimantan, Indonesia, are dominated by buffaloes with flat backlines.

The hoof shapes of the two regions also show similarities, with the bowl-type hoof shape dominating. Erdiansyah and Anggraeni (2008) reported that swamp buffaloes in Dompu, Indonesia, have a bowl hoof type. Buffaloes with a bowl hoof type are suitable for use as farming livestock. Buffaloes with bowl hoof types can be a selection criterion for labor buffaloes (Dudi et al. 2011).

The number of whorls on the head in both populations is one, while the number of whorls on the shoulder and hips is two. Yulianty et al. (2016) found that whorls on buffaloes in Jembrana, Indonesia, varied, such as on the face, left and right shoulders, and left and right gluteal regions. The variation of whorls in buffaloes in Bombana Island, Indonesia, was dominated by the face and waist (Nafiu & Asminaya 2023). Whorls are a parameter that can be used for the selection of temperament traits in animals. Aierqing et al. (2020) reported the results of a study on the relationship between whorls and temperament traits in Chinese cattle and horses (Encina et al. 2023).

Table 4 presents observations of body morphometrics for highland and lowland buffaloes. Body morphometric parameters such as SH, HH, BL, DC, and RW of highland buffaloes were significantly higher ($P<0.05$) than those of lowland buffaloes; all body morphometric parameters of highland buffaloes were higher than those of lowland buffaloes. Komariah et al. (2015) reported that swamp buffaloes in West Java, Indonesia, at high altitudes had greater body weight than those at low altitudes. Similar results were reported (Depison et al. 2022) in Balinese cattle research at different altitude locations. Many studies have found that the body size of buffaloes in Jambi province is the same as that of the Simeulue buffalo (Eriani et al. 2019), Serang Banten buffalo (Murni et al. 2020), Kuntu buffalo Yendraliza et al. 2021), and Kalimantan buffalo (Suhardi et al. 2022). However, they are smaller than buffaloes in Calayan, Philippines (Villamor et al. 2024), Murrah buffalo (Yadav & Vijh, 2022), and buffaloes in North India (Vohra et al. 2015). Different morphometric sizes in the two populations are likely due to the nutrient content of the available forage. Depison et al. (2021) reported that the crude protein and ether extract content of the highlands were better than those in lowland areas at the exact location. The content of minerals such as potassium and manganese in the highlands is relatively higher than in the lowlands, while the content of P, Fe, and Zn is comparatively lower and affects livestock performance (Komariah et al. 2015; Kumar et al. 2022).

Table 5 presents the morphometric indices of highland and lowland buffaloes. Analysis of morphometric indices revealed that only WS was significantly different ($P<0.05$), with higher values in highland buffaloes. In contrast, lowland buffaloes showed higher mean values in OI, HS, LI1, LI2, and BWI. HI, DI, FI, Bia, and Tp were higher in highland buffaloes. The morphometric indices of buffaloes showed that LI was not different from Kalimantan and Patalung buffaloes of Thailand (Suhardi et al. 2022), while HS, WS, FL, and Ba values were lower than those of these populations. In addition, HS, LI1, and DI were also not different from those of buffaloes in Sulawesi (Rusdin et al. 2022), but WS and Ba were recorded as lower than those populations. Body size is crucial because it can describe the productivity level of livestock (Kocu et al. 2017). Differences in body size can be influenced by sex, breed, and environmental factors (Syaiful & Maulida 2020; Wilastra et al. 2021; Lan et al.2022).

Phenotypic correlation

Pearson correlation between body morphometrics of highland and lowland buffaloes had low, medium ($P<0.05$), and high ($P<0.001$) correlation values Table 6. Highland buffalo had the lowest correlation (HW-SH) and the highest correlation (HH-SH), while the highland population had the lowest correlation (CC-RW) and the highest correlation (HH-SH). Murni et al. (2020) reported a similar finding, indicating that SH and HH had the highest correlation in swamp buffaloes in Banten, Indonesia. Singh et al. (2022) conducted a study on Indian dairy buffaloes. This report demonstrated a linear relationship between the SH and HH of buffaloes. Ağyar et al. (2022) reported that body weight had a significant correlation with SH, WH, and BL in Anatolian buffaloes. The highest correlation in this study was also found between HW and HH, as reported by Khan et al. (2022), who also noted a high correlation between (HW-HH) and (thigh width-chest width); this suggests that we can use Height and rump width as selection criteria for

Table 3. Percentage of qualitative traits in male and female buffalo

No	Qualitative traits		Highland		Lowland	
			Male (21)	Female (31)	Male (36)	Female (71)
1.	Skin color	a. Black	33.33	16.13	41.03	19.72
		b. Dark gray	52.38	51.61	41.03	56.34
		c. Light gray	14.29	32.26	12.82	16.90
		d. Albino	0.00	0.00	5.12	7.04
2.	Hair color	a. Black	9.52	16.13	30.77	30.99
		b. Dark gray	80.95	51.61	46.15	42.25
		c. Blonde	9.52	32.26	23.08	19.72
		d. White	0.00	0.00	0.00	7.04
3.	Horn shape	a. Tranga	57.14	51.61	28.21	57.75
		b. Pampang	0.00	0.00	23.08	7.04
		c. Siki	23.81	19.35	35.90	30.99
		d. Soko	4.76	6.45	7.69	2.82
		e. Langi	14.29	22.58	7.69	5.63
4.	Chevrons	a. Single	38.10	45.16	35.90	33.80
		b. Double	61.90	54.84	64.10	66.20
5.	Stocking color	a. Dark gray	33.33	25.81	0.00	11.27
		b. Light gray	38.10	58.06	53.85	43.66
		c. White	28.57	16.13	43.59	45.07
6.	Backline	a. Curved	66.67	58.06	41.03	47.89
		b. Flat	33.33	41.94	58.97	52.11
7.	Hoof Shape	a. Scissor type	14.29	45.16	41.03	30.99
		b. Bowl type	85.71	54.84	58.97	69.01
8.	Whorls on the head	a.1	38.10	41.94	61.54	53.52
		b.2	33.33	48.39	35.90	36.62
		c.>2	28.57	9.68	2.56	14.08
9.	Whorls on shoulders	a.1	4.76	9.68	25.64	29.58
		b.2	52.38	64.52	66.67	59.15
		c.>2	42.86	25.81	7.69	11.27
10.	Whorls on hips	a.1	19.05	16.13	23.08	16.90
		b.2	61.90	70.97	64.10	67.61
		c.>2	19.05	12.90	12.82	14.08

Taranga (horns grow upwards to form a half circle), Pampang (horns that grow sideways and tend to be very long, this pattern is usually observed from a castrated male buffalo), Sikki (horn growth is similar to Tarangga with points almost meeting), Sokko (horns grow downward with points under the neck), Langi (horns grow in opposite directions) (Suhardi et al. 2020).

Table 4. The average and standard deviation of buffalo body morphometric

Variables (cm)	Highland			Lowland		
	Male (21)	Female (31)	Total (52)	Male (36)	Female (71)	Total (107)
Shoulder Height	124.73±7.43	122.30±5.10	123.27±6.13*	121.19±3.83	118.76±4.34	119.57±4.29*
Hip Height	125.05±7.74	122.42±5.65	123.48±6.57*	121.24±5.20	119.77±4.07	120.26±4.49*
Body Length	130.34±7.42	123.52±7.68	126.27±8.15*	126.47±5.83	121.95±5.86	123.47±6.18*
Chest deep	77.68±5.67	76.91±4.96	77.22±5.17*	74.20±5.82	74.55±5.27	74.43±5.41*
Rump Length	45.75±4.03	41.28±1.78	43.08±3.59	43.23±5.01	42.28±3.43	42.6±4.01
Rump Width	31.78±1.92	28.84±2.08	30.02±2.45*	28.92±4.22	28.09±2.82	28.37±3.35*
Hip Width	46.33±5.72	47.83±3.94	47.22±4.70	48.09±8.14	45.29±5.70	46.23±6.68
Chest Width	49.76±5.21	46.74±4.71	47.95±5.04	45.49±6.52	44.26±5.10	44.67±5.59
Chest Circumference	180.96±7.71	174.28±6.05	176.97±7.40	176.55±7.27	175.10±7.15	175.58±7.15

*=Significant (P<0.001)

Table 5. The average and standard deviation of buffalo body morphometric indices

Variables	Highland			Lowland		
	Male (21)	Female (31)	Total (57)	Male (36)	Female (71)	Total (107)
Height index	95.72±2.96	99.23±4.92	97.81±4.51	95.94±3.43	97.51±4.05	96.98±3.89
Rump length index	35.10±2.45	33.52±2.06	34.15±2.32	34.17±3.42	34.69±2.56	34.51±2.86
Over-increase index	100.27±2.27	100.09±1.52	100.16±1.82	100.04±2.85	100.88±2.41	100.59±2.57
Height slope	0.33±2.78	0.12±1.85	0.20±2.23	0.05±3.43	1.00±2.81	0.68±3.04
Length index (1)	1.05±0.03	1.01±0.05	1.02±0.05	1.04±0.04	1.03±0.04	1.03±0.04
Width slope	0.93±0.10	1.03±0.12	0.99±0.12*	1.06±0.14	1.03±0.12	1.04±0.13*
Body weight index	327.35±18.07	315.29±24.29	320.15±22.39	323.85±19.46	325.23±22.79	324.76±21.55
Length index (2)	1.68±0.10	1.61±0.09	1.63±0.10	1.71±0.13	1.64±0.10	1.66±0.11
Balance:	0.10±0.01	0.08±0.01	0.09±0.01	0.09±0.018	0.09±0.01	0.09±0.02
Depth index:	0.62±0.05	0.63±0.04	0.62±0.04	0.61±0.04	0.62±0.04	0.61±0.04
Foreleg length:	47.37±7.38	45.50±5.40	46.25±6.21	47.04±5.23	45.22±5.19	45.82±5.22
Body index[a]	72.05±3.16	70.93±4.69	71.38±4.10	71.68±3.12	69.72±3.51	70.37±3.48
Body ratio	1.00±0.02	1.00±0.02	1.00±0.02	1.00±0.03	1.01±0.02	1.00±0.03
Transverse pelvis	25.53±1.58	23.60±1.73	24.38±1.89	23.84±3.20	23.66±2.31	23.72±2.62

*=Significant (P<0.001)

economically valuable livestock. The correlation between variables occurs due to the phenomenon of pleiotropy.

The highest correlation values were found between morphometric indices in highland buffaloes (OI-HS, Bre-HS, and Bre-OI) and those in lowland buffaloes (OI-HS, Bre-HS, and Bre-OI), as shown in Table 7. The correlation values between the morphometric indices match the body morphometry. The height slope value in livestock evaluation is an indication of excellent body posture quality (Sakar et al. 2023). Unfortunately, there have been no reports on the correlation of morphometric indices in buffaloes. Several reports on the correlation of morphometric indices were reported in Pasundan cattle (Putra et al., 2020), the highest correlation values (WS and Tp), Kacang goats (Depison et al., 2020), body weight, with the Dactyl thorax index, the Relative cannon index, and area index for Kacang goats in the lowland area. Body weight with a conformation index, area index, and index of body weight for Kacang goats in the highland area and thin-tailed sheep. The highest correlation between body weight and body index for thin-tailed sheep in the lowland area and body weight and area index for thin-tailed sheep in the highland area (Depison et al. 2021).

Principal component analysis

PCA of body morphometrics in buffaloes showed that highland buffaloes had three PCs and buffaloes in lowland areas had two PCs (Table 8). PCA of body morphometrics showed that 74.16% of the differences in highland buffaloes and 69.45% in lowland buffaloes can be explained, with the difference in the number of PCs suggesting that highland buffaloes have more complex body shapes and greater variation than lowland buffaloes. Several previous studies reported four PCs that explained 59.52% of the total variation in Murrah buffalo (Dahiya et al. 2021). Raut et al. (2023) identified five PCs that explained 61.90% of the total variation in Marathwadi buffaloes. Vohra et al. (2015) identified four PCs that explain 70.86% of the total variation of the lesser-known buffalo. Vohra et al. (2017) identified five PCs that accounted for 70.13% of the total variation in Chhattisgarhi buffalo. Five PCs were able to explain 67.4% of the total variation in Murrah females, while four PCs were obtained in Murrah bulls and explained 62% of the total variation (Vohra et al. 2017).

Meanwhile, the PCA of morphometrics indices in highland and lowland buffaloes had four PCs (Table 9). The extracted morphometrics index (PCA) accounted for 84.17% of the total variance of animal morphometrics indices in highland buffaloes and 84.02% in lowland buffaloes. However, there is currently no reported research on PCA for buffalo morphometric indices. Putra et al. (2020) found 4 PCs in Pasundan cattle based on 13 observed morphometric indices. The PCs could explain 89.38% of the total variation. Depison et al. (2021) found four PCs of thin-tailed sheep in the lowlands with a total variance of 78.23% and five PCs in the highlands with a total variance of 84.99%. Kacang goats have 4 PCs with a total variance of 85.82% in the lowlands and 86.19% in the highlands (Depison et al. 2020). In addition to genetic factors, this study shows that environmental factors also play a strong role in livestock performance. These environmental factors include disease, adaptability, and the nutritional quality of feed. Plots of morphometric components and morphometric indices of buffaloes are illustrated in Figures 3 and 4.

Discriminant analysis

Discriminant analysis was used to characterise the buffalo population based on body morphometrics and morphometric indices. In addition, about 48.09% of highland buffaloes and 51.92% in the lowlands can be characterised based on body morphometrics. Meanwhile, about 57.69% of highland buffaloes and 42.31% in the lowlands can be characterised based on morphometric indices (Table 10).. Previous studies have reported that breed characterization based on body size can distinguish buffaloes in three populations. Ali et al. (2024) Purnathadi (71.7%), Marathwadi (79.6%) and Ngapuri (61.7%); Anggraeni et al. (2011); West Nusa Tenggara buffalo (95.0%); North Sumatra (74.51%); South Sulawesi (74.9%); Central Java (67.33%); and NAD (66.0%). Salamena & Papilaja (2010) characterized the western (67.4%), central (53.6%), and eastern (50.8%) subpopulations of Moa buffaloes. Suhardi et al. (2022) North Kalimantan (100%), East Kalimantan (66%), and South Kalimantan (70%).

Hierarchical cluster analysis (HCA) based on body size and body index revealed three clusters. The first cluster was Merangin; Kerinci was in the second cluster, and Tanjung Jabung Barat, Tebo, and Batanghari were in the third cluster (Figure 5). The lowest Euclidean distance was Batanghari-Tebo (4.92), while the highest was Batanghari-Merangin (36.34) (Table 11). The highland buffalo populations (Merangin and Kerinci) are in close clusters, and the lowland buffalo populations are also in the same cluster. This evidence suggests that geographical and environmental factors play a significant role in shaping buffalo subpopulations. Clustering based on body size has also been reported for buffaloes in Banten (Murni et al. 2020), Indonesian local buffaloes (Anggraeni et al. 2011), and river buffaloes in India (Ali et al. 2024).

Table 6. Coefficient Correlation among body morphometrics of buffaloes in the Highland (below diagonal) and the lowland (above diagonal)

Traits	SH	HH	BL	CD	RL	RW	HW	CW	CC
SH	0	0.76**	0.64**	0.44**	0.40**	0.34*	0.54**	0.43**	0.60**
HH	0.94**	0	0.65**	0.46**	0.58**	0.61**	0.69**	0.55**	0.45**
BL	0.73**	0.74**	0	0.46**	0.45**	0.52**	0.64**	0.55**	0.40**
CD	0.59**	0.46	0.58**	0	0.51**	0.32	0.52**	0.51**	0.59**
RL	0.53**	0.56**	0.58**	0.25	0	0.54**	0.65**	0.57**	0.28
RW	0.38	0.37	0.42	0.11	0.54**	0	0.63**	0.56**	0.14
HW	0.07	0.15	0.24	0.01	0.14	-0.01	0	0.59**	0.38**
CW	0.30	0.26	0.39	0.19	0.31	0.43	0.39	0	0.42**
CC	0.47*	0.45	0.49	0.33	0.60**	0.35	0.25	0.31	0

*=Significant (P<0.005), **=Significant (P<0.001). SH= Shoulder height, HH= Hip height, HW= Hip width, BL= Body length, CC= Chest circumference, DC= Deep chest, CW= Chest width, RL= Buttock length, RW= Rump width

Table 7. Coefficient Correlation among body morphometrics index of buffaloes in the Highland (below diagonal) and the lowland (above diagonal)

Traits	HS	WS	RLI	HI	OI	LI1	LI2	BWI	DI	FL	Bia	Bre	Tp	Ba
HS	0	0.04	0.28	-0.37	0.99**	0.36*	-0.03	-0.43**	-0.17	0.30	0.21	0.99**	0.56**	0.48**
WS	0.26	0	0.07	-0.02	0.03	0.02	0.03	-0.12	-0.05	0.10	0.13	0.03	0.06	0.12
RLI	0.06	-0.07	0	0.17	0.26	-0.18	-0.42**	-0.05	0.18	-0.09	-0.16	0.26	0.28	0.52**
HI	-0.21	-0.07	0.32	0	-0.36	-0.99**	-0.33	0.14	-0.13	0.07	-0.73**	-0.36	-0.41**	-0.31
OI	0.99**	0.26	0.06	-0.21	0	0.35	-0.03	-0.43**	-0.18	0.30	0.20	1.00**	0.55**	0.47**
LI1	0.21	0.06	-0.32	-1.00**	0.21	0	0.32	-0.13	0.14	-0.08	0.72**	0.35	0.41**	0.30
LI2	0.48*	0.05	-0.01	-0.49*	0.47*	0.49*	0	-0.38**	-0.81**	0.74**	0.52**	-0.03	0.04	0.26
BWI	-0.27	0.02	0.24	-0.17	-0.26	0.17	-0.07	0	0.51**	-0.50**	-0.64**	-0.43**	-0.30	-0.52**
DI	-0.59**	-0.07	-0.23	-0.17	-0.59**	0.17	-0.74**	0.27	0	-0.95**	-0.18	-0.18	-0.03	-0.30
FL	0.60**	0.00	0.18	0.13	0.60**	-0.13	0.69**	-0.35	-0.93**	0	0.26	0.30	0.15	0.42**
Bia	0.16	-0.09	-0.47*	-0.59**	0.16	0.58**	0.36	-0.60**	0.01	0.19	0	0.20	0.37**	0.50**
Bre	0.99**	0.26	0.06	-0.21	1.00**	0.21	0.47*	-0.26	-0.59**	0.60**	0.16	0	0.55**	0.47**
Tp	0.04	-0.28	0.27	-0.21	0.04	0.23	0.27	0.20	-0.10	0.00	-0.08	0.04	0	0.84**
Ba	0.27	-0.22	0.58**	-0.10	0.27	0.11	0.59**	-0.09	-0.56**	0.53**	0.10	0.27	0.73**	0

*=Significant (p<0.005), **=Significant (P<0.001). HS= Heigh slope, WS= Width slope, RLI= Rump length index, HI= Height index, OI= Over increase index, LI1= Length index (1), LI2= Length index (2), BWI= Body weight index, DI= Depth index, FL=Foreleg length, Tp= Transverse pelvic, Bia= Body index[a], Bre= Body ratio[c]

Table 8. Eigenvalues, total variance, cumulative, communalities, Kaiser-Meiyer-Olkin (KMO) measure of sampling adequacy, and Bartlett's test of sphericity in the body morphometrics of buffalo

Body Measurement	Highland				Low land		
	PC1	PC2	PC3	EC	PC1	PC2	EC
Shoulder Height	0.82*	0.35	0.07	0.81	0.37	0.75*	0.71
Hip Height	0.76*	0.37	0.23	0.78	0.69*	0.51*	0.74
Body Length	0.82*	-0.07	0.03	0.68	0.61*	0.49	0.62
Chest deep	0.39	0.72*	0.11	0.70	0.35	0.67*	0.58
Rump Length	0.09	0.89*	0.00	0.81	0.75*	0.23	0.63
Rump Width	0.05	-0.07	0.92*	0.87	0.88*	0.00	0.78
Hip Width	0.10	0.45	0.64*	0.63	0.78*	0.37	0.75
Chest Width	0.41	0.48	0.31	0.51	0.67*	0.37	0.60
Chest Circumference	0.87*	0.33	0.03	0.88	0.04	0.91*	0.83
Eigenvalues	4.34	1.29	1.03	-	5.12	1.12	-
Variance (%)	48.24	14.42	11.49	-	56.93	12.51	-
Cumulative (%)	48.24	62.67	74.16	-	56.93	69.45	-
KMO	0.72				0.86		
Barlett’s test	*				*		

a = main component, *=Significant (P<0.005). PC=Principal component, EC= Eigenvalue contribution

Table 9. Eigenvalues, total variance, cumulative, communalities, Kaiser-Meiyer-Olkin (KMO) measure of sampling adequacy, and Bartlett's test of sphericity in the morphometrics indices of buffalo

	Highland					Lowland				
Variables	PC1	PC2	PC3	PC4	EC	PC1	PC2	PC3	PC4	EC
Height index	-0.09	-0.99	-0.06	-0.01	0.98	-0.24	0.05	-0.95	0.00	0.96
Rump length index	0.15	-0.42	0.53*	0.47	0.70	0.30	-0.31	-0.34	0.69*	0.77
Over-increase index	0.94*	0.15	-0.05	-0.04	0.91	0.97*	0.10	0.14	0.08	0.99
Height slope	0.94*	0.15	-0.05	-0.01	0.91	0.97*	0.10	0.15	0.10	0.99
Length index (1)	0.09	0.99*	0.07	0.00	0.98	0.25	-0.07	0.95*	0.00	0.97
Width slope	0.37	0.07	-0.52	0.31	0.51	-0.11	0.07	0.03	0.40	0.18
Body weight index	-0.24	0.16	0.09	0.84*	0.80	-0.31	-0.59	-0.18	-0.34	0.59
Length index (2)	0.59*	0.40	0.47	-0.19	0.77	-0.16	0.87*	0.39	-0.06	0.93
Balance:	0.33	0.02	0.90*	0.01	0.92	0.37	0.28	0.25	0.80*	0.87
Depth index:	-0.78*	0.27	-0.37	0.21	0.86	-0.11	-0.97	0.14	-0.01	0.97
Foreleg length:	0.76*	-0.22	0.33	-0.35	0.87	0.21	0.93*	-0.1	0.11	0.93
Body index[a]	0.07	0.59	0.00	-0.75	0.92	0.02	0.35	0.81	0.30	0.91
Body ratio	0.95*	0.14	-0.02	-0.01	0.92	0.97*	0.12	0.15	0.08	0.99
Transverse pelvic	-0.04	0.23	0.78*	0.24	0.72	0.50	0.00	0.35	0.58	0.71

Variables	Highland					Lowland				
	PC1	PC2	PC3	PC4	EC	PC1	PC2	PC3	PC4	EC
Eigenvalues	5.08	2.89	2.26	1.56	-	5.34	2.82	2.32	1.28	-
Variance (%)	36.27	20.62	16.12	11.16	-	38.12	20.14	16.59	9.18	-
Cumulative (%)	36.27	56.88	73.01	84.17	-	38.12	58.26	74.84	84.02	-
KMO			0.5					0.61		
Barlett's test			*					*		

a = main component, *=Significant (P<0.005). PC=Principal component, EC= Eigenvalue contribution

Table 10. Percentage (%) of individual classification per breed based on discriminant analysis

Factor	Prediction group membership (N)			
	Population	Highland (%)	Lowland (%)	Total
Morphometric	Highland	48.09 (25)	51.92 (27)	100 (52)
	Lowland	13.08 (14)	86.92 (93)	100 (107)
Morphometric index	Highland	57.69 (30)	42.31 (22)	100 (52)
	Lowland	16.82 (18)	83.18 (89)	100 (107)

Table 11. Euclidean morphometric distance matrix between five populations of Jambi buffalo based on body morphometry

Population	Batanghari	Kerinci	Merangin	Tanjabar	Tebo
Batanghari	0.00	77.65	90.82	133.71	89.63
Kerinci		0.00	83.86	65.22	48.96
Merangin			0.00	107.96	78.13
Tanjabar				0.00	51.96
Tebo					0.00

CONCLUSION

This study is the first report to characterise Jambi buffaloes in two different locations based on altitude using multivariate analysis. Buffaloes in both populations have high diversity in qualitative traits. Body morphometrics of highland buffaloes are larger than those of lowland buffaloes. There was a high correlation between the parameters observed in both populations. Highland buffaloes showed three principal components (PCs) based on body morphometrics, while lowland buffaloes showed two PCs. The four principal components are based on morphometric indices in both populations. We characterized about 48.09% of buffaloes in the highlands and 51.92% of those in the lowlands using body morphometrics. Meanwhile, 57.69% of highland buffaloes and 42.31% of lowland buffaloes could be characterized based on morphometric indices. Hierarchical cluster analysis (HCA) based on body size and body index revealed three clusters. The first cluster was Merangin; Kerinci was in the second cluster, and Tanjung Jabung Barat, Tebo, and Batanghari were in the third cluster. The government and breeders must collaborate to conserve the genetic resources of Jambi's local buffaloes and enhance their productivity through ecosystem-appropriate breeding programs that support population sustainability.

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REFERENCES

Abdullah NA, Mutawalli, Ammar M, Sari M. 2024. Identification of quantitative and qualitative traits diversity in local buffalo in Nagan Raya District. Indones J Vet Sci. 18:40–45. DOI:10.21157/j.ked.hewan.v18i2.34229.

Adebowale A, Soetan M, Adefabi A. 2023. Profile of morphological characters among male and female buffalo (*Bubalus bubalis*) populations in Kaliakah Village, Bali Province, Indonesia. AJAS. 8:135–142.DOI:10.13170/ajas.8.3.31935.

Ağyar O, Tırınk C, Önder H, Şen U, Piwczyński D, Yavuz E. 2022. Use of multivariate adaptive regression splines algorithm to predict body weight from body measurements of Anatolian buffaloes in Türkiye. Animals. 12:1–9. DOI:10.3390/ani12212923.

Aierqing S, Nakagawa A, Ouchi Y, Bungo T. 2020. The effect of facial hair whorl position and raising environment on the temperament of the Chinese Yellow cattle in Shinjang Uyghur Aptonom Rayoni, China. JAVAR. 7:477–481. DOI:10.5455/javar.2020.g444.

Alderson GL. 1999. The development of a system of linear measurements to provide an assessment of type and function of beef cattle. Anim Genet Resour Inf. 25:45–55.

Ali SS, Kuralkar SV, Kanadkhedkar HL, Bankar PS, Kataria RS, Vohra V. 2024. Discriminant analysis reveals differences in breed structure in river buffaloes of Maharashtra (India). Trop Anim Health Prod. 56:1–15. DOI:10.1007/s11250-024-03885-4.

Anggraeni A, Sumantri C, Praharani L, Dudi, Andreas E. 2011. Estimasi jarak genetik kerbau rawa lokal melalui pendekatan analisis morfologi. JITV. 16:199–210.

Badan pusat statistik (BPS) (2024). Populasi kerbau menurut provinsi (ekor), 2019-2021. bps.go.id. Avaliable at:https://jambi.bps.go.id/id/publication/2024/02/28/e697127048eeb86bba8d8d40/provinsi-jambi-dalam-angka-2024.html

Badan Standarisasi Nasional. Bibit kerbau - Bagian 1: Lumpur. 2011. SNI 7706.1:2011. BSN, Jakarta.

Barker JSF, Moore SS, Hetzel DJS, Evans D, Tan SG, Byrne K. 1997. Genetic diversity of Asian water buffalo (*Bubalus bubalis*): microsatellite variation and a compari- son with protein-coding loci. Anim Genet. 28:103–115. DOI:10.1111/j.1365-2052.1997.00085.x.

Bartlett MS. 1950. Tests of significance in factor analysis. Br J Psychol. 3:77–85.

Benerjee S, Ahmed BM, Tefere G. 2014. Studies on morphometrical traits of Boran bulls reared on two feedlots in Southern Ethiopia. Anim Genet Resour. 54:53–63. DOI:10.1017/S2078633614000095.

Corp IBM. 2017. IBM SPSS Statistics for Windows.

Curaudeau M, Rozzi R, Hassanin A. 2021. The genome of the lowland anoa (*Bubalus depressicornis*) illuminates the origin of river and swamp buffalo. Mol Phylogenet Evol. 161:107170. DOI:10.1016/j.ympev.2021.107170.

Dahiya SP, Kumar M, Dhillod S, Ratwan P. 2021. Principal component analysis of linear type traits to explain body conformation in Murrah buffaloes. Indian J Anim Sci. 90:1546–1550. DOI:10.56093/ijans.v90i11.111569.

Depison D, Putra WPB, Gushairiyanto G, Alwi Y, Suryani H. 2020. Morphometric characterization of Kacang goats raised in lowland and highland areas of Jambi Province, Indonesia. JAVAR. 7:734–743. DOI:10.5455/javar.2020.g475.

Depison, Putra WPB, Alwi Y, Suryani H. 2021. Morphometrics Characterization of Thin-Tail Sheep in Lowland and Highland Areas. Trop Anim Sci J. 44:386–398. DOI:10.5398/tasj.2021.44.4.386.

Depison, Putra WPB, Dika Irmaya H. 2022. Morphometric discrimination in Bali cattle (*Bos javanicus*) at two different altitude areas of Jambi province, Indonesia. Indian J Anim Sci. 92:222–225. DOI:10.56093/ijans.v92i2.122097.

Dudi, Sumantri C, Martojo H, Anang A. 2011. Keragaan sifat kualitatif dan kuantitatif kerbau lokal di Propinsi Banten. J Ilmu Ternak. 11:61–67. DOI:10.24198/jit.v11i2.371.

Encina A, Ligero M, Sánchez-Guerrero MJ, Rodríguez-Sainz de los Terreros A, Bartolomé E, Valera M. 2023. Phenotypic and Genetic study of the presence of hair whorls in Pura Raza Español Horses. Anim. 13:9–12. DOI:10.3390/ani13182943.

Erdiansyah E, Anggraeni A. 2008. Keragaman fenotipe dan pendugaan jarak genetik antara subpopulasi kerbau rawa lokal di Kabupaten Dompu, Nusa Tenggara Barat. Proc Semin Lokakarya Nas Usaha Ternak Kerbau. p.55–67.

Eriani K, Rahmi R, Jamil I, Rosnizar R, Azhar A. 2019. Body size characteristics and polymorphism in GH and GHRH genes of Simeulue Buffalo of Aceh, Indonesia. Biodiv. 20:236–242. DOI:10.13057/biodiv/d200127.

FAO. 2012. Phenotypic characterization of animal genetic resources. Rome.

Kaiser HF. 1958. The varimax criterion for analytic rotation in factor analysis. Psychometrika. 23:187–200.

Karabu MI, Aku AS, Nafiu Lode. 2021. Penampilan sifat-sifat kualitatif kerbau Rawa di Kabupaten Konawe. J Ilm Peternak Halu Oleo. 3. DOI:10.56625/jipho.v3i2.18029.

Khan N, Khan S, Israr M, Hilal MG, Ibrahim MNM, Khan NA. 2022. Comparing Azikheli buffaloes with Nili Ravi buffaloes for morphometry, productivity, and reproductivity. Pakistan J Agric Sci. 59:693–701. DOI:10.21162/PAKJAS/22.1453.

Khan M, Rahim I, Rueff H, Jalali S, Saleem M, Maselli D, Muhammad S, Wiesmann U. 2013. Morphological characterization of the Azikheli buffalo in Pakistan. Anim Genet Resour génétiques Anim genéticos Anim. 52:65–70. DOI:10.1017/s2078633613000027.

Khatke S, Mayekar A, Kadam N, Kokani D, Gavit M, Desai B. 2023. Morphological characterization of non-descript Buffalo in Konkan Region of Maharashtra State. Int J Vet Sci Anim Husb. 8:176–180. DOI:10.22271/veterinary.2023.v8.i3c.539.

Kocu O, Salundik S, Priyanto R, Prihantoro I. 2017. Produktivitas Sapi Bali di lahan pastura dan perkebunan kelapa sawit di Kabupaten Keerom Provinsi Papua. J Ilmu Produksi dan Teknol Has Peternak. 5:110–116. DOI:10.29244/jipthp.5.3.110-116.

Komariah K, Sumantri S, Nuraini H, Nurdiati S, Mulatsih S. 2015. Performans Kerbau Lumpur dan strategi pengembangannya pada daerah dengan ketinggian berbeda di Kabupaten Cianjur. J Vet. 16:606–615. DOI:10.19087/jveteriner.2015.16.4.606.

Krisnandi G, Rahmat D, Dudi. 2013. Identifikasi sifat kualitatif dan kuantitatif kerbau jantan dewasa. J Chem Inf Model. 53:1689–1699.

Kumar R, Joshi R, Kumar R, Srivatsan V, Satyakam, Chawla A, Patial V, Kumar S. 2022. Nutritional quality evaluation and proteome profile of forage species in the Western Himalaya. Grassl Sci. 68:214–225. DOI:10.1111/grs.12357.

Kurniadi MDK, Putri HM. 2021. Tradisi bantai adat: kearifan lokal menyambut bulan ramadhan masyarakat merangin jambi. J Lekt Keagamaan. 19:388–418. DOI:10.31291/jlka.v19i2.961.

Lan Q, Cao Z, Yang X, Gu Z. 2022. Physiological and proteomic responses of dairy buffalo to heat stress induced by different altitudes. Metabolites. 12. DOI:10.3390/metabo12100909.

Liang D, Zhao P, Si J, Fang L, Pairo-Castineira E, Hu X, Xu Q, Hou Y, Gong Y, Liang Z, Tian B, Mao H, Yindee M, Faruque MO, Konvongxay S, Khamphoumee S, Liu GE, Wu D, Barker JSF, Han J, Zhang Y. 2021. Genomic analysis revealed a convergent evolution of LINE-1 in coat color: a case study in Water Buffaloes (*Bubalus bubalis*). Mol Biol Evol. 38:1122–1136. DOI:10.1093/molbev/msaa279.

Maleo DAB, Nascimento MDI, Santos DATL, Lima DGL, Araujo DTCF, Rios SRR, Couto GDA, Fraga BA. 2018. Body morphometric measurements in murrah crossbred buffaloes (*Bubalus bubalis*). J Appl Anim Res. 46:1307–1312. DOI:10.1080/09712119.2018.1502669.

Maulana T, Iskandar H, Said S, Gunawan A. 2023. The current status and potential development of genetic resources of the indigenous Toraya spotted buffalo in Indonesia: a systematic review. World's Vet J. 13:617–625. DOI:10.54203/SCIL.2023.WVJ66.

Murni D, Lestari U, Indriwati SE, Efendi A, Maryani N, Amin M. 2020. Morphometric diversity and phenotypic relationship among indigenous buffaloes of Banten, Indonesia. Biodiv. 21:933–940. DOI:10.13057/biodiv/d210311.

Nafiu LO, Asminaya NS. 2023. Characteristics of the Backline and Unyeng-unyeng of Swamp Buffalo on Kabaena Island, Bombana Regency. Indones J Anim Agric Sci. 5:90–94.

Nafiu O, Saili T, Abadi M, Badaruddin R, Aba, AuzaA.F. 2023. External genetic characteristics of Swamp Buffalo Based on sex and age in South Konawe District. Int. J. Sci. Res. Sci. Eng. Technol. 10:301–310. DOI:10.32 628/ijsrset2310621.

Nur AE, Nugroho H, Kuswati K. 2018. Karakteristik fenotip Kerbau Rawa (*B. bubalis carabenesis*) di wilayah sentra pengembangan kerbau Desa Guosobokerto Kecamatan Welahan Kabupaten Jepara. J Ternak Trop. 19:156–166. DOI:10.21776/ub.jtapro.2018.019.02.10.

Paraguas AM, Cailipan TPC, Flores EB, Villamor LP. 2018. Morphology and phylogeny of swamp buffaloes (*Bubalus bubalis*) in Calayan Island, Cagayan. Philipp J Vet Anim Sci. 44:59–67.

Prihandini PW, Tribudi YA, Nur D, Hariyono H, Putri A, Nafsina Z, Sari L. 2023. Biodiversity of Indonesian indigenous buffalo : First review of the status, challenges, and development opportunities. Vet World. 16:2217–2229. DOI:10.14202/vetworld.2023.2217-2229.

Putra WPB, Said S, Arifin J. 2020. Principal component analysis (PCA) of body measurements and body indices in the pasundan cows. Black Sea J Agric. 3:49–55.

Putri ARI, Budiarto A, Ciptadi G, Saputra R. 2022. Relationship Distance Analysis of Swamp Buffalo Bubalus bubalis Phenotype in Three Regions in East Java, Indonesia. In. Proc 9th ISTAP. 18:227–230. DOI:10.2991/absr.k.220207.047.

Rahman M, Islam R, Hossain MK, Lucky NS, Zahan N. 2015. Paper phenotypic characterization of indigenous buffalo at Sylhet District. Int J Sci Res Agric Sci. 2:1–6.

Raut PB, Sajid Ali S, Nandedkar P V., Chopade MM, Siddiqui MBA, Wankhede SM, Naveeth K. 2023. Principal component analysis of body biometric traits in Marathwadi buffaloes. Indian J Anim Sci. 93:226–231. DOI:10.56093/ijans.v93i2.128668.

de Rezende MPG, Ferraz PC, Carneiro PLS, Malhado CHM. 2017. Phenotypic diversity in buffalo cows of the Jafarabadi, Murrah, and Mediterranean breeds. Pesqui Agropecu Bras. 52:663–669. DOOI:10.1590/S0100-204X2017000800012.

Rusdin M, Nafiu LO, Saili T, Aku AS, Rahman, Badaruddin R. 2022. The analysis of morphometric index of four buffalo populations in South East Sulawesi, Indonesia. In: Proc Int Conf Improv Trop Anim Prod Food Secur. 20. p. 51–55. DOI:10.2991/absr.k.220309.011.

Sakar ÇM, Ünal İ, Ergiden Y, Zülkadir U. 2023. Comparison of some biometric index values in Anatolian Black Cattle calves raised in different locations. Large Anim Rev.. 29:119–127.

Salamena JF, Papilaja BJ. 2010. Characterization and genetic relationships analysis of Buffalo Maluku Province. J Indones Trop Anim Agric. 35:75–82.

Saputraa F, Jakaria, Anggraeni A, Sumantri C. 2020. Genetic diversity of Indonesian Swamp Buffalo based on

microsatellite markers. Trop Anim Sci J. 43191–196. DOI:10.5398/tasj.2020.43.3.191.

Setiawan DB. 2022. Identifikasi sifat karakteristik ternak kerbau (*Bubalus bubalis*) yang dipelihara di Kecamatan Talang Empat. J Ilmu Pertan Kelingi. 2:158–165. DOI:10.58328/jipk.v2i1.66.

Singh, K R, Kumar B, Kumar R, Kumar S, Mandal K. 2022. Phenotypic characterization and multivariate analysis of biometric traits of Diara Buffalo in Bihar, India. Biol Forum – An Int J. 12:51–57.

Suhardi, Putra WP., Wibowo A, Ismanto A, Rofik, Haris M., Safitri A, Ibrahim, Summpum P. 2022. Canonical discriminant analysis of morphometric variables of Swamp Buffalo (*Bubalus bubalis*) in Kalimantan Island. Int J Vet Sci. 11:23–27. DOI:10.47278/journal.ijvs/2021 .067 Canonical.

Suhardi S, Summpunn P, Duangjinda M, Wuthisuthimethavee S. 2022. Buffalo (*Bubalus bubalis*) phenotypic diversity characterization reveals the need for improved performance based on quantitative and qualitative characteristics. Buffalo Bull. 41:373–390.

Suhardi, Summpunn P, Duangjinda M, Wuthisuthimethavee S. 2020. Phenotypic diversity characterization of Kalang and Thale Noi buffalo (*Bubalus bubalis*) in Indonesia and Thailand: Perspectives for the buffalo breeding development. Biodiv. 21:5128–5137. DOI:10.13057/ biodiv/d211118.

Syaiful L, Maulida S. 2020. Identifikasi ukuran tubuh Sapi Bali dan Simbal (Simmental-Bali) di Kecamatan Luhak Nan Duo Kabupaten Pasaman Barat. J Sains Peternak Indones. 15:219–226.

Thalkar M, Chavan S, Kedari V, Khirari P. 2018. Morphological characteristics of Purnathadi and Ellichpuri Strain of Nagpuri. Adv Life Sci. 4:4531–4537.

Vandre RK, Joshi S, Thakur MS, Tantia MS, Sharma R, Singh AP, Pandey A, Khare V, Kumar A, Sharma M, Khan A. 2022. Identification and morphological characterization of buffaloes in the Mahakaushal region of Madhya Pradesh. Indian J Anim Sci. 92:318–322. DOI:10.56093/ ijans.v92i3.122262.

Villamor LP, Cuanang AJE, Cailipan TPC, Paraguas AM, Flores EB, Villanueva MA, Balbin MM, Belotindos LP, Rellin FT. 2024. Population Structure, morphology, and health assessment of Philippine Swamp Buffalo (*Bubalus kerabau*, Fitzinger, 1860) in Calayan Island, Cagayan. Ruminants. 4:556–564. DOI:10.3390/ruminants4040 039.

Vohra V, Niranjan SK, Mishra AK, Jamuna V, Chopra A, Sharma N, Jeong DK. 2015. Phenotypic characterization and multivariate analysis to explain body conformation in lesser-known buffalo (*Bubalus bubalis*) from North India. AJAS. 28:311–317. DOI:10.5 713/ajas.14.0451.

Vohra V, Singh M, Das R, Chopra A, Kataria RS. 2017. Multivariate analysis of biometric traits and their shared variance in Chhattisgarhi buffalo. Indian J Anim Sci. 87:864–870. DOI:10.56093/ijans.v87i7.72255.

Wilastra AS, Gushairiyanto G, Erina S, Depison D. 2021. Analisis jarak genetik Sapi Bali pada Tiga Kecamatan di Kabupaten Merangin Provinsi Jambi. J Peternak. 18:1–12. DOI:10.24014/jupet.v18i1.10331.

Yadav DK, Vijh RK. 2022. Morphological standard of Murrah Buffalo of India based on multivariate analysis of the phenotypic traits. Indian J Anim Res. 56:109–115. DOI:10.18805/IJAR.B-4313.

Yeasmin MN, Khandokar MAMY, Tanni TSJ, Begum S. 2016. Comparative study of the morphometric characteristics of buffaloes in Institutional Herds of Bangladesh. Int J Innov Sci Eng Technol. 3:715–718.

Yendraliza, Rodiallah M, Zumarni, Elfawati, Hidayati, Kusnadi. 2021. Reproduction performance, morphometric and structural population of kuntu buffalo (*Bubalis bubalis* merr) in Kampar district, Riau, Indonesia. Biodiv. 22:3370–3377. DOI:10.13057/biod iv/d220644.

Yulianty S, Gede Soma I, Nengah Wandia I. 2016. Keragaman Fenotipe Kerbau Lumpur (*Bubalus bubalis*) di Kabupaten Jembrana Bali: Warna Kulit dan pusaran rambut. Indones Med Veterinus. 5:119–128.

Yunusa AJ, Salako AE, Oladejo OA. 2013. Morphometric characterization of Nigerian indigenous sheep using multifactorial discriminant analysis. Int J Biodivers Conserv. 5:661–665. DOI:10.5897/IJBC2013.0592.

Yusnizar Y, Wilbe M, Herlino AO, Sumantri C, Noor RR, Boediono A, Andersson L, Andersson G. 2015. Microphthalmia-associated transcription factor mutations are associated with white-spotted coat color in swamp buffalo. Anim Genet. 46:676–682. DOI:10.1111 /age.12334.

Zhang Y, Colli L, Barker JSF. 2020. Asian water buffalo: domestication, history and genetics. Anim Genet. 51:177–191. DOI:10.1111/age.12911.

Identification of Protein A and Capsular Structures in *Staphylococcus aureus* Isolates from Milk of Cows with Subclinical Mastitis

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ABSTRAK

Putri RY, Windria S, Cahyadi AI. 2025. Identifikasi Struktur Protein A dan Capsular pada isolate *Staphylococcus aureus* susu sapi dengan mastitis subklinis. JITV 30(2): 82-91. DOI: http://dx.doi.org/10.14334/jitv.v30i2.3471.

Staphylococcus aureus merupakan salah satu penyebab masalah utama mastitis subklinis. Bakteri ini merupakan kelompok mikrobiota normal dan bisa menjadi bakteri patogen, karena kemampuannya menghasilkan toksin, invasif, dan ketahanan terhadap agen antimikroba. Proses patogenisitas *Staphylococcus aureus* salah satunya melibatkan faktor virulensi yang berada pada pemukaan bakteri dengan cara menghambat proses fagositosis, seperti protein A dan kapsul polisakarida. Identifikasi keberadaan protein A dan kapsul polisakarida dilakukan pada 28 isolat *Staphylococcus aureus* yang berasal dari bahan biologis tersimpan (BBT) dan diisolasi dari sapi penderita mastitis subklinis dengan hasil *California Mastitis Test* (CMT) ++ (positif 2). Keberadaan protein A dapat diidentifikasi menggunakan metode *serum soft agar* (SSA), sedangkan keberadaan kapsul polisakarida dapat diidentifikasikan menggunakan metode *salt aggregation test* (SAT). enelitian ini dilakukan untuk mendeteksi keberadaan faktor virulensi keduanya secara fenotipik. Hasil uji identifikasi protein A pada metode SSA menghasilkan 25 dari 28 isolat positif protein A dan kapsul polisakarida pada metode SAT menghasilkan seluruh isolat positif kapsul. Maka dari itu, kedua faktor virulensi tersebut dapat berkontribusi pada patogenisitas.

Kata Kunci: Kapsul, Polisakarida, Protein A, *Staphylococcus aureus*, Mastitis Subklinis, Virulensi

ABSTRACT

Putri RY, Windria S, Cahyadi AI. 2025. Identification of protein A and capsular structures in *Staphylococcus aureus* isolates from milk of cows with sublinical mastitis. JITV 30(2):82-91. DOI: http://dx.doi.org/10.14334/jitv.v30i2.3471.

Staphylococcus aureus is a primary etiological agent of subclinical bovine mastitis. While commonly part of the normal microbiota, it can transition into a pathogen through its capacity to produce toxins, invade host tissues, and resist antimicrobial agents. Its virulence is primarily linked to surface-expressed factors such as Protein A and polysaccharide capsules, which facilitate immune evasion by inhibiting phagocytosis. In this study, 28 *S. aureus* isolates were obtained from preserved biological materials (BBT) and assessed for the presence of Protein A and capsular structures. Protein A was identified using the serum soft agar (SSA) method, and polysaccharide capsules were detected via the salt aggregation test (SAT). Results showed that 25 of the 28 isolates expressed Protein A, while all exhibited capsule formation. These findings suggest that both virulence factors play a crucial role in the pathogenic potential of *S. aureus* in subclinical mastitis.

Key Words: Capsule, Polysaccharide, Protein A, *Staphylococcus aureus*, Subclinical Mastitis, Virulency

INTRODUCTION

Staphylococcus aureus is an infectious agent with high mortality and morbidity rates (Cheung et al. 2021). According to the study by Rao et al. (2023), livestock in India, especially in the Madurai district of Tamil Nadu, has recently experienced an increasing prevalence of *S. aureus* bacterial infections, with prevalence rates of approximately 42.63% in cattle and 28.57% in small ruminants. *Staphylococcus aureus* is a significant cause of subclinical mastitis (Windria et al. 2023; Ayuti et al. 2023). This statement is supported by the study conducted by Belay et al. (2022), which shows that bacterial pathogens isolated from mastitis cases are predominantly *Staphylococcus aureus* (42.6%), followed by *Streptococcus* spp. (26.2%) and other *Staphylococcus* species (26.2%). The incidence of *Staphylococcus aureus* infection has become a significant and critical issue in the dairy cattle industry (Pumipuntu et al. 2017).

Staphylococcus aureus infections are characterized by prolonged healing processes due to the persistence of the bacterium and its ability to evade the host immune system (Zhu et al. 2024). According to Windy et al. (2023), *S. aureus* exhibits complex pathogenicity within the host, facilitated by its virulence factors. The activity of these virulence factors is regulated by the accessory

gene regulator (*agr*) and the alternative transcriptional sigma factor SigB. The virulence factors of *S. aureus* include surface cell components and secreted proteins (Jenul & Horswill, 2019). Surface cell components, such as polysaccharide capsules and protein A, play a crucial role during the exponential growth phase by promoting bacterial adhesion and evading the host immune response (Wang & Muir 2016).

Protein A and polysaccharide capsules are the key virulence factors of *S. aureus*, playing crucial roles in evading the host immune response. Protein A inhibits opsonophagocytosis, acts as a B cell superantigen, and mediates inflammation. It interferes with the recognition of bacteria by phagocytic cells through its interaction with IgG (Fcγ immunoglobulin G), disrupting the opsonization process (Thammavongsa et al. 2015). Polysaccharide capsules further enhance immune evasion by obstructing the attachment of C3b to the bacterial surface, thereby inhibiting complement activation and phagocytosis. They also promote bacterial colonization during the adhesion phase, facilitate biofilm formation, and contribute to biofilm dispersion (Gao et al. 2024). The combined actions of protein A and polysaccharide capsules significantly enhance the pathogenicity of *S. aureus* by impeding phagocytosis (Husna 2018).

The identification of both virulence factors is necessary to determine their presence, as insufficient studies have been carried out on this topic. *S. aureus-producing* Protein A can be detected qualitatively using the serum soft agar (SSA) method (Evan et al. 2021), while polysaccharide capsules can be detected using the salt aggregation test (SAT) method (Khusnan & Kusmanto 2019). Both methods aim to determine the presence of virulence factors on the bacterial surface that contribute to the pathogenicity of subclinical mastitis. This study aims to identify *S. aureus* bacteria that produce the virulence factors Protein A and polysaccharide capsules.

MATERIALS AND METHODS

Sample

The samples used in this study are biological materials, specifically 28 preserved isolates of *Staphylococcus aureus*. The samples were derived from the milk of dairy cows diagnosed with mastitis subclinis, confirmed by a positive result (2++) on the California Mastitis Test (CMT). The isolates used as samples are preserved biological materials in the Microbiology Laboratory of the Faculty of Medicine, Universitas Padjadjaran, preserved in 30% glycerol medium at -20°C.

Re-identification of preserved isolates

Staphylococcus aureus was cultured on a blood agar plate (BAP) to observe colonies with a round shape and white to golden color. The procedure for culturing on BAP involved sampling with a loop, followed by incubation for 24 hours at 37°C. A hemolysis zone will form around the bacterial colonies, as the pathogenic bacteria produce a hemolysin toxin that causes lysis of the cytoplasmic membrane in the blood (Windria et al. 2023).

The Gram staining procedure involves preparing a smear of the sample on a glass slide, inoculated from BAP media using a loop, and then heating it with a Bunsen flame. Then, the smear was stained with crystal violet, allowed to stand for 2 minutes, and rinsed with water. Next, Lugol's iodine solution is applied to the smear, left for 30 seconds, and rinsed with water. The smear is then dipped in 96% alcohol to decolorize the stain, followed by immediate rinsing with water. Finally, the smear is counterstained with safranin, left for 2 minutes, and rinsed with water. A drop of immersion oil is applied to the dried smear, and the sample is observed under a microscope at 100x Magnification. *Staphylococcus aureus* is a Gram-positive bacterium characterized by its purple color (forming grape-like clusters) and coccal shape (Hayati et al. 2019).

Bacterial isolation on mannitol salt agar (MSA) was performed by taking a single loop of the sample and inoculating it onto the mannitol medium, followed by incubation at 37°C for 24 hours. The color change of *Staphylococcus aureus* from red to yellow is attributed to its ability to ferment mannitol (Thakur et al. 2017; Darmawi et al., 2019).

The coagulase test is performed to detect the presence of the coagulase enzyme. The test was conducted by inoculating bacteria into 1 mL of Nutrient Broth using a loop, followed by incubation at 37°C for 24 hours. Subsequently, rabbit plasma containing citrate was added to the Nutrient Broth with the bacteria, and the mixture was incubated again for 24 hours. The presence of clots at the bottom of the Eppendorf tube indicates a positive result (Hayati et al. 2019).

The catalase test is a method used to differentiate between the genera of *Staphylococcus* and *Streptococcus*. The catalase test was performed by taking a bacterial colony from MSA media using a loop and placing it on a glass slide. Then, 1–2 drops of H_2O_2 were added to the slide and mixed with the bacteria. The presence of gas bubbles (O_2) indicates a positive result (Yanto et al. 2021).

The DNase test is used to identify pathogenic *Staphylococcus* species, such as *Staphylococcus aureus*. The procedure begins with inoculating the culture onto a DNase agar plate and incubating at 37°C. After incubation, the agar plate is flooded with HCl and left for

approximately 5 minutes. The formation of a clear zone around the colonies indicates a positive result (Karimela et al. 2018).

Identification of Protein A Presence by Serum Soft Agar (SSA) Method

The test procedure involves inoculating bacteria into Todd Hewitt Broth (THB) medium and incubating it at 37°C for 18–24 hours. Soft agar (SA) preparation consists of 0.15% agar base in 10 mL of Brain Heart Infusion (BHI) medium. The soft agar (SA) is heated until the liquid becomes homogeneous and then cooled to 37–40°C. Bacteria from the THB medium are collected using a loop, inoculated into 10 mL of physiological NaCl, and homogenized until the bacterial cells are evenly mixed. Next, 100 µL of rabbit serum is added to the prepared soft agar (SA). A bacterial suspension from the THB medium is then transferred using a loop into the serum soft agar (SSA) mixture, homogenized with a vortex, and incubated at 37°C for 18–24 hours. The formation of compact colonies in the tube indicates a positive result (Djannatun et al. 2016).

Identification of Polysaccharide Capsule Presence by Salt Aggregation Test (SAT)

The test begins by inoculating bacteria into 5 mL of Brain Heart Infusion (BHI) medium and incubating at 37°C for 24 hours. The culture is vortexed and transferred into a tube, followed by centrifugation for 5 minutes at 5000 rpm. The supernatant is discarded, and the resulting pellet is washed three times with Phosphate Buffer Saline (PBS). The bacterial suspension in BHI is adjusted to a concentration of 10^8 cells/mL by matching it to a $BaSO_4$ standard. Once the desired concentration is achieved, 50 µL of the bacterial suspension is mixed with 50 µL of ammonium sulfate at concentrations of 1.2 M, 1.6 M, 2 M, 2.4 M, and 3.2 M on a glass slide, then homogenized using a sterile toothpick. The absence of aggregation on the glass slide will identify *Staphylococcus aureus* with polysaccharide capsules (Khusnan & Kusmanto 2019).

Data analysis

The presence of virulence factors, such as Protein A and polysaccharide capsules, in *Staphylococcus aureus* bacteria is qualitatively detected using the serum soft agar (SSA) method and the salt aggregation test (SAT), which are performed after re-identification of preserved bacterial isolates. The data obtained from these methods show phenotypic changes in the media used. The research results are presented in a table that contains the positive and negative outcomes of the tests, along with an interpretation of their characteristics. Data analysis will be conducted descriptively to describe the results.

Ethical Approval

This study received ethical approval from the Research Ethics Committee of the Faculty of Medicine (Approval No. 903/UN6.KEP/EC/2024).

RESULT AND DISCUSSION

Re-identification of preserved isolates

Staphylococcus aureus cultured on a blood agar plate (BAP) medium showed that 24 isolates exhibited β-hemolysin activity, while four isolates exhibited α-hemolysin activity (Table 1). The characteristics of hemolysis types can be observed based on the zones formed (Sodiq et al. 2019). β-hemolysis produces a clear zone, referred to as complete hemolysis (Figure 1A), while α-hemolysis forms a dark zone with partial clearing, referred to as partial hemolysis (Figure 1B). *Staphylococcus aureus* can exhibit four types of hemolysins: β, α, γ, and δ (Artursson et al. 2016; Turista & Puspitasari 2019; Divyakolu et al 2019; Wang et al. 2020). According to Turista & Puspitasari (2019), β and α-hemolysins are the most critical determinants in the pathogenic process of *Staphylococcus aureus*. Hemolysins exert cytotoxic effects, inducing cell lysis (Pakshir et al., 2017). Specifically, these bacteria contribute to mastitis by exacerbating necrosis in mammary gland tissues (Pérez et al. 2020; Campos et al. 2022). According to Abril et al. (2020), hemolysin in *S. aureus* plays a dominant role in the development of subclinical mastitis in dairy cows. Hemolysin is responsible for inflammation and injuries to the mammary gland epithelium, which increases tissue loss and leads to the manifestation of mastitis (Demontier et al. 2020).

The results of testing 28 isolates on mannitol salt agar (MSA) showed that 25 isolates produced positive results, while 3 isolates were negative (Table 1). Similar findings were reported in a study by Santos et al. (2015), which noted that 9 out of 59 *S. aureus* isolates yielded negative MSA results. Positive results are characterized by a color change from red to yellow (Figure 1B (a)), while negative results retain the red color (Figure 1B (b)). The color change occurs due to the fermentation of mannitol into organic acids, which causes the pH indicator phenol red in the medium to change. *Staphylococcus aureus* possesses an enzyme critical for mannitol metabolism, namely Mannitol-1-phosphate dehydrogenase (M1PDH) (Nguyen et al. 2019).

In this study, the results of Gram staining indicated that all isolates exhibited the characteristic traits of *S.*

Table 1. Re-identification results of 28 isolates preserved from the milk of dairy cows causing subclinical mastitis with CMT results 2++ (positive 2)

No.	Hemolysis Type	Gram staining	MSA	Catalase	Coagulase	DNAse	Interpretation
1.	β	coccus +	+	+	+	+	*S. aureus*
2.	α	coccus +	+	+	+	+	*S. aureus*
3.	β	coccus +	+	+	+	+	*S. aureus*
4.	β	coccus +	+	+	+	+	*S. aureus*
5.	β	coccus +	+	+	+	+	*S. aureus*
6.	β	coccus +	+	+	+	+	*S. aureus*
7.	β	coccus +	+	+	+	+	*S. aureus*
8.	β	coccus +	+	+	+	+	*S. aureus*
9.	β	coccus +	-	+	+	+	*S. aureus*
10.	β	coccus +	+	+	+	+	*S. aureus*
11.	β	coccus +	+	+	+	+	*S. aureus*
12.	β	coccus +	+	+	+	+	*S. aureus*
13.	α	coccus +	+	+	+	+	*S. aureus*
14.	β	coccus +	-	+	+	+	*S. aureus*
15.	β	coccus +	-	+	+	+	*S. aureus*
16.	β	coccus +	+	+	+	+	*S. aureus*
17.	β	coccus +	+	+	+	-	*S. aureus*
18.	α	coccus +	+	+	+	+	*S. aureus*
19.	β	coccus +	+	+	+	-	*S. aureus*
20.	α	coccus +	+	+	+	+	*S. aureus*
21.	β	coccus +	+	+	+	+	*S. aureus*
22.	β	coccus +	+	+	+	+	*S. aureus*
23.	β	coccus +	+	+	+	+	*S. aureus*
24.	β	coccus +	+	+	+	+	*S. aureus*
25.	β	coccus +	+	+	+	+	*S. aureus*
26.	β	coccus +	+	+	+	+	*S. aureus*
27.	β	coccus +	+	+	+	+	*S. aureus*
28.	β	coccus +	+	+	+	+	*S. aureus*
Total	α = 4 β = 24	28	+ = 25 - = 3	28	28	+ = 26 - = 2	

α= Alpha, β= Beta,γ= Gamma, Coccus += Gram Positive, += Positive, - = Negative

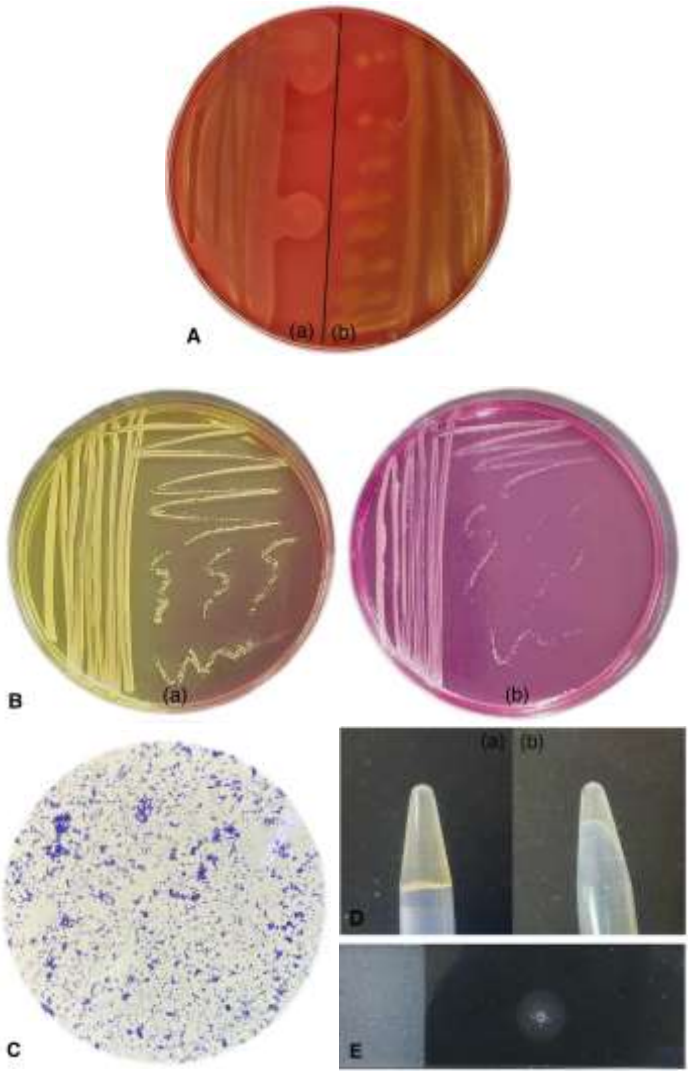

Figure 1. Identification Results of Staphylococcus aureus. (A) BAP Showing Hemolysis Zones (a) β-hemolysis (b) α-hemolysis, (B) MSA Showing (a) Positive mannitol fermentation (b) Negative mannitol fermentation, (C) Gram staining Results at 100x Magnification, (D) Coagulase Test (a) Clump formation (b) No clump formation, (E) Catalase Test: Presence of gas bubbles, (F) DNase Test (a) Presence of a clear zone around the colony (b) No clear zone formation. (Personal Documentation)

aureus, including a coccoid shape, a clustered arrangement, and a purple coloration (Figure 1C). These findings are consistent with those reported by Darmawi et al. (2019), which described similar traits and categorized *S. aureus* as a Gram-positive bacterium. The bacterial cell wall consists of layers of peptidoglycan, lipoteichoic acid (LTA), wall teichoic acids (WTA), and surface proteins (Wang et al. 2022). The ability to retain the stain is attributed to the thick peptidoglycan layer, which dehydrates upon alcohol exposure, causing the cell wall pores to close and trap the crystal violet dye within the cell (Yanto et al. 2021). Gram-positive bacteria such as *Staphylococcus aureus* tend to elicit a slower immune and inflammatory response. However, in mastitis cases, they are more likely to cause chronic infections compared to Gram-negative bacteria (Günther et al. 2017).

Staphylococcus aureus has a clotting factor called protein coagulase (*coa*). The results of this study showed that 28 samples produced the coagulase enzyme, resulting in the formation of a clot at the bottom of the Eppendorf tube (Figure 1D(a)). The process of interaction between the host cell plasma and coagulase activates thrombin to form fibrin, a clotting factor (Hayati et al. 2019). The clotting factor formed provides an opportunity for *S. aureus* bacteria to evade the immune response, as the immune system does not detect the fibrin formed, thereby preventing the phagocytosis process (Crosby et al. 2016). Staphylococcus aureus, which causes mastitis, produces coagulase as its virulence factor (Suwito et al. 2024).

The catalase test results showed that all isolates were positive for catalase enzyme production, as indicated by the formation of gas bubbles (Figure 1E). These findings

align with studies by Azis et al. (2022), which demonstrated that *Staphylococcus aureus* possessing the catalase enzyme produces gas bubbles in the test. This enzyme breaks down hydrogen peroxide (H_2O_2) into oxygen (O_2) and water, providing bacterial defense against reactive oxygen species (ROS) (Yanto et al. 2021). Catalase is one of the factors that enable bacteria to survive during immune responses (Karimela et al. 2019).

Staphylococcus aureus produces an extracellular enzyme known as deoxyribonuclease (DNase) (Subathra et al. 2016). This study yielded positive DNase test results in 25 isolates and negative results in 3 isolates. Positive results were indicated by the formation of a clear zone around the colonies, while negative results showed no clear zone (Figure 1F). These findings align with the study conducted by Pumipuntu et al. (2017), which reported 26 positive samples and 2 negative samples. Negative results in this test may indicate the occurrence of false negatives (Windria et al. 2023), consistent with the findings of Pumipuntu et al. (2017), who also observed false negatives in DNase tests.

Mastitis is an inflammation of the mammary gland, characterized by its complexity, particularly in terms of origin, severity, and outcomes (Thompson-Crispi et al., 2014; Saleem et al., 2024). According to Haxhiaj et al. (2022) and Huma et al. (2022), *Staphylococcus aureus* is a common pathogenic bacterium responsible for mastitis.

Positive results occur because Protein A interacts by forming bonds with the Fc receptor on IgG, which is found in various mammalian species (Lestari & Salasia 2017). The compact colonies formed on SSA can provide insight into the persistence and chronicity of this infection in subclinical mastitis (Ningrum et al. 2016). Negative results have two possible explanations: either the bacteria lack Protein A, or Protein A is present but concealed by the bacterial capsule (Lestari & Salasia 2017).

Factors affecting the results of the SSA test include the dilution process using 9% NaCl and the homogenization process during vortexing, as the SSA method is sensitive to the homogenization process. The release activity of Protein A on the surface of

In this study, the identification of polysaccharide capsules using the salt aggregation test (SAT) showed that all 28 isolates were positive for polysaccharide capsules. Positive results were indicated by the absence of aggregation and hydrophilic properties at specific concentrations of ammonium sulfate. A study by

S. aureus possesses various virulence factors that contribute to subclinical mastitis infection and support pathogenesis in response to the host's immune system (Pérez et al. 2022)

Identification of the presence of protein A and polysaccharide capsules

Identification of Protein A using the serum soft agar (SSA) method in 28 phenotypically cultured isolates resulted in 25 positive isolates and 3 negative isolates for Protein A. Positive results were indicated by compact colonies. In contrast, negative results showed diffuse colonies (Figure 6). In the study by Djannatun et al. (2016), 7 out of 15 *Staphylococcus aureus* isolates showed positive results, characterized by a change in colony morphology from diffuse to compact following interaction with rabbit serum on SSA.

Staphylococcus aureus is influenced by the presence of sortase A (srtA). If sortase A is absent, the attachment of surface proteins, including Protein A, will be disrupted (Ningrum et al. 2016).

The virulence factor Protein A is classified as a structural surface component that contributes to the progression of mastitis (initiating infection in the mammary gland) caused by *Staphylococcus aureus* (Tegegne et al. 2021). The interaction between Protein A and the Fc region of IgG interferes with the immune response by inhibiting opsonization and phagocytosis. This response creates favorable conditions for *Staphylococcus aureus* to proliferate and cause infection (Lestari & Salasia, 2017). Protein A is responsible for intramammary infection (Demontier et al. 2020). Khusnan et al. (2016) found that *Staphylococcus aureus* isolates were 85.7% hydrophilic and 14.3% hydrophobic.

The use of ammonium sulfate at specific concentrations serves as an indicator for determining the degree of hydrophobicity, with its activity determined by

Table 2. Identification results of the presence of protein A and polysaccharide capsules in *Staphylococcus aureus* bacteria from the milk of bovine subclinical mastitis

	Protein A in serum soft agar (SSA)	Polysaccharide Capsules in salt aggregation test (SAT)
Positive	25	28
Negative	3	0
Total	28	

Protein A-positive isolates exhibit compact colony morphology, while polysaccharide capsule-positive isolates are hydrophilic, with aggregation occurring at concentrations >2 M

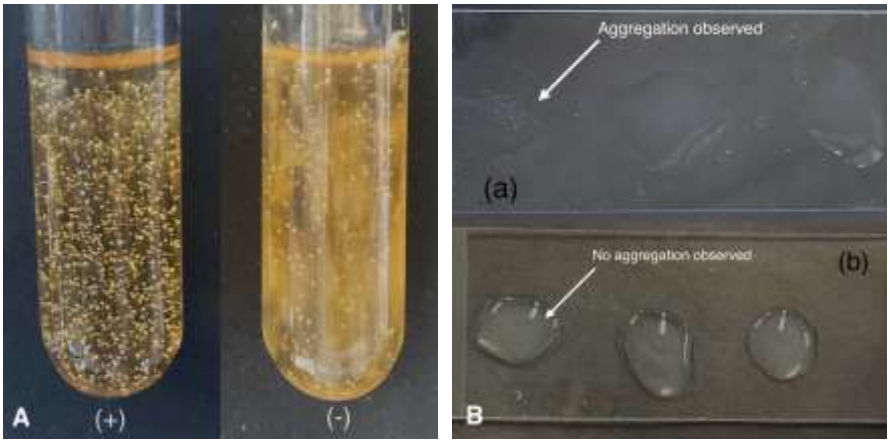

Figure 2. Identification results on *Staphylococcus aureus* (A) The presence of protein A (+) Positive for protein A with compact colonies (-) Negative for protein A with diffuse colonies, (B) The presence of Polysaccharide Capsule (a) Aggregation was observed (b) No aggregation was observed. (Personal Documentation)

water attraction and protein precipitation (Prasiddhanti & Wahyuni 2015). Farid et al. (2021) further explained that the degree of hydrophobicity is categorized into two types: hydrophilic, if aggregation occurs at ammonium sulfate concentrations greater than 2.0 M, and hydrophobic, if aggregation occurs at ammonium sulfate concentrations between 1 and 2 M. All isolates showed positive results for polysaccharide capsules, as none formed aggregates during observation on a glass slide. Three isolates formed aggregates only at a concentration of 3.2 M (Figure 2B(a)), while no aggregation was observed at lower concentrations. According to the explained theory, if aggregation occurs at concentrations greater than 2 M, the isolate is classified as hydrophilic, indicating that the *Staphylococcus aureus* bacteria in the isolates from this study possess capsules on their surface (Khusnan & Kusmanto 2019).

There is a correlation between the hydrophobicity properties and virulence of *S. aureus* bacteria, where hydrophilic bacteria are more pathogenic compared to hydrophobic ones (Khusnan & Kusmanto 2019). The polysaccharide capsule of *S. aureus* is responsible for the adhesin process, or the attachment of the bacterium to the host, particularly to epithelial cells (Khusnan & Kusmanto 2019). According to Salimena et al. (2016), the polysaccharide capsule is responsible for chronic and persistent infections in bovine mastitis. The polysaccharide capsule facilitates the initial adhesion/attachment process of biofilm formation (Vidlund et al. 2021). This biofilm activity causes *S. aureus* to further spread the infection process in mastitis cases.

CONCLUSION

The presence of virulence factors, including protein A and polysaccharide capsules, was identified phenotypically in 28 preserved isolates of *Staphylococcus aureus* bacteria. *Staphylococcus aureus* in this study has a protein A virulence factor (25 isolates out of 28 isolates) and a polysaccharide capsule (28 isolates). *Staphylococcus aureus* bacteria, identified by the virulence factor protein A and polysaccharide capsules on their surface, are more pathogenic. The resulting impact is the expression of pathogenicity. Both virulence factors contribute to mastitis infection in cows.

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REFERENCES

G. Abril A, G. Villa T, Barros-Velázquez J, Cañas B, Sánchez-Pérez A, Calo-Mata P, Carrera M. 2020. *Staphylococcus aureus* Exotoxins and Their Detection in the Dairy Industry and Mastitis. Toxins (Basel). 12:537. DOI:10.3390/toxins12090537.

Artursson K, Söderlund R, Liu L, Monecke S, Schelin J. 2016. Genotyping of *Staphylococcus aureus* in bovine mastitis and correlation to phenotypic characteristics. Vet Microbiol. 193:156–161. DOI:10.1016/j.vetmic.2016.08.012.

Ayuti SR, Hidayati WN, Admi M, Rosmaidar R, Zainuddin Z, Hennivanda H, Makmur A. 2023. Isolasi dan identifikasi bakteri gram positif *Staphylococcus aureus* dan micrococcus pada peternakan sapi yang terindikasi mastitis. JPI. 25:98. DOI:10.25077/jpi.25.1.98-109.2023

Aziz F, Lestari FB, Indarjulianto S, Fitriana F. 2022. Identifikasi dan karakterisasi resistensi antibiotik terduga *Staphylococcus aureus* pada susu mastitis subklinis asal sapi perah di Kelompok Ternak Sedyo Mulyo, Pakem,

Sleman Yogyakarta. J Ilmu Peternak Vet Trop. 12. DOI:10.46549/jipvet.v12i1.226.

Belay N, Mohammed N, Seyoum W. 2022. Bovine mastitis: prevalence, risk factors, and bacterial pathogens isolated in lactating cows in Gamo Zone, Southern Ethiopia. Vet Med Res Reports. Volume 13:9–19. DOI:10.2147/VMRR.S344024.

Campos B, Pickering AC, Rocha LS, Aguilar AP, Fabres-Klein MH, de Oliveira Mendes TA, Fitzgerald JR, de Oliveira Barros Ribon A. 2022. Diversity and pathogenesis of *Staphylococcus aureus* from bovine mastitis: current understanding and future perspectives. BMC Vet Res. 18:1–16. DOI:10.1186/s12917-022-03197-5.

Crosby HA, Kwiecinski J, Horswill AR. 2016. *Staphylococcus aureus* aggregation and coagulation mechanisms, and their function in host–pathogen interactions. 1–41. DOI:10.1016/bs.aambs.2016.07.018.

Darmawi D, Zahra AF, Salim MN, Dewi M, Abrar M, Syafruddin S, Adam M. 2019. 6. Isolation, identification and sensitivity test of *Staphylococcus aureus* on post surgery wound of local dogs (*Canis familiaris*). J Med Vet. 13:37–46. DOI:10.21157/j.med.vet.v13i1.4122.

Demontier E, Dubé-Duquette A, Brouillette E, Larose A, Ster C, Lucier J-F, Rodrigue S, Park S, Jung D, Ruffini J, et al. 2021. Relative virulence of *Staphylococcus aureus* bovine mastitis strains representing the main Canadian spa types and clonal complexes as determined using in vitro and in vivo mastitis models. J Dairy Sci. 104:11904–11921. DOI:10.3168/jds.2020-19904.

Divyakolu S, Chikkala R, Ratnakar KS, Sritharan V. 2019. Hemolysins of *Staphylococcus*: an update on their biology, role in pathogenesis, and as targets for anti-virulence therapy. Adv Infect Dis. 09:80–104. DOI:10.4236/aid.2019.92007.

Djannatun T, Wibawan IWT, Kunci K. 2016. Keberadaan protein A pada permukaan sel bakteri *Staphylococcus aureus* menggunakan teknik "Serum Soft Agar" Jj Kedokteran Yarsi. 24:148–156.

Evan Y, Indrawati A, Pasaribu FH. 2021. Pengembangan uji cepat metode koaglutinasi untuk mendeteksi antigen *Vibrio parahaemolyticus* penyebab penyakit vibriosis pada Udang Vaname (*Litopenaeus vannamei*). Biodidaktika Biodidaktika. 16. DOI:10.30870/biodidaktika.v16i1.10784 .

Lestari FB, Salasia SIO. 2017. Karakterisasi *Staphylococcus aureus* isolat susu sapi perah berdasar keberadaan protein-A pada Media Serum Soft Agar terhadap aktivitas fagositosis secara *in vitro*. J Sain Vet. 33:2. DOI:10.22146/jsv.17888.

Nguyen T, Kim T, Ta HM, Yeo WS, Choi J, Mizar P, Lee SS, Bae T, Chaurasia AK, Kim KK. 2019. Targeting mannitol metabolism as an alternative antimicrobial strategy based on the structure-function study of mannitol-1-phosphate dehydrogenase in *Staphylococcus aureus.* Torres VJ, editor. MBio. 10. DOI:10.1128/mBio.02660-18.

Ningrum SG, Arnafia W, Oscarina S, Soejoedono RD, Latif H, Ashraf M, Wibawan IWT. 2016. Phenotypic and serotypic characterization of *Staphylococcus aureus* strains from subclinical mastitis cattle. J Vet. 17:112–118. DOI:10.19087/jveteriner.2016.17.1.112.

Pakshir K, Bordbar M, Zomorodian K, Nouraei H, Khodadadi H. 2017. Evaluation of CAMP-like effect, biofilm formation, and discrimination of *Candida africana* from vaginal Candida albicans species. J Pathog. 2017:1–5. DOI:10.1155/2017/7126258.

Pérez VKC, Custódio DAC, Silva EMM, de Oliveira J, Guimarães AS, Brito MAVP, Souza-Filho AF, Heinemann MB, Lage AP, Dorneles EMS. 2020. Virulence factors and antimicrobial resistance in *Staphylococcus aureus* isolated from bovine mastitis in Brazil. Brazilian J Microbiol. 51:2111–2122. DOI:10.1007/s42770-020-00363-5.

Prasiddhanti L, Wahyuni AETH. 2015. Karakter permukaan *Escherichia coli* yang diisolasi dari susu kambing Peranakan Ettawah yang berperan terhadap kemampuan adesi pada sel epitelium ambing. J Sain Vet. 33:29–41.

Pumipuntu N, Kulpeanprasit S, Santajit S, Tunyong W, Kong-ngoen T, Hinthong W, Indrawattana N. 2017. Screening method for *Staphylococcus aureus* identification in subclinical bovine mastitis from dairy farms. Vet World. 10:721–726. DOI:10.14202/vetworld.2017.721-726.

Rao RT, Madhavan V, Kumar P, Muniraj G, Sivakumar N, Kannan J. 2023. Epidemiology and zoonotic potential of Livestock-associated *Staphylococcus aureus* isolated at Tamil Nadu, India. BMC Microbiol. 23:1–12. DOI:10.1186/s12866-023-03024-3.

Saleem A, Saleem Bhat S, A. Omonijo F, A Ganai N, M. Ibeagha-Awemu E, Mudasir Ahmad S. 2024. Immunotherapy in mastitis: state of knowledge, research gaps and way forward. Vet Q. 44:1–23. DOI:10.1080/01652176.2024.2363626.

Saleem A, Saleem Bhat S, A. Omonijo F, A Ganai N, M. Ibeagha-Awemu E, Mudasir Ahmad S. 2024. Immunotherapy in mastitis: state of knowledge, research gaps and way forward. Vet Q. 44:1–23. DOI:10.1080/01652176.2024.2363626.

Salimena APS, Lange CC, Camussone C, Signorini M, Calvinho LF, Brito MAVP, Borges CAV, Guimarães AS, Ribeiro JB, Mendonça LC, Piccoli RH. 2016. Genotypic and phenotypic detection of capsular polysaccharide and biofilm formation in *Staphylococcus aureus* isolated from bovine milk collected from Brazilian dairy farms. Vet Res Commun. 40:97–106. DOI:10.1007/s11259-016-9658-5.

Santos DCM dos, Costa TM da, Rabello RF, Alves FA, Mondino SSB de. 2015. Mannitol-negative methicillin-resistant *Staphylococcus aureus* from nasal swab specimens in Brazil. Brazilian J Microbiol. 46:531–533. DOI:10.1590/S1517-838246220140179.

Sodiq AH, Setiawati MR, Santosa DA, Widayat D. 2019. Potensi mikroba asal mikroorganisme lokal dalam meningkatkan perkecambahan benih paprika. J Agroekoteknol. 11:214. DOI:10.33512/jur.agroekotetek.v11i2.7694.

Subathra Devi C, Mohanasrinivasan V, Subramaniam V, Parashar M, Vaishnavi B, Jemimah Naine S. 2016. Molecular characterization and potential assessment of extracellular DNase producing *Staphylococcus aureus* VITSV4 isolated from bovine milk. Iran J Sci Technol Trans Sci. 40:191–199. DOI:10.1007/s40995-016-0090-z.

Suwito W, Andriani, Wahyuni AETH, Nugroho WS, Sumiarto B. 2024. Coagulase and clumping factor in *Staphylococcus aureus* from Ettawa-Crossbreed goat (PE) mastitis in Yogyakarta Indonesia. 070026. DOI:10.1063/5.0184959

Tegegne DT, Mamo G, Waktole H, Messele YE. 2021. Molecular characterization of virulence factors in *Staphylococcus aureus* isolated from bovine subclinical mastitis in central Ethiopia. Ann Microbiol. 71:28. https://doi.org/10.1186/s13213-021-01639-3

Thakur P, Nayyar C, Tak V, Saigal K. 2017. Mannitol-fermenting and tube coagulase-negative *Staphylococcal* isolates: unraveling the diagnostic dilemma. J Lab Physicians. 9:065–066. DOI:10.4103/0974-2727.187926.

Thammavongsa V, Kim HK, Missiakas D, Schneewind O. 2015. S*taphylococcal* manipulation of host immune responses. Nat Rev Microbiol. 13:529–543. DOI:10.103 8/nrmicro3521.

Thompson-Crispi K, Atalla H, Miglior F, Mallard BA. 2014. Bovine mastitis: frontiers in immunogenetics. Front Immunol. 5. DOI:10.3389/fimmu.2014.00493.

Turista DDR, Puspitasari E. 2019. The growth of *Staphylococcus aureus* in the blood agar plate media of sheep blood and human blood groups A, B, AB, and O. J Teknol Lab. 8:1–7. DOI:10.29238/teknolabjournal.v 8i1.155.

Vidlund J, Deressa Gelalcha B, Swanson S, costa Fahrenholz I, Deason C, Downes C, Kerro Dego O. 2022. Pathogenesis, diagnosis, control, and prevention of bovine *Staphylococcal* mastitis. In: Mastit dairy cattle, sheep goats. IntechOpen. DOI:10.5772/intechopen. 101596.

Wang M, Buist G, van Dijl JM. 2022. *Staphylococcus aureus* cell wall maintenance – the multifaceted roles of peptidoglycan hydrolases in bacterial growth, fitness, and virulence. FEMS Microbiol Rev. 46. DOI:10.1093/femsre/fuac025.

Wang L-J, Yang X, Qian S-Y, Liu Y-C, Yao K-H, Dong F, Song W-Q. 2020. Identification of hemolytic activity and hemolytic genes of Methicillin-resistant *Staphylococcus aureus* isolated from Chinese children. Chin Med J (Engl). 133:88–90. DOI:10.1097/CM9.00000000000 00571.

Wang B, Muir TW. 2016. Regulation of virulence in *Staphylococcus aureus*: Molecular mechanisms and remaining puzzles. Cell Chem Biol. 23:214–224. DOI:10.1016/j.chembiol.2016.01.004.

Windria S, Cahyaningtyas AA, Cahyadi AI, Wiraswati HL, Ramadhanti J. 2023. Identifikasi fenotip dan genotip *Staphylococcus aureus* isolat asal susu sapi perah mastitis subklinis di wilayah Pamulihan, Kabupaten Sumedang Jawa Barat. J Sain Vet. 41:215. DOI:10.22146/jsv.76052.

Yanto RB, Satriawan NE, Suryani A. 2021. Identifikasi dan resistensi *Staphylococcus aureus* terhadap antibiotik (Chloramphenicol dan Cefotaxime Sodium) dari pus infeksi piogenik di Puskesmas Proppo. J Kim Ris. 6:154. DOI:10.20473/jkr.v6i2.30694

Zhu Z, Hu Z, Li S, Fang R, Ono HK, Hu DL. 2024. Molecular characteristics and pathogenicity of *Staphylococcus aureus* exotoxins. Int J Mol Sci. 25. DOI:10.3390 /ijms25010395.

Strengthening Breeding Stock Supply: A Study of Community-Based Duck Farming in Magelang under Good Breeding Practice Guidelines

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ABSTRAK

Rahayu A, Hartatik T, Sasongko H, Guntoro B, Wihandoyo, Ariyadi B, Maharani D. 2025. Memperkuat pasokan bibit: kajian peternakan itik berbasis masyarakat di Magelang berdasarkan standar *Good Breeding Practice*. JITV 30(2):93-107. DOI:http//dx.doi.org/10.13443/jitv.v30i2.3528.

Rencana pelaksanaan *breeding* dianggap penting dalam meningkatkan produktivitas ternak. Penelitian ini bertujuan mengkaji (1) pelaksanaan breeding Itik Magelang dengan membandingkan *existing breeding* di masyarakat dengan standar *Good Breeding Practice* (GBP), dan (2) kelayakan Kabupaten Magelang sebagai sumber bibit. Penelitian melibatkan 30 peternak itik Magelang yang dipilih secara *purposive sampling*. Data dikumpulkan melalui wawancara dan dianalisis secara deskriptif. Variabel meliputi pemilihan bibit; prasarana dan sarana; kesehatan hewan; pelestarian fungsi lingkungan; sumber daya manusia; pendampingan dan pengawasan oleh pemerintah. Penilaian kelayakan menggunakan data primer dari peternak dan data sekunder dari Dinas Peternakan, dianalisis dengan skoring dan SWOT. Hasil penelitian diperoleh bahwa jika dibandingkan dengan aspek GBP maka aspek pada peternak itik di Magelang masih di bawah standar GBP terlihat dari *skoring* (skala 1-5) untuk pemilihan bibit sebesar 2,67; prasarana dan sarana 2,85; kesehatan hewan 2,67; pelestarian fungsi lingkungan 2,16; SDM 3,33; pendampingan dan pengawasan oleh pemerintah 3. Kelayakan Kabupaten Magelang sebagai sumber bibit ditunjukkan dari hasil analisis SWOT dengan nilai internal dan eksternal berturut-turut sebesar 1,55; 1,05. Hasil ini menempatkan Magelang pada kuadran I yang menunjukkan adanya kekuatan dan peluang untuk dikembangkan. Kesimpulannya, meskipun belum memenuhi standar GBP, Kabupaten Magelang masih menunjukan potensi yang kuat sebagai sumber bibit itik.

Kata Kunci: *Good Breeding Practice*, Itik Magelang, Kelayakan Wilayah, Sumber Bibit

ABSTRACT

Rahayu A, Hartatik T, Sasongko H, Guntoro B, Wihandoyo, Ariyadi B, Maharani D. 2025. Strengthening breeding stock supply: A study of community-based duck farming in Magelang under Good Breeding Practice guidelines. JITV 30(2): 93-107. DOI:http//dx.doi.org/10.13443/jitv.v30i2.3528.

Breeding implementation is crucial to improve livestock productivity. This study evaluated (1) Magelang duck breeding practices compared to Good Breeding Practice (GBP) standards, and (2) the feasibility of Magelang District as a breeding stock source. Thirty Magelang duck farmers were selected purposively. Data were collected through direct interviews and analyzed descriptively. Variables observed included breeding stock selection, infrastructure and facilities, animal health, preservation of environmental functions, human resources, government assistance, and supervision. A feasibility analysis was conducted using primary farmer data and secondary data from the Animal Husbandry Department, and assessed through scoring and SWOT analysis. Results showed that current practices remain below GBP standards, with average scores (scale 1-5) for breeding stock selection (2.50); infrastructure and facilities (2.85); animal health (2.67); environmental function preservation (2.16); human resources (3.33); government assistance and supervision (3,00). SWOT analysis indicated Magelang has potential as a breeding stock source, with internal and external scores of 1.55 and 1.05, placing it in Quadrant I (supporting growth strategies). In conclusion, although current practices have not fully met GBP standards, Magelang District still shows strong potential as a producer of quality duck breeding stock.

Key Words: Breeding Stock Source, Good Breeding Practice, Magelang Duck, Region's Feasibility

INTRODUCTION

Breeding is a series of cultivation activities to produce livestock breeding stock. A breed stock source area is an agroecosystem area that is not limited by government administrative boundaries and has the potential to develop breed stock of specific types, breeds, or strains of livestock (Peraturan Menteri Pertanian Republik Indonesia 2014). Based on the Decree of the Minister of Agriculture No. 701/Kpts/PD.410/2/2013 on the Determination of Magelang Duck Breeds, it is stated that Magelang ducks are local Indonesian duck breeds

that have been cultivated for generations, thus becoming a valuable resource of local Indonesian genetic diversity (Ricardo et al. 2024). It is hoped that Magelang ducks can become a national breed stock source, as the Minister of Agriculture has identified twenty-two breed stock source areas with twenty-four kinds of livestock commodities (Direktorat Jenderal Peternakan dan Kesehatan Hewan 2002) in Indonesia, and Magelang ducks are omitted. The decree on the Magelang duck breeds is expected to become a source area for Magelang ducks in the Magelang district and surrounding areas.

Magelang ducks are among the most productive local poultry for producing meat and eggs (Rahayu et al. 2022). Along with domestic activity, ducks make various adaptations to adapt to the new environment. Adaptations, such as very active behavior, either in the cage or outside the cage (Rahayu et al. 2020). Magelang ducks have a characteristic white necklace around their neck. These ducks act as a source of egg production, ranging from 48-70%. If they are intensively maintained, the production can reach 80% a notable improvement for Magelang ducks, which are typically rejected for use as meat ducks (Yuwono, 2012). Magelang ducks have a large body proportion that can reach 1.5 kg, with relatively high egg production and varied plumage colors compared to other local ducks (Rahayu et al. 2015). According to (Luthfiana et al. 2020), the difference between male and female Magelang ducks is evident in their posture, with male ducks being slimmer than female ducks. According to Rahayu et al. 2019), the body weight of Magelang ducks is approximately 1.9 kg, the body length is approximately 30.67 cm, the neck length is approximately 14.18 cm, and the chest circumference is approximately 39.09 cm. These ducks have been bred and raised for many years in the Magelang district and surrounding areas. However, many duck varieties in Indonesia are not considered pure breeds and still exhibit significant genetic and phenotypic diversity. This situation partly arises from the changing rearing practices, such as the herding system, which leads to random crossbreeding and potentially alters the genetic makeup of the duck population; this is evident in the wide variation observed in both body morphology and productivity levels. People tend to prefer raising ducks over native chickens due to their higher egg production (Rahayu et al. 2019). Therefore, it is essential to enhance breeding practices, both genetically and phenotypically, to produce high-quality breeding stock.

Ducks, once primarily raised for egg production, are now also valued for their meat. As their role expands, the demand for duck breeding stock has also grown. Duck farming in Indonesia remains mostly traditional and small-scale, with no specialized businesses focused on producing hatching eggs (Rahayu et al. 2020). To meet the demand for ducks as both egg and meat producers, it is essential to have high-quality hatching eggs available in sufficient quantities. Therefore, a breeding business is needed to produce screaming breed stock. The breeding stock source area is integral to the breeding program, which in Indonesia lags behind those in developed countries. Key challenges include inadequate farm infrastructure resulting from traditional methods, limited facilities, poor market access, and varying levels of farmer education. Additionally, there is a lack of long-term, government-supported breeding programs. Therefore, an effective implementation plan for breeding programs is necessary.

A breeding implementation plan is crucial for enhancing livestock productivity. To develop this plan, key information required encompasses breeding stock selection, infrastructure and facilities, animal health, environmental conservation, human resources, and government assistance and supervision. The current state of the breeding program in the community is suspected to be substandard and has not been formally evaluated. Additionally, the suitability of the Magelang district as a breeding source area remains unclear. There has been no in-depth research on the implementation of Magelang duck breeding, making it essential to study its practices in comparison to existing breeding and Good Breeding Practice (GBP) standards. Therefore, this study aims to (1) evaluate the breeding practices of Magelang duck farmers in comparison with Good Breeding Practice (GBP) standards; and (2) assess the feasibility of Magelang District as a breeding stock source based on SWOT analysis.

MATERIALS AND METHODS

Materials

The study is divided into two parts: (1) comparing existing breeding practices with GBP standards and (2) assessing the feasibility of the Magelang district as a source of breeding stock. This research was conducted for three months (June-August 2023) in Magelang Regency. The study involved 30 respondent farmers in Magelang. Interviews were conducted to gather data on the implementation of breeding among Magelang duck farmers. The questionnaire validation was carried out by obtaining expert opinions, specifically from the academic supervisor and experts in the fields of poultry farming and socio-economics, to review the drafted questionnaire. The expert assessment included: (1) clarity of language and sentences in each question item, (2) suitability of the questions with the research objectives, and (3) relevance and coverage of the content in each question item to the variables being studied. Suggestions and input from experts were utilized to revise the questionnaire, resulting in a more precise and more targeted document (Sugiyono, 2019). The revised

questionnaire was then tested on 5-10 respondents who had similar characteristics to the primary research respondents. This trial aimed to: (1) assess the extent to which respondents understood the meaning of each question, (2) identify whether there were any ambiguous or difficult-to-understand questions, and (3) estimate the time required to complete the questionnaire. Based on the validity test results, the questionnaire was further revised and refined before being used for data collection in the main study. This research phase took place at smallholder farms in the Magelang district and at the local Animal Husbandry Department.

Ethical approval

The Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, approved this experimental work (Approval Number: 005/EC-FKH/Eks./2023).

Methodology

Comparing existing breeding with GBP standards

Thirty farmers were interviewed in person, selected based on the criteria of having at least 10 years of breeding experience, raising ducks from day-old ducklings (DOD) to culling, and having a solid understanding of duck breeding practices. The survey took place among farmers in the Magelang district who specialize in raising Magelang ducks. The farmers were provided with a structured questionnaire designed to assess the breeding system of Magelang ducks within the community. The survey covered several variables, including breed stock selection, infrastructure and facilities, animal health, environmental preservation, human resources, and government assistance and supervision. The assessment data will be compared with GBP standards and analyzed using a SWOT analysis, as detailed in the data analysis section. Data from the farmer interviews were analyzed descriptively and scored according to GBP standards.

Feasibility of Magelang ducks as a viable source of breeding stock

The tools used to collect data on breeding practices included a structured questionnaire and stationery. The study utilized both primary and secondary data. Primary data was gathered through direct interviews with breeders in the field, while secondary data was collected from the local office. The data, presented in a scoring system from 1 to 5, was processed to determine weight and rating values. The scoring was based on the combination of strengths and weaknesses, as well as opportunities and threats. The rating of questionnaire data obtained was established through discussions with Magelang duck farmers in the Magelang district. The weight is derived from the balance of strengths and weaknesses, as well as opportunities and threats. The weight value is determined based on interviews conducted between researchers and Magelang duck farmers in Magelang District. The score for each aspect is then totaled and averaged by dividing it by the number of respondents. As stated by David (2009) livestock development strategies can be formulated using a SWOT analysis, which involves identifying internal and external factors summarized in the IFAS (Internal Factors Analysis Summary) and EFAS (External Factors Analysis Summary) matrices. According to (Rangkuti F, 2006), strategic factors can be identified by combining external strategic factors (opportunities and threats) with internal strategic factors (strengths and weaknesses) into a strategic factors analysis summary.

Data analysis

This study employs quantitative descriptive analysis to determine the score for each aspect of good breeding practice in Magelang duck farms. Data obtained from the questionnaire were processed to obtain an average score for each aspect, then interpreted based on categories (low, medium, high). Data were analyzed using descriptive quantitative analysis with Microsoft Excel and SPSS version 25 to calculate mean scores, frequency distributions, and to present the data in tables for more precise interpretation.

RESULTS AND DISCUSSION

Comparing existing breeding with GBP standards

The study examined the condition of Magelang duck farms across several aspects, including breeding stock selection, infrastructure and facilities, animal health, environmental preservation, human resources, and government assistance and supervision. These findings are detailed in the following sub-chapters. The study results were then compared to the GBP standard using a scoring system ranging from 1 to 5, where (1) indicates poor, (2) sufficient, (3) moderate, (4) good, and (5) very good.

Breeding stock selection

Breeding stock selection is a crucial element of any breeding program. The aspects involved in breeding stock selection encompass criteria, objectives, methods, and crossbreeding practices. The scoring outcomes for breeding stock selection at Magelang duck farms are presented in Table 1.

Table 1. Aspects of breed stock selection in Magelang duck farms, Magelang district

Aspect	Score	Description
a. Breedstock selection criteria	3.00	As per SNI, as many as 2 criteria
b. Purpose	3.94	Layers and broilers
c. Selection method	2.37	Not understand
Average	2.77	

Based on the results obtained by examining three aspects of breeding stock selection, the scores for breeding stock selection criteria, objectives, and selection methods were 3.00, 3.94, and 2.37, respectively, with an average score of 2.77; this is because several factors in GBP cannot be found directly in the field. For example, there should be several criteria for selecting minimum technical requirements (MTR or PTM : Persyaratan Teknis Minimal) breed stock; however, in the field, there are only two criteria: the age of 5-6 months, and a brownish fur color with light to dark brown variations, accompanied by a white collar on the neck. According to Keputusan Menteri Pertanian Republik Indonesia Nomor 701/Kpts/PD.410/2/2023, Magelang ducks are defined by both qualitative and quantitative traits. Qualitative characteristics include feather and eggshell color as well as body shape. Magelang ducks typically have brown feathers, ranging from light to dark brown or blackish, and may sometimes be entirely black. They also feature a distinctive white necklace around their neck. The eggshell color is bluish-green (Rahayu et al. 2015).

Male Magelang ducks have a slim body shape and maintain an upright posture when standing and walking, perpendicular to the ground. The body shape of female Magelang ducks is upright, and they do not incubate their eggs. Quantitative characteristics of Magelang ducks include male ducks weighing between 1.8 and 2.5 kg, while females weigh between 1.5 and 2.0 kg. The average weight of their eggs ranges from 60 to 70 grams, with hatching eggs weighing approximately 67.1±4.7 g. These ducks produce between 200 and 300 eggs per year, with a peak egg production rate of 55.1%. They reach sexual maturity at 5 to 6 months of age, have an egg production period of 9 to 10 months, and a feed conversion ratio of 4 to 5. The width of the white necklace around their neck ranges from 1 to 2 cm. The score for the objective is 3.94, which is based on the purpose of the Magelang duck itself, as determined by the criteria in the GBP scoring, specifically as a commercial egg and meat producer.

The purpose of raising Magelang ducks is for egg and meat production, where the ducks produced will lay eggs daily. After being culled, they will be sold for use as meat ducks. Therefore, a proper selection method is needed to achieve this goal. The selection method is important in achieving breeding goals. The scoring result for the selection method was 2.37, indicating that farmers still do not fully understand the selection methods. Selection can be carried out using both quantitative and molecular approaches. Quantitative selection involves measuring and comparing observable traits in livestock to identify those that are most beneficial to the breed.

On the other hand, molecular selection utilizes DNA markers. However, most breeders are familiar only with quantitative selection and have not engaged in molecular methods; many are even unfamiliar with the concept of molecular selection. Breeders typically practice crossbreeding within a single breed to preserve the original characteristics. According to Maharani et al. (2017), the objectives of crossbreeding are: (1) creating a new breed by combining traits from two or more breeds; (2) achieving heterosis, which aims to produce crossbred livestock with performance exceeding the average of the parent breeds; (3) developing a commercial cross; and (4) grading up, which involves crossing local livestock with imported studs using the backcross method to enhance the traits of the animals.

Based on the research results (Table 1), the average score for the breeding stock selection aspect among Magelang duck farmers was categorized as low. Most farmers did not perform optimal selection of breeding stock and tended to use ducklings hatched by themselves or purchased from local markets without considering genetic quality. This low breeding stock selection can be attributed to several factors (1) Lack of knowledge regarding superior breeding stock criteria. According to interviews, most farmers did not understand the importance of selecting breeding stock based on body weight, body conformation, beak and leg color, or the production history of the parents. They only chose ducklings that appeared healthy without considering their productivity traits. According to North & Bell (1990) selecting superior breeding stock based on genetic traits and body conformation is a key factor in improving egg production and growth performance in meat ducks. (2) Limited access to superior breeding stock. Magelang duck farmers often face difficulties in obtaining high-quality breeding stock due to the limited availability in the local area. They frequently used ducklings from their hatchings or from small local

hatcheries, which did not necessarily have good genetic quality. Wibowo TB et al. (2020) also noted that limited access to superior breeding stock is a significant obstacle to the development of Tegal ducks, (3) Limited business capital. Purchasing superior breeding stock requires higher costs compared to ordinary ducklings. Farmers with constrained financial resources tend to select more affordable ducklings or rely on home hatching, even if the resulting quality is not optimal. Syafwan et al. (2019) reported that the relatively high price of superior breeding stock is one reason why smallholder farmers are reluctant to replace or refresh their breeding stock. When compared to Alabio duck farmers in South Kalimantan, the breeding stock selection practices of Magelang duck farmers were relatively lower. Syafwan et al. (2019) reported that Alabio duck farmers routinely performed breeding stock selection based on lineage and production performance to maintain duck productivity and quality.

Infrastructure and facilities

Data for the infrastructure and facilities aspect was gathered by descriptively comparing the current field conditions with the GBP standard. The scoring was derived from interviews with farmers and direct observations in the field, which were then compared to the standards outlined in Minister of Agriculture Regulation No. 32/Permentan/OT.140/2/2014. The scores for the infrastructure and facilities aspects of Magelang duck farms are presented in Table 2. The use of these facilities and infrastructure is intended to maximize the effectiveness of tools and items that meet the farm's needs. Key considerations include the objectives to be achieved through their use, the characteristics of the users, and the availability of supporting facilities and infrastructure.

Infrastructure

The results in Table 2 show a score of 4.14 for land and location, while water and energy sources have a score of 4.17. This value is close to the GBP (Peraturan Menteri Pertanian Republik Indonesia 2014), where the land and location are easily accessible and can be reached using transport. The assessment is conducted descriptively, involving the scoring of interview results and direct observation of field conditions from the researcher's perspective. Land surveying refers to the physical environment, including climate, terrain, soil, water systems, and vegetation. These factors affect the potential and ability of land to support livestock development. Land resource factors are categorized into land units. Magelang district covers a total area of 108573 hectares, accounting for approximately 3.34% of Central Java province. This land is divided into 34.05% paddy fields, 38.61% dry land, and 27.34% non-agricultural land. The district's strategic location and remarkable soil fertility are enhanced by its surrounding natural features, including the mountains of Merapi, Merbabu, and Sumbing, as well as the Menoreh hills. These geographical features create a diverse landscape, with elevations ranging from 153 to 3065 meters above sea level, and an average altitude of 360 meters (BPS 2022).

Magelang District has potential for developing both large and small-scale livestock, due to its advantageous tropical climate, marked by distinct rainy and dry seasons, with temperatures ranging from 20 to 27°C. The area receives substantial rainfall and has plentiful water resources, supported by the Progo and Bogowonto watersheds, which encompass springs and rivers. The district features 10 significant rivers, with peak flow rates of up to 2314 cubic meters per second during the wet season and a minimum of 110.3 cubic meters per second in the dry season, as well as 55 springs that collectively discharge 9509 liters per second.

Facilities

The results presented in Table 2 indicate that the buildings have received a score of 2,22 as they do not meet the GPB standards (Peraturan Menteri Pertanian Republik Indonesia 2014). For an effective local duck breeding operation, the buildings should adhere to specific criteria, including the type, construction, and layout of the structures. However, the facilities on duck farms in Magelang District fail to meet these standards. Specifically, the existing buildings are not compliant with the GPB requirements; they use a single type of cage that serves multiple functions, such as housing both starter and finisher ducks, rather than having specialized cages for different stages of growth. Sick ducks are usually still housed in the same cage as healthy ducks, with no isolation cage. Feed is stored near the cage, without allocated storage room. The farm is typically situated behind the house, occupying an area of approximately 3 x 6 meters. Water and energy sources are usually obtained by allowing the ducks to access rice fields or rivers located some distance away from the house. The score for the farm equipment and machinery used is 2.17, which is because it is still below the GBP standard, as shown in Figure 1. It can be seen that only a basin is provided for feed, and there is also a box near the door for egg storage. The score for breeding stock is 3. The breed stock used is close to the average GBP standard. DOD is obtained by hatching or buying from larger farmers, with attention to the good condition of the breed stock.

Based on the research results (Table 2), the average score for the facilities and infrastructure aspect among Magelang duck farmers was categorized as moderate. This aspect includes the availability of housing, feed and

Table 2. Aspects of Infrastructure and Facilities in Magelang Duck Farms, Magelang District

Aspect	Score	Description
a. Infrastructure:		
1. Land and location	4.14	Strategic location, supported by transportation and road access
2. Water and Energy Sources	4.17	Sufficient clean water and energy sources are available
b. Facilities:		
1. Buildings (type of building, building construction, and building layout)	2.22	There are buildings, but the type, construction, and layout are not appropriate
2. Equipment and devices for animal farming and veterinary care	2.17	Equipment and devices for animal farming and veterinary care are quite appropriate
3. Breed stocks	3.06	Breed stock is by SNI
4. Feed	3.00	Feed by SNI
5. Veterinary medicine	2.37	Medicine is given traditionally
Average	3.02	

Figure 1. Magelang duck farm in Magelang district

drinking containers, sanitation tools, and other supporting facilities that play an important role in optimizing duck farming. A moderate score in this aspect indicates that most farmers already have the basic facilities needed, but the quality and quantity are not yet fully adequate. This condition can be attributed to several factors: (1) Limited business capital to improve existing facilities. The majority of Magelang duck farmers are small-scale farmers with limited capital. According to interviews, although farmers already have permanent or semi-permanent housing, the size remains minimal, with simple constructions made from bamboo, wood, and basic roofing materials. Feed and drinking containers are partly standard, but some farmers still use recycled items to save production costs. Syafwan et al. (2019) stated that limited capital is one of the main factors hindering the provision of better facilities and infrastructure in smallholder poultry farms in Indonesia. (2) Farmers' awareness of the importance of facilities and infrastructure is not matched with access to technology and financial assistance. Based on interviews, farmers realize that the quality of housing and equipment affects duck productivity. However,

most are not yet able to renovate their housing completely or replace traditional equipment with modern tools due to financial limitations and the absence of assistance from local governments. (3) Limited land area hinders the development of supporting facilities. Duck farming in Magelang typically occurs in home yards with limited space, so farmers only build housing as needed. This results in the unavailability of supporting facilities such as feed storage, hatching rooms, or waste processing areas. North & Bell (1990) stated that adequate facilities and infrastructure support livestock health, comfort, and management efficiency. When compared to Tegal duck farmers, the facilities and infrastructure of Magelang duck farmers were categorized as moderate, whereas those of Tegal duck farmers were better. Wibowo et al. (2020) reported that Tegal duck farmers generally have semi-intensive housing with permanent constructions, standard feed and drinking facilities, and access to government development programs for Tegal ducks, resulting in more optimal facilities and infrastructure to support duck farming productivity.

Animal health

Addressing several key animal health aspects is crucial in Magelang duck farms, including monitoring the occurrence of animal diseases, implementing effective disease prevention strategies, and enforcing comprehensive biosecurity measures. These factors are detailed in Table 3, highlighting the importance of maintaining overall farm health and safety.

It is crucial to address several key animal health aspects in Magelang duck farms, including monitoring the occurrence of animal diseases, implementing effective disease prevention strategies, and enforcing comprehensive biosecurity measures. These factors are detailed in Table 3, highlighting the importance of maintaining overall farm health and safety. Scores were determined by comparing interview responses with the GBP standard. The animal disease situation received a score of 2.91, reflecting the common occurrence of diseases like Newcastle disease (ND) and coccidiosis in the field. The score for disease prevention measures was 2.74, primarily because of traditional remedies. Practices giving garlic to sick ducks by placing one clove in their mouths daily are commonly used. The biosecurity score was 2.26, reflecting the minimal biosecurity practices typically implemented, such as basic cage cleaning and feed replacement, without any disinfection or sanitization processes. According to GBP standards, comprehensive disinfection and sanitation should be applied to every person, piece of equipment, and vehicle entering or leaving the farm; this includes providing disinfectant containers and handwashing stations near the farm entrance, which are changed daily. Additionally, strict controls should be placed on the movement of items such as livestock products, feed, manure, bedding, and litter that could carry viruses, with all goods disinfected before entering the farm, except for feed and medicine (Peraturan Menteri Pertanian Republik Indonesia 2014).

Based on the research results (Table 3), the score for the animal health aspect among Magelang duck farmers was categorized as low, as evidenced by the low implementation of vaccination programs, the use of deworming medication, and the lack of routine disease control among farmers. The low score for animal health can be attributed to several factors: (1) Lack of knowledge about animal health. Most farmers do not understand the importance of animal health programs, such as regular vaccination and vitamin supplementation. Based on interviews, farmers only provide medication to ducks when they show signs of illness. However, North & Bell (1990) stated that preventive measures, such as vaccination and good sanitation, are more effective in maintaining the health and productivity of poultry. (2) Absence of regular animal health assistance. Farmers rarely receive visits from veterinary paramedics or livestock health extension officers, which limits their knowledge, and treatments are often based on trial and error or personal experience. Syafwan et al. (2019) also stated that the lack of visits from animal health officers is a significant constraint in disease control among smallholder poultry farmers. (3) Limited funds for treatment and vaccination. Vaccinations and medications are considered additional expenses that burden small-scale farmers. Therefore, some farmers choose not to vaccinate their ducks and instead rely on traditional methods to treat diseases. When compared to Tegal duck farmers, the implementation of animal health practices among Magelang duck farmers is relatively lower. Wibowo et al. (2020) reported that most Tegal duck farmers administer at least one vaccination during the starter period to prevent infectious diseases. According to North & Bell (1990), animal health is a crucial aspect of poultry farming, as diseases can reduce production performance, egg quality, growth, and even increase mortality, leading to economic losses for farmers.

Preservation of environmental functions

The scoring data for the aspect of environmental function preservation was derived by descriptively comparing the current field conditions with the GBP standard. This evaluation was based on interviews with farmers and direct observations in the field, which were then compared to the criteria outlined in the GBP standard, as specified in Peraturan Menteri Pertanian Republik Indonesia Number 32/Permentan/OT.140/2/2014, as shown in Table 4.

Table 4 shows a score of 2.91 for point a, indicating that the Magelang duck farm has an average level of envi-

Table 3. Aspects of Animal Health in Magelang Duck Farms, Magelang district

Aspect	Score	Description
a. Animal Disease Situation	2.91	There is an animal disease, and it is pretty dangerous
b. Animal Disease Safety Measures	2.74	Animal disease safety measures are adequate
c. Implementation of Biosecurity	2.26	Less implementation of biosecurity
Average	2.64	

Table 4. Aspects of Preserving Environmental Functions in Magelang Duck Farms, Magelang District

Aspect	Score	Description
a. Avoiding environmental pollution and erosion	2.91	Avoiding environmental pollution and erosion
b. Avoiding noise, foul odor, and water pollution	2.26	Lessening noise, foul odor, and water pollution
c. Making a waste treatment unit with a production capacity to produce organic fertilizer	2.26	Less making a waste treatment unit by the production capacity to produce organic fertilizer
d. Make sewage channels and disposal sites	2.26	Lack of sewage channels and disposal sites
e. Make a place for burning or burying duck carcasses	2.14	There is burning or burying of duck carcasses
f. Make air circulation	2.29	Lack of air circulation
Average	2.35	

ronmental pollution. Point b scores 2.26, as the cages are still located behind the house without considering noise levels. Point C also scores 2.26 due to the absence of a waste treatment facility capable of producing organic fertilizer. Duck carcasses are typically burned or buried near the house without a designated disposal site, resulting in a score of 2.14 for point e. Additionally, air circulation is poor due to the open cage, resulting in a score of 2.29 for point f. Overall, the conditions observed in the field do not align with the GBP standards.

Based on the research results (Table 4), the average score for the preservation of environmental functions aspect among Magelang duck farmers was categorized as low. This aspect encompasses waste management, odor control, and efforts to maintain the cleanliness of the surrounding area. The low score for preservation of environmental functions can be attributed to several factors: (1) Lack of knowledge and environmental awareness. Most farmers do not understand the impact of livestock waste on the environment and public health. According to interviews, farmers perceive duck waste, including manure and wastewater from cleaning duck houses, as posing no serious problems. However, North & Bell (1990) stated that improperly managed livestock waste can contaminate soil and water, causing foul odors that disturb nearby communities. (2) Limited land and waste management facilities. Magelang duck farmers typically raise ducks in their home yards, often with limited space, and therefore do not have dedicated areas to process or store waste. They usually dispose of manure directly into gardens or water channels without prior treatment. Syafwan et al. (2019) reported that limited land area is one of the main constraints in waste management for small-scale poultry farms. (3) Absence of strict regulations or environmental supervision. There are no strict regulations or sanctions related to environmental pollution by smallholder farms, causing farmers to be less concerned about implementing environmentally friendly waste management practices. When compared to Alabio duck farmers in South Kalimantan, the preservation of environmental functions practices of Magelang duck farmers was relatively lower. Syafwan et al. 2019 reported that Alabio duck farmers processed duck manure into compost fertilizer for their gardens or sold it to local farmers, which reduced environmental pollution while also increasing their income. According to North & Bell (1990), maintaining the preservation of environmental functions is essential in poultry farming to avoid causing health

problems for both the livestock and the surrounding community, and to ensure the sustainability of the farming business itself.

Human resources

Human resources on Magelang duck farms in Magelang district are scored based on the condition of farmers in the field compared to the GBP standard. The score for the human resource aspect in Magelang duck farms, as per Peraturan Menteri Pertanian Republik Indoneisa Nomor 32/Permentan/OT.140/2/2014, is presented in Table 5.

Based on the scoring results, the score for the physical and spiritual health aspect is 4.06; this means that the breeders found in the field are physically and mentally healthy. The score for having skills relevant to their field is 2.80, indicating that they possess sufficient skills in their field. The score for applying work safety and security according to the provisions is 3.00. When compared with the GBP score, the average score for human resources is 3.29, as not all aspects listed in the GBP can be implemented in the field; several factors, such as age, education level, and breeding experience, influence this. Human resources involved in duck farming must meet GBP standards, among others, as follows: 1. Able-bodied; 2. Have skills in their field and understand the risks associated with their work; and 3. Implementing occupational safety and security by the provisions of laws and regulations in the field of labor (Peraturan Menteri Pertanian Republik Indonesia 2014).

Human resources are individuals who work in organizations, set general goals and priorities, plan work processes, produce goods and/or services, track efficiency, allocate financial resources, and sell goods and/or services (Bratton & Gold 2017). The results showed that the organization had efficient and talented human resources (Obeidat et al. 2017). The factor considered most likely to achieve a competitive advantage is the provision of human resources related to management (Tahir et al. 2015, 2019). The issue of human resources essentially refers to the reassessment of labor capabilities (Hall 2008; Wright & McMahan, 2017). Hasibuan (2003) argues that human resources encompass the abilities possessed by everyone, including physical and mental capacities. The environment and heredity are natural factors, while the desire to fulfill their satisfaction is supported by work motivation. (Fathoni 2006) revealed that human resources are the wealth and capital of every most important human activity.

The study included 30 farmers, who were then used as research informants. Characteristics of Magelang duck farmers include age, education level, and farming experience. Age significantly impacts the condition of the farmer and their physical ability to perform work or activities. The productivity level of a young person is significantly faster compared to breeders who have entered old age, commonly referred to as non-productive. The results of interviews with breeders revealed that as many as 63% of breeders were dominated by the age group of 51-60 years. Maryam et al. (2016) stated that when reaching a certain age, such as 55, 60, or 65 years, a worker must either enter retirement or cease to be productive. Age has an impact on work productivity in jobs that require physical labor (Makatita et al.2014) Farmers in Magelang are more dominated by older individuals because breeding is only a secondary business and is also less attractive to young people. Usually, those who have retired or are approaching retirement make breeding their business after retirement.

The education level of farmers in Magelang is predominantly comprised of high school graduates (70%). This level of education reflects that most farmers are ready to receive knowledge about how to breed Magelang ducks properly. The farming experience of Magelang duck farmers in Magelang district is dominated by 10-20 years (86%). This experience is long enough to understand how to properly raise Magelang ducks. Farmers usually understand farming procedures because of their long-standing habit of raising livestock, so they are already familiar with the characteristics of the ducks.

Based on the research results (Table 5), the average score for the human resources (HR) aspect among Magelang duck farmers was categorized as moderate. This aspect includes educational level, farming experience, training attended, and technical knowledge of duck farming. The quality of human resources among Magelang duck farmers can be attributed to several factors, namely: (1) Low level of formal education. Most Magelang duck farmers only completed elementary or junior high school. This low level of education affects their ability to read, understand, and apply information related to modern livestock technology and management. (Handayani et al. 2020) stated that formal education has a significant influence on farmers' ability to adopt technological innovations. (2) Lack of training or technical extension. According to interviews, the majority of farmers had never participated in specific duck farming training in recent years. As a result, their knowledge of feed management, health, and biosecurity is only based on traditional experience passed down from previous generations. Syafwan et al. (2019) emphasized that regular training and extension services will enhance farmers' human resource capacity, thereby increasing livestock productivity. (3) Relatively old age of farmers. The majority of farmers are between 45 and 60 years old. This relatively old age reduces their motivation to learn new things. In addition, the regeneration of duck farmers in Magelang is low, as

younger generations are less interested in continuing the family duck farming business. Wibowo et al. (2020) reported a similar phenomenon among Tegal duck farmers, where younger generations prefer jobs in the non-agricultural sector. (4) Lack of awareness regarding the importance of HR development. Many farmers view duck farming merely as a side business or family tradition, and thus do not have a mindset oriented towards business growth and sustainability. According to North & Bell (1990), the quality of human resources is one of the key factors determining the success of poultry farming, particularly in terms of implementing effective management practices, cost efficiency, and animal health management. When compared to Alabio duck farmers in South Kalimantan, the human resource quality of Magelang duck farmers is relatively lower. Syafwan et al. (2019) demonstrated that Alabio duck farmers have access to regular training from local governments and non-governmental organizations, leading to improved knowledge and skills in duck farming.

Assistance and supervision by the Government

Aspects of assistance and supervision by the government in Magelang duck farms, according to Peraturan Mneteri Pertanian Republik Indonesia Nomor 32/Permentan/OT.140/2/2014, as stated in Table 6.

Based on the scoring results obtained, the scores for the assistance, supervision, and reporting aspects are 3.00, 3.00, and 3.00, respectively, due to the lack of assistance and supervision by the relevant Livestock Service Office. The scores for training, supervision, and reporting are each 3.00; this means that all three have sufficient values. Coaching is conducted to enhance effectiveness and efficiency in managing duck farming. Coaching is conducted within the context of implementing sustainable and environmentally friendly cultivation practices through education, training, and counseling (Purwanto, 2003) The central government undertakes development, while provincial and regional governments, as well as district/city local governments, do so within their respective jurisdictions. Supervision is carried out to ensure the quality and safety of duck products, and is carried out both directly and indirectly. Direct supervision is carried out at the cultivation site on site suitability, cultivation management, feeding, animal disease security, and preservation of environmental functions. Supervision of duck farming is carried out by a supervisory officer appointed by the head of the local district/city office, which oversees livestock and animal health functions.

Based on the research results (Table 6), the average score for government assistance and supervision among Magelang duck farmers is categorized as moderate, indicating that while some government programs have been implemented, they are not yet optimal across all farmers. Several factors contribute to this moderate score: (1) Existing programs are present but not evenly distributed. Interviews revealed that some farmers have received assistance, such as ducklings or feed, from the Livestock Service Office, but not every year, and often only for certain farmer groups. Some farmers have also received extension visits, but the frequency is low, so the impact of these programs is not widespread. (2) Limited number of livestock extension workers. The number of livestock extension officers in Magelang District

Table 5. Aspects of Human Resources in Magelang Duck Farms, Magelang District

Aspect	Score	Description
a. Physically and mentally healthy	4.06	Physically and mentally healthy
b. Have skills according to their field and understand the risks of the job	2.80	Fairly skilled in their respective field.
c. Able to apply work safety and security by the provisions of laws and regulations in the field of labor	3.00	Moderately able to apply work safety and security
Average	3.29	

Table 6. Aspects of Assistance and Supervision by Government in Magelang Duck Farms, Magelang District

Aspect	Score	Description
a. Assistance	3.00	Enough coaching
b. Supervision	3.00	Enough supervision
c. Reporting	3.00	Reporting is sufficient
Average	3.00	

is limited, resulting in low visitation intensity to duck farmers. Syafwan et al. (2019) note that the ratio of extension workers to farmers in rural areas is often unbalanced, which affects the effectiveness of technical support in the field. (3) Government programs are not explicitly focused on Magelang ducks. Most local government livestock programs still prioritize dairy cattle and goats, while local duck development has not become a primary focus. As a result, assistance and supervision for duck farmers are only a small part of general poultry programs. (5) Farmers' limited access to government program information. Many farmers are unaware of existing government training or assistance programs due to poor access to information and a lack of socialization. Wibowo et al. (2020) reported that low information literacy among farmers is one reason they rarely participate in training or apply for government assistance.

Feasibility of Magelang ducks as a viable source of breeding stock

Magelang district is one of the districts in Central Java province, located adjacent to several districts and cities, including Temanggung, Semarang, Boyolali, Purworejo, Wonosobo, and Magelang City, as well as the Yogyakarta Special Region Province. This condition is very favorable for trade routes from areas around the Magelang district. Magelang District is famous for its distinctive duck, known as the Magelang duck. This duck has been designated as the Magelang duck family based on Keputusan Menteri Pertanian Nomor 701/Kpts/PD.410/2013. Farmers favor Magelang ducks due to their good adaptability and relatively high egg productivity (200-300 eggs/year), with excellent egg quality. Male ducks weighing 1.8-2.5 kg can be used in culinary dishes that are popular with the public. In Magelang, ducks are raised by farmers who typically manage between 10 and 50 ducks, with an average of around 20. These farms are generally operated on a subsistence or part-time basis. Livestock are usually kept in the rice fields in the morning to feed on rice residue, snails, and worms, and in the afternoon, they are confined to a cage. Ducks also often get additional feed in the form of household food scraps and rice bran to support good egg production.

Magelang ducks have many advantages over other ducks, which is why they have spread to 21 sub-districts in the Magelang district. Another advantage is that it has a large body shape with a heavier body weight (Rahayu et al. 2021), so its meat is more marketable as a slaughtered duck and has high egg production. According to David (2009), breeding implementation strategies can be formulated through a SWOT analysis, utilizing the results of internal and external factor identification presented in the IFAS (Internal Factors Analysis Summary) matrix and the EFAS (External Factors Analysis Summary) matrix. A SWOT diagram is obtained by comparing internal and external factors to describe the position of the Magelang duck business. The Internal Factors Analysis Summary (IFAS) outlines the internal strengths and weaknesses of Magelang duck farmers. These factors are identified through a questionnaire and analyzed using the data analysis methods described. The results of the calculation of the strengths and weaknesses of Magelang Duck farmers, as listed in Table 7, are then used to give the weight of each internal factor by summing the data from each question on the aspects of strengths and weaknesses, and then dividing by the number of respondents. The IFAS matrix is presented in Table 7.

Several key factors influence the viability of Magelang as a source of duck breeding. On the strength side, Magelang's advantages include speedy livestock growth, high demand for eggs, and the potential to increase income. However, significant weaknesses include the relatively small number of ducks raised and the inactivity of livestock groups. Externally, the Analysis Summary of External Factors (EFAS) highlights opportunities and threats faced by Magelang duck farmers. Significant opportunities include easy access to duck breeds and a more consumptive society. Conversely, the primary threats are the high demand for livestock and competition from other commodities. These factors are detailed and weighted in the EFAS matrix presented in Table 8.

The weighting is determined by evaluating the combination of strengths and weaknesses, as well as opportunities and threats. The weight values are derived from interviews conducted between the research enumerator and Magelang duck farmers in the Magelang district. Determination of rating based on discussion with Magelang duck farmers in the Magelang district. The score is obtained by multiplying the weight and rating. The SWOT analysis indicates that Magelang duck farms in the Magelang district effectively capitalize on available opportunities and mitigate potential threats. The analysis positions the development plan for Magelang duck breeding in Quadrant I (Figure 2) (1.55; 1.05), as it falls on the x-axis at 1.55 and the y-axis at 1.05.

A growth strategy, which means maximizing strengths and opportunities to achieve growth, indicates that opportunities and strengths are key points that must be developed in the current situation. The strategy recommendation is that a progressive approach must be implemented, meaning that Magelang duck farms require guidance from various institutions, such as universities and local livestock services, to optimize their operations further. Opportunities and Strengths of Magelang Duck Farming that need to be developed, such as: (1) Adaptive genetic potential of Magelang ducks, Magelang ducks have good adaptability to local

environmental conditions, resilience to weather changes, and relatively good reproductive ability. These characteristics are fundamental strengths for population development and improvement in production. (2) Stable local market demand, Magelang duck eggs and meat already have their market in Magelang and surrounding areas, both for household consumption and traditional market traders. This stable demand is a key strength in ensuring the sustainability of the farming business. (3) Availability of alternative local feed resources. In Magelang, various local feed resources such as rice bran, bran, and leftover vegetables from markets are available and can be used as supplementary feed, thereby reducing feed costs. (4) Development of processed products based on Magelang ducks. Processing duck products such as salted eggs, shredded duck meat (abon itik), or crispy duck skin chips can increase product value and expand the market beyond the local area. (5) Implementation of Good Breeding Practices and biosecurity to improve productivity. (6) Applying modern management practices, good biosecurity, and using superior breeding stock will increase production efficiency and product quality, thus enhancing the competitiveness of Magelang duck products. (7) Support from local government programs for local duck development, Livestock development programs, training, and capital assistance from district or provincial livestock services are opportunities that can be utilized to improve farmers' human resources, facilities, and infrastructure, and livestock productivity. (8) Increasing trend of animal protein consumption. Public awareness of animal protein consumption continues to increase, making the market prospects for Magelang ducks, both for meat and eggs, more promising in the future.

Philipsson et al. (2003) suggested that the components that must be considered in a breeding program include the role of livestock, breeding objectives, recording, and building infrastructure. Methods to create a long-term breeding plan include (1) defining the production system, (2) defining the purpose and direction of breeding, (3) collecting the information needed, (4) determining selection criteria, (5) carrying out selection and mating, (6) dissemination, and (7) evaluation (Oldenbroek et al. 2014) .

This research is currently limited to defining the purpose and direction of breeding and collecting the necessary information to develop a breeding program.

Table 7. IFAS Matrix Calculation Results

No	Internal Factors	Weight	Rating	Score
	Strength	(a)	(b)	(c)
1	Speedy livestock growth	0.11	5.00	0.55
2	High demand for eggs	0.12	4.00	0.48
3	High farmer motivation to return to business	0.11	4.00	0.44
4	Long farming experience	0.12	5.00	0.60
5	Strategic business location	0.12	4.00	0.48
6	Business risk is relatively small	0.11	3.00	0.33
7	Low production cost	0.09	4.00	0.36
8	Private capital	0.11	3.00	0.33
9	Can increase income	0.11	4,00	0.44
	Total Strengths	1.00		4.01
	Weaknesses			
1	Difficulty of maintenance	0.14	3.00	0.43
2	The small number of ducks raised	0.17	2.00	0.34
3	Ducks raised are often consumed personally	0.12	3.00	0.36
4	Inactive livestock group	0.20	2.00	0.40
5	Fluctuating sales	0.19	3.00	0.57
6	Income contribution from the business is relatively small	0.18	2.00	0.36
	Total Weaknesses	1.00		-2.46

The results of the IFE matrix analysis on breeders in Magelang District obtained a total score of 1.55

Table 8. EFAS Matrix calculation results

No	External Factors	Weight	Rating	Score
		(a)	(b)	(c)
	Opportunities	0.17	4.00	0.70
1	Duck breeds are easy to obtain	0.17	4.00	0.70
2	An increasingly consumptive society	0.18	3.00	0.54
3	High interest in egg consumption	0.17	4.00	0.68
4	Almost every community has a duck business	0.15	4.00	0.60
5	The demand for duck eggs is starting to get high	0.16	3.00	0.48
6	The market is relatively open	0.16	4.00	0.64
	Total Opportunities	1.00		3.64
	Threats			
1	The many needs of farmers	0.27	2.00	0.53
2	The number of competitors in other livestock commodities	0.16	3.00	0.48
3	Lack of attention from the local livestock office	0.16	2.00	0.32
4	The complexity of internal and/or external problems of livestock groups	0.22	3.00	0.66
5	A large number of Magelang duck farmers	0.20	3.00	0.60
	Total Threat	1.00		-2.59

The results of the EFE matrix analysis on farmers in Magelang obtained a total score of 1.05

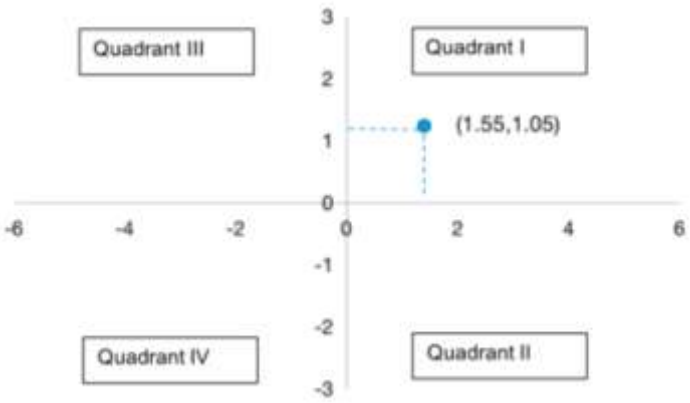

Figure 2. SWOT Diagrams

For the next stage, further research, including phenotypic and genotypic studies, will be carried out to fulfill all the required methods.

CONCLUSION

In conclusion, the data indicate that duck farming practices in Magelang District currently fall below the established Good Breeding Practice standards, such as breeding stock selection, animal health, and preservation of environmental functions. Nonetheless, the district remains a viable source of breeding stock. The SWOT analysis further reveals that Magelang duck farms are at an early stage of development. To enhance their practices and achieve higher standards, these farms must focus on leveraging identified opportunities and strengthening their existing capabilities such as adaptive genetic potential of Magelang ducks, stable local market demand, availability of alternative local feed resources, development of processed products

based on Magelang ducks, implementation of Good Breeding Practices and biosecurity to improve productivity, applying modern management practices, support from local government programs for local duck development, increasing trend of animal protein consumption.

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REFERENCES

[BPS] Badan Pusat Statistik. 2022. statistik prasarana dan sarana pertanian 2017–2021. Jakarta (Indones): Kementerian Pertanian Republik Indonesia.

Bratton J, Gold J. 2017. Human resource management: Theory and practice. London (UK): Palgrave Macmillan.

David FR. 2009. Manajemen Strategis. Jakarta (Indones): Salemba Empat.

Direktorat Jenderal Peternakan dan Kesehatan Hewan. 2002. Penetapan Jumlah Induk Ternak Berdasarkan Wilayah. [Diakses 27 Desember 2024] https://ditjenpkh. pertanian.go.id.

Fathoni A. 2006. Manajemen sumber daya manusia. Jakarta (Indones): Rineka Cipta.

Hall CM. 2008. Tourism planning: Policies, processes and relationships. London (UK): Pearson Education.

Handayani D, Suryani E, Hadi S. 2020. Analysis of factors influencing the application of innovation in community livestock technology in Indonesia. JPI. 22: 45–54. DOI:10.25077/jpi.22.1.45-54.2020.

Hasibuan M. 2003. Organization and motivation for productivity improvement. Jakarta (Indones): Bumi Aksara.

Luthfiana NA, Santoso B, & Rahayu A. (2020). Korelasi Genetik Bobot Telur Dengan Indeks Telur Itik Magelang di Dusun Sempu Desa Ngadirojo Kecamatan Secang Kabupaten Magelang. Agrista: Jurnal Ilmiah Agribusiness UNS, 4(1), 382–387.

Maharani D, Hariyono DNH, Cho S, et al. 2017. Genetic diversity among Indonesian local duck populations in Java Island assessed by microsatellite markers. JABG, 1:136-142. DOI:10.12972/jabng. 20170013

Makatita J, Isbandi S, Dwidjatmiko. 2014. Tingkat efektivitas penggunaan metode penyuluhan pengembangan ternak sapi potong di Kabupaten Buru Provinsi Maluku . Agromedia. 32:64-74. DOI:10.47728 /ag.v32i2.95.

Maryam et al. 2016. Pengaruh umur, pendidikan, pengalaman dan jumlah ternak peternakan kambing terhadap perilaku sapta usaha beternak kambing di desa Wonosari Kecamatan Patebon. 34 (1): 1-6. DOI:10.47728/ag. v34i1.134.

North MO, Bell DD. 1990. Commercial Chicken Production Manual (4th ed.). New York (USA): Van Nostrand Reinhold.

Obeidat BY, Abdallah AB, Aqqad NO, Akhoershiedah AHOM, Maqableh M. 2017. The effect of intellectual capital on organizational performance: The mediating Role of Knowledge Sharing. Comm Netw. 9: 1–27. DOI:10.4236/cn.2017.91001.

Oldenbroek K, Waaij LVD. 2014. Animal breeding and genetics for BSc students. WOT CGN: Animal Breeding and Genomics.

Peraturan Menteri Pertanian Republik Indonesia Nomor 32/Permentan/OT.140/2/2014 tentang Pedoman budi daya itik pedaging dan itik petelur yang baik. Jakarta (Indones): Kementerian Pertanian Republik Indonesia.

Philipsson J, Rege JEO. 2003. Sustainable breeding programmes for tropical farming systems. Nairobi, Kenya: ILRI.

Purwanto E. 2003. The effect of beck and mckeown model geography text organization on reading ability and learning style on high school students' learning acquisition (dissertation). Malang (Indones): Universitas Nasional Malang.

Rahayu A, Purwantini D, Maharani D, Hartatik T. 2015. Single nucleotide polymorphisms identification and genotyping analysis of melanocortin 1 receptor gene in various plumage colours Magelang Ducks. Int J Poult Sci. 14:207-212.

Rahayu A, Rahayu TP. 2020. Management of intensive and extensive Magelang duck maintenance in Secang District, Magelang Regency. Bul App Anim Res. 2: 38-43.

Rahayu A, Ratnawati S, Idayanti RW, Hartati L, Pramono PB. 2022. Phenotypic characterization of the quantitative traits of Magelang Duck in Sempu Hamlet, Magelang Regency. KLS. p.440–446. DOI 10.18502/kls.v0i0. 11829.

Rahayu A, Ratnawati S, Idayanti RW, Santoso B, Luthfiana NA. 2020. Effect of intensive and semi-intensive maintenance systems on Magelang Ducks. JSPI. 15:355–359. DOI:10.31186/Jspi.Id.15.4.355-359.

Rahayu A, Ratnawati S, Idayanti RW, Septian MH, Santoso B, Luthfiana NA. 2021. Phenotypic correlation of quantitative traits of Magelang ducks in Magelang District. JITRO. 8:98-103. DOI:10.33772/Jitro. V8i2.12 567.

Rahayu A, Santoso B, Luthfiana NA. 2019. Identification of Magelang Ducks to analyze morphological diversity in Ngadirojo Village, Secang District, Magelang Regency. JALSPRO. 3:179-185. DOI:10. 31002/Jalspro.V3i2. 2034.

Rangkuti F. 2006. Measuring Techniques and Strategies to Improve Customer Satisfaction. Jakarta (Indones): Gramedia.

Ricardo JO, Noriega JG, Cardona H. 2024. Assessment of population structure and genetic diversity in Colombian Indigeneous Hair Sheep. LRRD. 36.

Sugiyono. 2019. Quantitative, qualitative, and R&D research methods. Bandung (Indones): Alfabeta.

Syafwan W, Nasrullah N, Zainuddin D. 2019. Constraints faced by small-scale poultry farmers in West Sumatra, Indonesia. IJPS. 18:63-68. DOI:10.3923 /Ijps.2019. 63.68.

Tahir R, Rudianto A, Prayitno D. 2015. Strategic human capital. Bandung (Indones): Alfabeta.

Tahir R, Rudianto A, Prayitno D, Amiruddin T, Rosita. 2019. Employee competencies and compensation strategies as company's strategic effort to escalate employee performance. IJRTE. 8:7200-7208. DOI:10.35940/i jrte.C6220.098319.

Wibowo TB, Prabowo S, Nuraini N. 2020. Analysis of duck farming management in Tegal Regency. JPN. 6:12-21. DOI:10.25015/Jpn.V6i1.2020.

Wright PM, McMahan GC. 2017. Theoretical perspective for strategic human resources management. J Man. 18:295-320. DOI:10.1177/014920639201800 205.

Yuwono MD. 2012. Budidaya tenak itik petelur. Pryono TJ, Ernawati, Ed. Semarang (Indones): Balai Pengkajian Teknologi Pertanian Jawa Tengah.

The Impact of Adding Egg Yolk in Various Concentrations into The Tyrode Extender on Muscovy Duck *(Cairina moschata)* Sperm Quality

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ABSTRAK

Rasad SD, Widyastuti R, Setiawan I, Sujana E, Setiawan R, Solihati N, Nadzir MNHMM. 2025. Pengaruh penambahan berbagai konsentrasi kuning telur ke dalam ekstender Tyrode terhadap kualitas spermatozoa itik (*Cairina Moschata*). JITV 30(2):107-113. DOI:http://dx.doi.org/10.14334/jitv.v30i2.3513.

Penelitian ini bertujuan untuk melihat pengaruh konsentrasi kuning telur yang berbeda dalam ekstender Tyrode terhadap kualitas semen Itik setelah pengawetan. Sampel semen dikoleksi dari Itik berusia 1,5 tahun. Sampel dievaluasi untuk motilitas sperma, integritas membran plasma, integritas akrosom, dan morfologi sperma sebelum dipisahkan menjadi tiga bagian yang sama dan diencerkan dalam ekstender Tyrode yang mengandung kadar kuning telur yang berbeda: T_1 (5%), T_2 (10%), dan T_3 (15%). Setelah 21 jam pengawetan pada suhu 4-5ºC, setiap sampel dievaluasi untuk parameter yang sama seperti semen segar. Hasil penelitian menunjukkan penurunan yang nyata pada motilitas sperma, mebran plasma dan akrosom pada T_1, T_2, dan T_3 dibandingkan semen segar yaitu T_1 (53.34%; 63.5%; 60.04% vs 84.16%), T_2 (48.7%; 57.8%; 55.3% vs. 85.6%), and T_3 (50.6%; 57.6%; 56.1% vs 85.4%), respectively ($P<0.05$). Sebaliknya, morfologi sperma abnormal meningkat secara signifikan pada T_1, T_2, dan T_3 dibandingkan dengan semen segar (6,69%, 6,89%, 7,89% vs 2,2%) ($P<0,05$). Lebih jauh, penambahan 10% kuning telur ke dalam ekstender Tyrode menghasilkan persentase terbaik dari motilitas sperma, integritas membran sperma, dan integritas akrosom setelah pembekuan. Akan tetapi persentase sperma dengan morfologi abnormal tidak berbeda secara signifikan ($P>0.05$) antara T_1, T_2, dan T_3 setelah periode pembekuan. Dapat disimpulkan bahwa menambahkan 10% kuning telur ke dalam ekstender secara substansial meningkatkan motilitas sperma, integritas membran, dan integritas akrosom pada sperma Itik setelah 21 jam pembekuan.

Kata Kunci : Kuning Telur, Pembekuan, Kualitas Semen, Tyrode

ABSTRACT

Rasad SD, Widyastuti R, Setiawan I, Sujana E, Setiawan R, Solihati N, Nadzir MNHMM. 2025. The impact of adding egg yolk in various concentrations into the Tyrode extender on the quality of muscovy duck *(Cairina moschata)* spermatozoa. JITV 30(2):107-113. DOI:http://dx.doi.org/10.14334/jitv.v30i2.3513.

This study investigated the impact of varying egg yolk concentrations in Tyrode extender on the quality of *Muscovy duck* semen after preservation. A sample of semen was collected from 1.5-year-old *Muscovy ducks*. The sample was evaluated for sperm motility, plasma membrane integrity, acrosome integrity, and sperm morphology before being separated into three equal parts and diluted in Tyrode extender containing increasing levels of egg yolk: T_1 (5%), T_2 (10%), and T_3 (15%). After 21 hours of preservation at 4-5ºC, each sample was evaluated for the same parameters as fresh semen. The study found a significant decrease in sperm motility, membrane integrity, and intact acrosome cap following preservation in T_1, T_2, and T_3 compared to fresh ejaculate (53.34%; 63.5%; 60.04% vs 84.16%), (48.7%; 57.8%; 55.3% vs. 85.6%), and (50.6%; 57.6%; 56.1% vs 85.4%), respectively ($P<0.05$). In contrast, abnormal sperm morphology increased considerably in T_1, T_2, and T_3 compared to fresh semen (6.69%, 6.89%, 7.89% vs 2.2%) ($P<0.05$). Furthermore, the addition of 10% egg yolk to the Tyrode extender resulted in the best percentages of sperm motility, sperm membrane integrity, and intact acrosome cap following preservation. Surprisingly, the percentage of sperm with abnormal morphology did not differ significantly ($P>0.05$) between T_1, T_2, and T_3 after the preservation period. It can be concluded that adding 10% egg yolk to the extender substantially improved sperm motility, membrane integrity, and intact acrosome cap in Muscovy duck sperm after 21 hours of preservation.

Key Words: Egg Yolk, Preservation, Sperm Quality, Tyrode

INTRODUCTION

The Muscovy duck (*Cairina moschata*) has the potential to improve its genetic quality by utilizing a genetic resource (*germplasm)* obtained from Central and South America and developed as indigenous livestock in Indonesia (Lan & Worowan 2021). Muscovy ducks have the potential to be developed as meat, egg, and feather-producing birds because of their excellent body and egg

weights and thicker feathers compared to chickens and ducks. However, the population is decreasing year after year. A reduced population of productive ducks has led to a decline in the number of ducks compared to the number slaughtered. Efforts must be made to accelerate the transmission of genetic potential and enhance the genetic quality of ducks by applying Artificial Insemination (AI) Technology with semen from superior males. The utilization of limited superior males can be achieved by preservation or cryo-preservation, which involves storing genetic material as chilled or frozen sperm.

A critical factor during sperm preservation or cryopreservation is the choice of the extender. The extender maintains sperm quality during cryopreservation or preservation by providing spermatozoa with an optimal environment and nutrition (Bustani & Baiee 2019). The extender could also preserve the integrity of the spermatozoa cell membrane during cryopreservation, thereby minimizing sperm injury caused by the ice crystal formation (Kumar et al. 2019). The extender combines a buffer, an energy source, and an anti-cold shock agent. Lipoprotein and lecithin act as anti-cold shock agents capable of maintaining and protecting the integrity of sperm cell membranes during preservation or freezing. Egg yolk is one of the sources of lipoproteins (Salmani et al. 2014). Moreover, egg yolk, as a cryoprotectant, functions as a medium for providing food, energy sources, and protection for extracellular spermatozoa from cold shock when freezing, a protective agent, provides the effect as a buffer against spermatozoa (Upadhyay et al. 2021)

Currently, there is a lack of information regarding the precise concentration of egg yolk that should be applied to the Tyrode extender for Muscovy *duck* sperm. This study aims to evaluate the quality of Muscovy duck sperm diluted with a Tyrode extender containing egg yolk at different concentrations.

Tyrode's diluent has several advantages in freezing poultry semen, primarily due to its ability to maintain sperm motility and viability. The addition of egg yolk to Tyrode′s diluent, for example, has been shown to increase sperm motility and reduce abnormality rates in frozen Muscovy duck semen. Tyrode diluents help maintain the ability of sperm to move actively. The correct use of Tyrode diluents can reduce in the abnormalities of sperm. It leads to a higher success rate of artificial insemination. Tyrode diluents can be combined with egg yolk for more optimal results. It is also relatively easy to use in the semen freezing process of poultry.

It is essential to note that selecting the correct diluent and freezing technique is crucial for maintaining the quality of poultry sperm. The use of Tyrode diluents combined with egg yolk, for example, has shown promising results in studies. The findings from this study can be utilized to enhance the quality of preserved and frozen duck sperm, hence improving the success rate of Artificial Insemination.

MATERIAL AND METHODS

Ethical approval

The Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran, No. 297/UN6, approved all the procedures in this study.KEP/EC/2023. Semen samples were collected from *Cairina moschata* at the Indigenous Ducks Breeding Station of Universitas Padjadjaran. A *Chairina moschata* was given 0.5 kg of mixed feed consisting of concentrate, bran, and ground corn.

Semen collection and sperm dilution

Semen collection from one male duck was carried out twice a week, at 08:00–09:00 pm on Tuesdays and Thursdays, using an Artificial Vagina based on the procedure in a previous study (Watanabe & Sugomiri 1957). The collected semen was transferred into a 1.5 mL ampule using a pipette and immediately evaluated for sperm progressive motility, membrane integrity, intact acrosome caps, and abnormal morphology. Furthermore, each ejaculate is divided into three equal parts and diluted in a Tyrode extender with a different egg yolk concentration: T1 (5%), T2 (10 %), and T3 (15%). The sample semen was mixed with extender and various egg yolk concentrations and stored at 3-5°C for 21 hours, and then the sperm evaluation was performed (Hidayat et al. 2022).

Sperm evaluation

Sperm progressive motility

The percentage of progressive sperm motility was observed under a binocular microscope (CX21 Olympus, Japan) at 400x magnification and calculated based on a previous study (Iskandar et al. 2022). Sperm motility was calculated by calculating the total sperm concentration and the concentration of dead sperm by placing one drop of semen in the *Neubauer* counting chamber. Sperm motility (Y) in percentage (%) was calculated by the formula below.

$$Y = \frac{\sum Total\ Sperm - \sum Dead\ sperm}{Total\ of\ Spermatozoa}\ x\ 100\%$$

Sperm membrane intact

The procedure was carried out to assess the integrity of the plasma membrane, as per previous research. Thirty grams of Sodium Chloride were dissolved in 100

ml of distilled water as a Hypo osmotic Swelling Test (HOS-test) solution. The HOS-test solution was added at a 1:6 or 10 µl: 60 µl ratio. The semen that had been mixed with the HOS-Test solution was then incubated at 38°C for 30 minutes. After incubation, the sample was prepared for review on an object glass, and 200 spermatozoa were counted. A circular or bent tail marked the intact Plasma Membrane, while a straight tail indicated that the plasma membrane was incomplete or damaged (Widyastuti et al. 2022).

Intact acrosome cap of sperm

A 100 µl NaCl physiological solution containing 1% formalin was mixed with 25 µl of cement. The mixture was gently shaken to ensure homogeneity and left to stand for 5 minutes. A light microscope was utilized to examine the thin stain preparation on a glass object at a magnification of 400x in the presence of at least 200 spermatozoa. Spermatozoa that have an intact acrosome cap have been identified by an enormous black point on their heads; conversely, spermatozoa that have been damaged lack this characteristic (Rizal & Herdis 2005).

Sperm abnormal morphology

Spermatozoa abnormalities were evaluated using an eosinnigrosine-stained smear under 1000x magnification. Furthermore, the evaluation of sperm morphology following the previous research with categories: a) Pear-shaped, b) Macrocephalus, c) Microcephalus, d) Detached Head, e) Head only, f) Circular Tail, g) Tail, and h) Stump Tail (Arifiantini 2012; Susilawati 2011).

Statistical analysis

Data was analyzed using GraphPad Prism (version 10)(La Jolla, USA). The normality of values distribution was first tested with the Shapiro-Wilk test. Sperm data were subjected to two-way factorial analysis of variance (ANOVA) followed by multiple pairwise comparisons using a post-doc (Tukey test). The threshold of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The findings revealed that the presence of egg yolk in Tyrode extender significantly reduced the progressive motility of Muscovy duck spermatozoa in all treatment groups. Moreover, adding 5% egg yolk to Tyrode extender significantly inhibits the progressive motility of Muscovy duck spermatozoa after 21 hours of preservation by more than 20% compared to fresh semen. Interestingly, adding 10% egg yolk to Tyrode solution is capable of maintaining the progressive motility of Muscovy duck spermatozoa after 21 hours of preservation, as opposed to the other treatments (Figure 1a).

Enhancing spermatozoa quality can be achieved by incorporating egg yolk into the extender, which serves as an energy source for the spermatozoa (Allai et al. 2018). Egg yolk provides nourishment, energy, and protective substances for sperm during storage. The study demonstrated that supplementing egg yolk in different concentrations can help preserve progressive motility, membrane integrity, and intact acrosome cap and reduce abnormal morphology in Muscovy duck spermatozoa preserved for 21 hours at 4-5ºC.

The Tyrode extender has several functions, including increasing sperm motility and reducing abnormality rates in frozen Muscovy duck semen. Another function that we can contribute to a higher success rate of artificial insemination is relatively easy to prepare and use in the poultry semen freezing process.

Our findings indicate that adding 10% egg yolk to the extender significantly improves the maintenance of spermatozoa's progressive motility compared to other concentrations. The results showed that adding 10% egg yolk to the extender adequately provides nutrients for preserving *Muscovy duck* spermatozoa during preservation. Egg yolk helps maintain *Muscovy duck* spermatozoa motility by providing proteins as an alternative energy source. Egg yolk contributes ATP and ADP, serving as an energy source for spermatozoa (Bilodeau et al. 2002). Amino acids found in egg yolks, like L-phenylalanine, L-tryptophan, and L-tyrosine, are involved in oxidative deamination to produce hydrogen peroxide, which helps sustain spermatozoa motility (Aitken et al. 2015).

Preservation at 4-5°C for 21 hours significantly decreased the percentage of intact membranes in *Muscovy duck* spermatozoa across all treatment groups ($P<0.05$, respectively). Comparing preserved semen to fresh semen, the percentage of intact spermatozoa membranes is reduced by 20-30%. In addition, 10% egg yolk added to the extender results in approximately 60% spermatozoa with an intact acrosome cap, whereas 5% egg yolk added results in only around 55% (Figure 1b).

The number of intact acrosome caps in *Muscovy duck* spermatozoa decreased by 25–30% after 21 hours of preservation at 4–5°C compared to fresh sperm. After 21 hours of preservation, the acrosomes remained intact when 10% and 15% egg yolk were added to the extender, compared to the 5% egg yolk addition. The proportion of intact acrosome caps in Muscovy duck spermatozoa preserved in extenders containing 10% and 15% egg yolk is approximately 60%. In contrast, the proportion is approximately 55% in spermatozoa preserved in extenders containing 5% egg yolk (Figure 2).

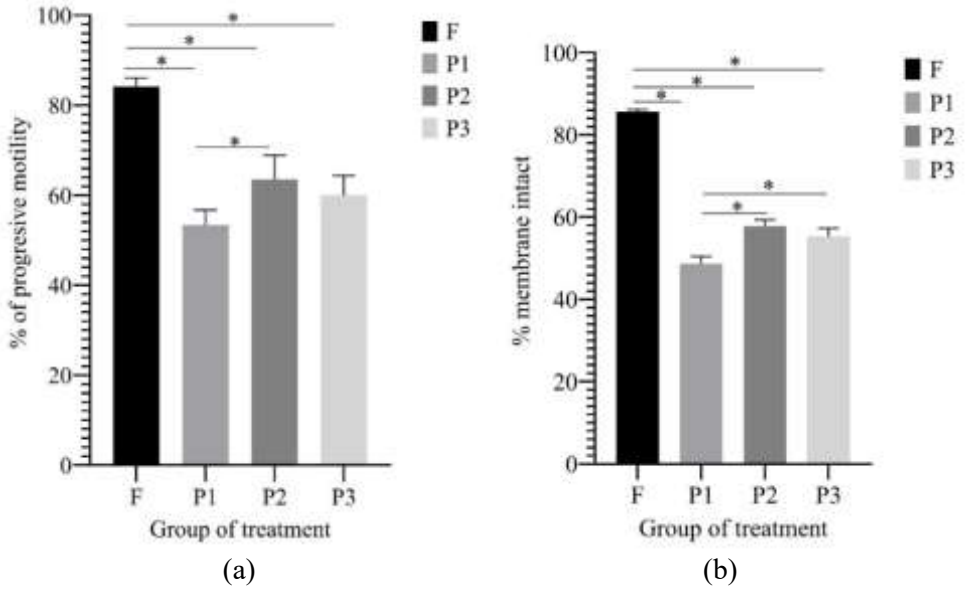

Figure 1. Spermatozoa progressive motility (a) and Spermatozoa membrane intact (b). F= fresh semen, P1= Addition of 5% Egg Yolk in Tyrode extender, P2= Addition of 10 % Egg Yolk in Tyrode extender, and P3= Addition of 15% Egg Yolk in Tyrode extender. Data presented as average bars with * means significant differences among the experimental groups (P<0.05).

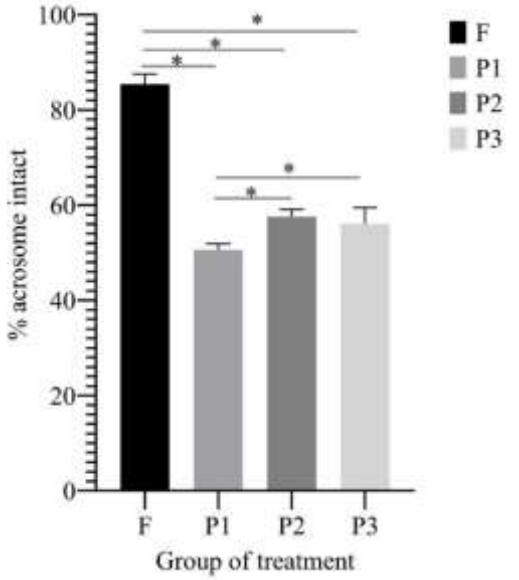

Figure 3. Intact acrosome cap of sperm. F= fresh semen, P1= Addition of 5% Egg Yolk in Tyrode extender, P2= Addition of 10 % Egg Yolk in Tyrode extender, and P3= Addition of 15% Egg Yolk in Tyrode extender. Data presented as average bars with * significant differences among the experimental groups (P<0.05)

Furthermore, there was a significant increase in the percentage of *Muscovy duck* spermatozoa showing abnormal morphology after being preserved at 4-5ºC for 21 hours compared to fresh semen. There was no significant difference in the increase of spermatozoa with abnormal morphology across all treatment groups (P>0.05, respectively). However, Muscovy duck spermatozoa preserved in the extender with the addition of 15% egg yolk showed the most significant percentage of spermatozoa with abnormal morphology (Figure 4).

A functioning membrane plays a role in the fertilizing ability of spermatozoa, as it is essential for spermatozoa capacitation, acrosome reaction, and adherence of the spermatozoa to the oocyte surface (Gadella & Luna 2014). Throughout this research, we utilize an HOS test to predict membrane integrity by assessing the spermatozoon's membrane ability to maintain equilibrium between the spermatozoon cell and its surrounding environment. Generally, preservation-induced stress on sperm occurs by altering the structure

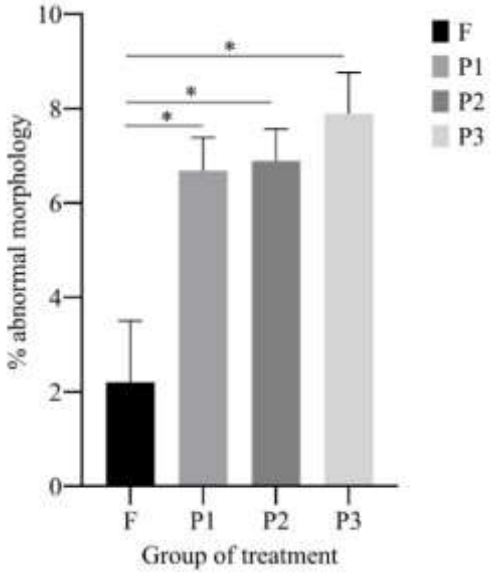

Figure 4. Spermatozoa with abnormal morphology. F= fresh semen, P1= Addition of 5% Egg Yolk in Tyrode extender, P2= Addition of 10 % Egg Yolk in Tyrode extender, and P3= Addition of 15% Egg Yolk in Tyrode extender. Data presented as average bars with * significant differences among the experimental groups (P<0.05)

of plasma membrane phospholipids, resulting in malfunction and increased membrane permeability (Cotter et al. 2005; Wongtawan et al. 2006). Tyrode diluent, which is adding egg yolk, will undoubtedly contain cholesterol, which comes from the egg yolk, provides a denser plasma membrane structure to shield spermatozoa from cold shock and help retain fluidity (Bathgate et al. 2006). The research found that adding 10% egg yolk to the extender provided better protection for the membranes of Muscovy duck spermatozoa during preservation. Moreover, adding 10% egg yolk to the skim milk extender resulted in better sperm motility than adding 15% egg yolk (Yuniar et al. 2021). Adding 10% egg yolk to Ringer Lactate diluent in Pelung's chicken sperm (Hidayat et al. 2022) and spermatozoa in Boer goats improved motility more than adding 15% or 20% egg yolk (Ihsan 2011).

The acrosome reaction is essential for mammalian spermatozoa to penetrate the zona pellucida (Gerton, 2002). Spermatozoa undergo the acrosome reaction before binding to the zona pellucida in particular species, whereas in others, binding to the zona can only be initiated by spermatozoa that possess an intact acrosome cap (Fazeli et al. 1997). Based on our findings, the percentage of intact acrosome caps in Muscovy duck spermatozoa was substantially higher when 10% and 15% egg yolk were added to the extender during preservation at 4-5ºC, as compared to the addition of 5% egg yolk. The findings suggested that a 10% egg yolk addition was adequate to prevent the capitulation of Muscovy duck spermatozoa after the spontaneous acrosome reaction while preserving the spermatozoa, in contrast to the addition of 5% or 15% egg yolk in the extender.

Moreover, current research results indicate that adding egg yolk at 5%, 10%, and 15% of the extender significantly decreases the number of spermatozoa with abnormal morphology in Muscovy duck spermatozoa following 4-5ºC preservation. Moreover, adding 15% egg yolk in the extender resulted in a higher percentage of spermatozoa with abnormal morphology. The results indicated that the concentration of egg yolk is too high, causing an imbalance in osmotic pressure, which can damage the shape of spermatozoa. The addition of 5% and 10% egg yolk to Tyrode diluent can prevent abnormalities by protecting and maintaining the protein coat of spermatozoa from incoming fluids, which have low osmotic pressure. Furthermore, recent research findings have shown that integrating egg yolk at 5%, 10%, and 15% in the extender significantly reduces the percentage of Muscovy duck spermatozoa with abnormal morphology after preservation at 4-5ºC. The results indicated that the concentration of egg yolk is too high, causing an imbalance in osmotic pressure, which can damage the shape of spermatozoa.

The current research found that preserving Muscovy duck spermatozoa for 21 hours decreased the progressive motility, membrane integrity, acrosome intactness, and normal morphology of the spermatozoa. However, adding egg yolk to the extender helped slow down this decrease. The presence of lecithin and lipoprotein in egg yolk acts as an antioxidant capable of protecting spermatozoa and fighting free radicals so that damage to spermatozoa due to oxidative stress can be reduced by the Reactive Oxygen Species (ROS) process (Alvarez-Rodriguez et al. 2013; Mehdipour et al. 2018; Yustiti et al. 2021). The decrease in spermatozoa motility, intact membrane, intact acrosome cap, and aberrant

morphology in the high concentration of egg yolk extender is due to excess fat granules that hinder sperm movement by increasing energy consumption; this leads to a depletion of energy reserves in spermatozoa cells, causing a buildup of lactic acid due to metabolism, resulting in damage to the sperm cell membrane and a rise in abnormalities (Zamira & Aitken. 2016).

Moreover, spermatozoa undergo anaerobic metabolism due to an insufficient energy supply, resulting in the production of lactic acid and a reduction in pH and motility. Disruption of the plasma membrane causes damage to metabolic reactions within the cell membrane (Santoso et al. 2020). Plasma membrane damage results in the disappearance of the aspartate aminotransferase enzyme, leading to a lack of energy reshuffling and subsequent loss of motility in spermatozoa (Septiyani et al. 2013). Lastly, adding egg yolk into the extender can be beneficial and may be suggested as a standard practice for preserving *Muscovy duck* spermatozoa.

CONCLUSSION

It can be concluded that adding 10% egg yolk to the extender substantially improved sperm motility, membrane integrity, and acrosome integrity in *Muscovy duck* sperm, because it is cheaper, especially when applied to small farmers.

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REFERENCES

Aitken JB, Naumovski N, Curry B, Grupen CG, Gibb Z, Aitken RJ. 2015. Characterization of an L-amino acid oxidase in equine spermatozoa. Biol Reprod. 92:1–13. DOI:10.1095/biolreprod.114.126052.

Allai L, Benmoula A, Marciane da Silva M, Nasser B, El Amiri B. 2018. Supplementation of ram semen extender to improve seminal quality and fertility rate. Anim Reprod Sci. 192:6–17. DOI:10.1016/j.anireprosci.2018.03.019.

Alvarez-Rodríguez M, Alvarez M, Anel-López L, Martínez-Rodríguez C, Martínez-Pastor F, Borragan S, Anel L, De Paz P. 2013. The antioxidant effects of soybean lecithin- or low-density lipoprotein-based extenders for the cryopreservation of brown-bear (*Ursus arctos*) spermatozoa. Reprod Fertil Dev. 25:1185–1193. DOI:10.1071/RD12181.

Arifiantini R. 2012. Semen collection and evaluation techniques in animals. Bogor (Indones): IPB Press.

Bathgate R, Maxwell WMC, Evans G. 2006. Studies on the effect of supplementing boar semen cryopreservation media with different avian egg yolk types on in vitro post-thaw sperm quality. Reprod Domest Anim. 41:68–73. DOI:10.1111/j.1439-0531.2006.00623.x.

Bilodeau J, Blanchette S, Cormier N, Sirard M. 2002. Reactive oxygen species-mediated loss of bovine sperm motility in egg yolk Tris extender: protection by pyruvate, metal chelators and bovine liver or oviductal ¯uid catalase Jean-FrancËois. Theriogenology. 57:1105–1122. DOI:10.1016/s0093-691x(01)00702-6.

Bustani GS, Baiee FH. 2021. Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. Vet World. 14:1220–1233. DOI:10.14202/vetworld.2021.1220-1233.

Cotter PZ, Goolsby HA, Prien SD. 2005. Preliminary evaluation of a unique freezing technology for bovine spermatozoa cryopreservation. Reprod Domest Anim. 40:98–99. DOI:10.1111/j.1439-0531.2004.00540.x.

Fazeli A, Hage WJ, Cheng FP, Voorhout WF, Marks A, Bevers MM, Colenbrander B. 1997. Acrosome-intact boar spermatozoa initiate binding to the homologous zona pellucida *in vitro*. Biol Reprod. 56:430–438. DOI:10.1095/biolreprod56.2.430.

Gadella BM, Luna C. 2014. Cell biology and functional dynamics of the mammalian sperm surface. Theriogenology. 81:74–84. DOI:10.1016/j.theriogenology.2013.09.005.

Gerton GL. 2002. Function of the sperm acrosome. Fertilization. Fertilization. Hardy DM, editor. Academic Press. p.265–302. DOI:10.1016/B978-012311629-1/50010-3.

Hidayat N-, Ismoyowati I, Tugiyanti E, Suswoyo I, Rosidi R, Sulistyawan IH, Nugroho AP. 2022. Date extract supplementation in ringer lactate-egg yolk extender on post-thawing quality of Pelung chicken spermatozoa. J Kedokt Hewan. Indones J Vet Sci. 16:110–114. DOI:10.21157/j.ked.hewan.v16i3.24317.

Ihsan MN. 2011. Penggunaan telur itik sebagai pengencer semen kambing. J Ternak Trop. 12:10–14.

Iskandar H, Sonjaya H, Arifiantini RI, Hasbi H. 2022. The quality of fresh and frozen semen and its correlation with molecular weight of seminal plasma protein in Bali cattle. Trop Anim Sci J. 45:405–412. DOI:10.5398/tasj.2022.45.4.405.

Kumar A, Prasad JK, Srivastava N, Ghosh SK. 2019. Strategies to minimize various stress-related freeze-thaw damages during conventional cryopreservation of mammalian spermatozoa. Biopreserv Biobank. 17:603–612. DOI:10.1089/bio.2019.0037.

Lan L, Worowan L. 2021. Variations in morphometric traits of Muscovy ducks measured at PNGUNRE Poultry Farm. Int J Poult Sci. 20:243–248. DOI:10.3923/ijps.2021.243.248.

Mehdipour M, Daghigh Kia H, Moghaddam G, Hamishehkar H. 2018. Effect of egg yolk plasma and soybean lecithin on rooster frozen-thawed sperm quality and fertility. Theriogenol. 116:89–94. DO:10.1016/j.theriogenology.2018.05.013.

Rizal M, Herdis. 2005. Viability of frozen-thawed epididymal sperm of Garut ram cryopreserved with modified tris extender. HAYATI J Biosci. 12:61–66. DOI:10.4308/hjb.12.2.61.

Salmani H, Towhidi A, Zhandi M, Bahreini M, Sharafi M. 2014. *In vitro* assessment of soybean lecithin and egg yolk based diluents for cryopreservation of goat semen. Cryobiol. 68:276–280. DOI:10.1016/j.cryobiol.2014.02.008.

Santoso BI, Saleh DM, Mugiyono S. 2020. The effect of egg yolk levels in skim milk diluents and storage time to the motility and abnormalities of kampung rooster spermatozoa Kampung. J Anim Sci Technol. 2:1–11.

Septiyani R, Arifiantini R, Susnawati T. 2013. Hubungan antara Viabilitas, motilitas dan keutuhan membran plasma spermatozoa semen beku Sapi Limousin. In: Pros Semin Nas Asos Reproduksi Hewan Indones. Bogor (Indones): Bogor Agricultural University.

Upadhyay VR, Ramesh V, Dewry RK, Kumar G, Raval K, Patoliya P. 2021. Implications of cryopreservation on structural and functional attributes of bovine spermatozoa: An overview. Andrologia. 53:1–16. DOI:10.1111/and.14154.

Watanabe M, Sugomiri Y. 1957. Studies on the artifical insemination of duck. Zootech e Vet. 12:119–124.

Widyastuti R, Haq NMD, Pristihadi DN, Wahyudin W, Maheshwari H, Sumantri C, Boediono A. 2022. The viabilities of freeze-thaw Pasundan-Bull sperms after a short-term exposure to media with different pHs. Trop Anim Sci J. 45:270–276. DOI:10.5398/tasj.2022.45.3.270.

Wongtawan T, Saravia F, Wallgren M, Caballero I, Rodríguez-Martínez H. 2006. Fertility after deep intra-uterine artificial insemination of concentrated low-volume boar semen doses. Theriogenol. 65:773–787. DOI:10.1016/j.therioge nology.2005.07.003.

Yuniar TU, Saleh DM, Mugiyono S. 2021. Pengaruh Penambahan Kuning Telur pada Pengencer Susu Skim dan Lama Penyimpanan pada Suhu 5^{o}C Terhadap Kualitas Spermatozoa Ayam Pelung. J Anim Sci Technol. 3:29–46.

Yustiti A, Husmaini, Jaswandi. 2021. The effect of giving honay end egg yolk mixture to the quality of spermatozoa, fertility and hatchability of Sentul chicken. Biospecies. 14:36–45. DOI:10.22437/biospecies.v14i1.10963.

Zamira G, Aitken RJ. 2016. The Impact of Sperm Metabolism during in Vitro Storage: The Stallion as a Model. Biomed Res Int. DOI:10.1155/2016/9380609.

Relationship Between Growth Performance and Metabolite Profile of Broiler Chickens Supplemented with Probiotics *Bacillus coagulans* and *Lactobacillus plantarum*

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ABSTRAK

Pradhika EI, Astuti RI, Meryandini A. 2025. Hubungan performa tubuh dan profil metabolit pada ayam broiler dengan suplementasi probiotik *Bacillus coagulans* and *Lactobacillus plantarum*. JITV 30(2):115-125. DOI:http://dx.doi.org/10.14334/jitv.v30i1.3453.

Suplementasi probiotik merupakan alternatif dari Antibiotic Growth Promotor. Probiotik L. plantarum dan B. coagulans diketahui dapat meningkatkan performa pertumbuhan ayam broiler. Informasi mengenai hasil metabolit kedua probiotik dengan inangnya masih terbatas. Penelitian ini bertujuan untuk mengidentifikasi metabolit pembeda antara Bacillus coagulans dan Lactobacillus plantarum dan metabolit yang berhubungan dengan peningkatan performa pertumbuhan ayam broiler dengan hasil suplementasi probiotik . Sebanyak 120 ekor Ayam Broiler unsexRoss 308 diberikan pakan perlakuan dengan Kontrol Negatif (NC), L. plantarum (LP), B. coagulans (BC), dan B. coagulans dicampur dengan L. plantarum (BCLP). Parameter kinerja pertumbuhan yang dievaluasi adalah rata-rata bobot badan (average Body Weight/ avg BW), konversi pakan (adjustment Feed Conversion Ratio/adjFCR), konsumsi pakan kumulatif(cumulative Feed Intake/cumFI) dan faktor efisiensi performa (Performance Efficiency Factor/PEF). Analisis metabolik dilakukan dengan metode pemprofilan metabolit tidak tertarget pada sampel sekum yang terdiri dari analisis senyawa spektrum luas dan analisis senyawa volatil. Hasil penelitian menunjukkan bahwa kinerja pertumbuhan (avg BW) yang berbeda nyata ($p \leq 0,05$). Sedangkan parameter lainnya yaitu, adjFCR, cumFI, dan PEF, tidak memberikan perbedaan yang signifikan ($P > 0,05$). Metabolit pembeda yang penting antar perlakuan adalah asam asetat, asam laktat, asam butanoat, 1-oktadekanol, dan asam palmitat. Metabolit yang dapat dinyatakan sebagai metabolit pembeda antara LP dan BC adalah asam asetat, asam laktat, dan asam butanoat. Sedangkan metabolit yang dapat dinyatakan sebagai metabolit pembeda adalah asam laktat sebagai pembeda kinerja avgBW yang baik dan 1-oktadekanol dan asam palmitat sebagai pembeda tanpa suplementasi probiotik. Kesimpulan dari studi ini adalah asam asetat, asam laktat dan asam butanoat adalah metabolit pembeda antara probiotik B. coagulans dan L. plantarum dan asam laktat sebagai metabolit pembeda kinerja yang baik.

Kata Kunci: Ayam Broiler, Metabolit Pembeda, Metabolit, Performa, Probiotik

ABSTRACT

Pradhika EI, Astuti RI, Meryandini A. 2025. Relationship of growth relationship between growth performance and metabolite profile of broiler chickens supplemented with probiotics *Bacillus coagulans* and *Lactobacillus plantarum*. JITV 30(2): 115-125. DOI:http://dx.doi.org/10.14334/jitv.v30i1.3453.

Probiotic supplementation is an alternative to Antibiotics Growth Promotor. The probiotics L. plantarum and B. coagulans are known to improve the growth performance of broiler chickens. Information regarding the metabolite results of these two probiotics with their hosts is still limited. This study aims to identify differentiating metabolites between Bacillus coagulans and Lactobacillus plantarum and metabolites associated with enhanced growth performance in chickens supplemented with probiotics. A total of 120 unsexed Ross 308 Broilers were given a treated diet with Negative Control (NC), L. plantarum (LP), B. coagulans (BC), and B. coagulans mixed with L. plantarum (BCLP). The growth performance parameter evaluated was the average Body Weight (average BW), adjustment Feed Conversion Ratio (adjFCR), cumulative Feed Intake (cumFI), and Performance Efficiency Factor (PEF). Metabolomic analysis was carried out using the untargeted metabolite profiling method on cecum samples, consisting of broad-spectrum and volatile compound analyses. The study shows that analysis of differences in growth performance resulted in only the avgBW parameter being significantly different ($P \leq 0.05$). Meanwhile, other performance parameters, adjFCR, cumFI, and PEF, do not provide any significant difference ($P > 0.05$). The important differentiating metabolites between treatments are acetic acid, lactic acid, butanoic acid, 1-octadecanol, and palmitic acid. Metabolites that can be stated as differentiating metabolites between LP and BC are acetic acid, lactic acid, and butanoic acid. Meanwhile, metabolites that can be declared differentiating metabolites are lactic acid as a differentiator for good avgBW performance and 1-octadecanol and palmitic acid as differentiators without probiotic supplementation. It can be concluded that

acetic acid, lactic acid, and butanoic acid are the metabolites that differentiate the probiotics *B. coagulans* and *L. plantarum* and lactic acid as a differentiating metabolite of good performance.

Key Words: Broiler Chicken, Differentiating Metabolites, Metabolite, Performance, Probiotics

INTRODUCTION

The chicken farming industry continues to develop to meet the increasing need for animal protein. One important factor in achieving optimal growth and health of chickens is using feed enriched with AGP (Antibiotic Growth Promoter). The prohibition of AGP dramatically influences the productivity of broiler chickens in Indonesia as an implementation of Law (UU) Number 41 of 2014 concerning Amendments to Law Number 18 of 2009 concerning Livestock and Animal Health concerning the ban on using antibiotics and certain hormones as feed additives; this encourages researchers to develop safe and effective AGP alternatives. One solution to this problem, called AGP replacer, is probiotic supplementation. Probiotics have been widely used in the feed industry today as AGP replacers. Some of the advantages of using probiotics in the digestive tract are stimulating beneficial microbes, preventing pathogen colonization by competition for attachment to the epithelium, reducing pH conditions, producing organic acids, forming compounds with systemic effects, and modulating the immune system (Abd El-Hack et al. 2020; Tran et al. 2022).

One of the LAB (Lactic Acid Bacteria) based probiotics is the *L. plantarum* strain. Feed supplemented with *L. plantarum* strain B1 was shown to reduce the number of *E. coli*, increase other LAB bacteria, produce SCFA (Short Chain Fatty Acid), and improve broiler performance (Peng et al. 2016). One type of SFB (Spore Former Bacteria) used as probiotics is *B. coagulans* (Gu et al. 2015). *B. coagulans* strain R11 was shown to prevent oxidative damage and reduce the abundance of pathogens such as *E. coli*, *P. aeruginosa*, and Salmonella (Xing et al. 2020). One approach to studying the efficacy of probiotics on the health of their hosts is through the metabolomics approach. Metabolite profiling of untargeted compounds allows for identifying compounds that undergo significant concentration changes under different treatment conditions (Frainay and Jourdan 2017).

Liu et al. (2020) explained that the metabolite results from probiotics could generally be lactic acid, hydrogen peroxide, secreted proteins (extracellular proteins), organic acids, indole, bacteriocins, and antimicrobial peptides. Wang et al. (2021) provide an overview of the characteristics of LAB metabolites as probiotic functions, including being able to produce short-chain fatty acids, amines, bacteriocins, vitamins and exopolysaccharides. According to Elshaghabee et al. (2017), Bacillus spp. stimulate the immune system and produce several antimicrobial substances, e.g., bacteriocins like inhibitory substances and antibiotics.

Probiotics based on *L. plantarum* and *B. coagulans* improve broiler growth performance (Khajeh Bami et al. 2020; Peng et al. 2016). However, this characteristic is unclear in identifying metabolite compounds that distinguish the two types of probiotics. The metabolomic analysis provides an overview of the diversity of metabolite compounds from probiotics. Therefore, it is necessary to know the profile of metabolite diversity between the two probiotics, which is associated with growth performance. This study aimed to identify the differences in metabolite profiles between *B. coagulans* and *L. plantarum* and to determine the metabolites that distinguish good growth performance.

MATERIALS AND METHODS

This research was conducted at the research farm (AME House/Digestibility Assay Unit, House 7, Room A & B, closed house) of PT Nugen Bioscience Indonesia, Malingping, Banten and applied chemistry department laboratory of PT Nugen Bioscience Indonesia, Ancol, North Jakarta. The Animal Ethics Committee School of Veterinary Medicine and Biomedical Science IPB University approved the experiment with approval number 070/KEH/SKE/VII/2023.

Treatment, experimental design and poultry management

One hundred and twenty DOC Broiler (46±0.1g) Ross 308 grade A3 unsex (PT Charoen Pokphand Jaya Farm, Tangerang, Indonesia) were randomly divided into four dietary treatments: LP (*L. plantarum*), BC (*B. coagulans*), BCLP (*B. coagulans* and *L. plantarum*) and NC (no probiotics) with 6 replicates per treatment and 5 bird per replicates. Twenty-four cages were arranged on racks randomly divided into two rooms (rooms A and B). Each room contained 12 cages with three replications of each treatment. Each cage (0.61×0.61×0.39 m, 0.145 m^3) consists of 2 nipple drinkers/cage, 1 bell drinker/cage, 1 feeder/cage, and 1 lamp/cage. Each room (8×5×2.5 m, 100 m^3) contains 4 cage racks/room, 1 fan unit/room, and 1 water tank unit/room. Cages were cleaned with disinfectant [peracetic acid-hydrogen peroxide-acetic acid (Cid 2000, PT SHS International, Jakarta, Indonesia)].

Rooms were fumigated (75 g peroxyacetic acid (Forcent Fumigant, PT Indovetraco Makmur Abadi, Jakarta, Indonesia) added to 150 ml formaldehyde 37 % (Formac, PT Indovetraco Makmur Abadi, Jakarta, Indonesia) for an area of 5 m^3) before use to prevent contamination. DOCs were weighed before being put into the cages. Feeding was done using an ad libitum feeding system according to the treatment. In the starter/brooding phase (0-10 days), DOC was fed with S10 feed, and in the grower and finisher phases (11-30 days), they were fed with S11 feed, which has been supplemented with probiotic products according to the treatment. The nutritional content of the feed can be seen in Table 1. Room temperature was maintained according to Ross 308 guidelines (Aviagen, 2018) by adding a heat source lamp in the brooding phase, adjusting the frequency of ventilation opening, and setting the fan switch. Chicken performance was determined by measuring avgBW (average Body Weight at 10, 21, and 30 days), cumFI (cumulative Feed Intake), adjFCR (adjusted Feed Conversion Ratio), PEF (Performance Efficiency Factor), and mortality parameters calculated according to Ross 308 Aviagen (2018) and Martínez & Valdivié (2021) standard guidelines which can be seen in the Table 2. All chickens were then slaughtered, and cecum content samples were aseptically removed from the chickens using scissors and tweezers and placed into labeled sterile tubes. Samples were frozen with dry ice in an ice box and then stored in an ultra-low temperature freezer (Kaltis 390, Taipei, Taiwan) at -80°C, according to Zhou et al. (2021).

Feed preparation

Probiotic products consist of *L. plantarum* N1A1 or *B. coagulans* BR04 mixed in a carrier (corn starch and $CaCO_3$) (PT Nugen Bioscience Indonesia, Jakarta, Indonesia) with concentration >10^6 CFU/g. Starter (S10) and grower-finisher (S11) phase feeds (PT Charoen Pokphand Indonesia, Balaraja, Indonesia) were each mixed with 1.5 % probiotic product to produce a minimum concentration of 10^8 CFU/kg feed as recommended by Wang et al. (2021). Feed is mixed with a mini-feed mixer for 15 minutes at room temperature. The NC treatment was supplemented with products without probiotics (only carriers).

Metabolomic analysis

. The extraction and derivatization step for metabolomic analysis of untargeted broad-spectrum compounds was adopted from Fiehn (2016) of standard mix QC. Acetonitrile:Isopropanol: Water (AIW) solution (Merck, Darmstadt, Germany) with a ratio of 3:3:2 was purged with N_2 gas from gas generator (Proton N341M, Proton OnSite, USA) for 5 min and then cooled at -20 °C. 25-30 mg of cecum content sample was weighed with analytical balance (Precisa XB 220A, Dietikon, Switzerland) into a 2 ml microtube, and 1 ml of AIW was added. The microtube was mixed with a vortex (Heidolph REAX control, Schwabach) for 10 s followed by one h sonication (Elmasonic P300H, Singen, Germany) at 35°C and then centrifuged (Biofuge Fresco Sorvall, Thermo Fisher Scientific, Waltham, USA) at 13,000 ×*g* for 2 min. 450 µl of supernatant was separated into a new microtube and concentrated with a vacuum concentrator (Concentrator 5301, Eppendorf, Hamburg, Germany) for 2 hours at 45°C. MeOX solution was prepared by mixing 20 mg methoxyamine HCl (Sigma Aldrich, Massachusetts, USA) and 1 ml pyridine (Merck, Darmstadt, Germany), then sonicated for 15 min at 60 °C. The concentrated microtube was added with 50 µl MeOX, followed by 1.5 hours of sonication at 30°C. Then 100 µl of MSTFA (N-methyl-N-(trimethylsilyl)-trifuoroacetamide, Merck, Darmstadt, Germany) was added and sonicated for one h at 37°C and then centrifuged at 13,000 ×*g* for 10 min at 18°C. The supernatant obtained was then transferred to a vial insert and placed into a GC vial. The samples in the vials were then randomly arranged and analyzed using a gas chromatography system with specifications according to Jain et al. (2019) below. GC system: Agilent 7000C Triple Quadrupole GC/MS System (Agilent, Santa Clara, USA); column: HP-5MS Ultra

Table 1. Nutritional content of S10 and S11 feed

Parameter	S10 feed	S11 feed
Moisture (%)	10.59	10.79
Fat (%)	5.56	6.04
Fiber (%)	4.94	6.32
Protein (%)	20.22	19.31
Ash (%)	5.44	5.39
ME (kkal/kg)	3155	3122

ME= Metabolism Energy

Table 2. Calculation formula for performance parameter

Parameter	Formula
avgBW (g/bird)	total bird weight/number of birds.
cumFI (g/bird)	Average daily Feed Intake (avgDFI) × number of birds × number of days
adjFCR	actFCR + (target body weight – actual body weight / 4500 g).
actFCR	total feed consumed / total bird weight
PEF	livability × bird weight (kg) / age (days) × FCR
livability (%)	The final number of birds/initial number of birds × 100
mortality (%)	total death or culling/number of birds × 100

Inert (30 m×0.25 mm×0.25 µm); gas: He (2.25 ml/min); injection volume: 1 µl; delay: 4 min; inlet: splitless, 250°C, 14.7 psi; oven: 75°C, 280°C (4°C/min, 1.56 min); detector: MS, source: 230°C, 40-600 m/z, scan time: 0.2 s.

Extraction and derivatization methods in metabolomics analysis for volatile targeted compounds were adopted from Hsu et al. (2019). Partially frozen samples stored at -80°C were freeze-dried with a freeze dryer for 24 hours. Samples that were not analyzed immediately could be stored again at -80°C. A dry sample of 0.02 g was weighed in a microtube, and 1 ml of 0.5% phosphoric acid (Merck, Darmstadt, Germany) was added. The sample was vortexed for 30 s and then centrifuged at 3,000×*g* for 10 min. 60 µl of supernatant was removed, and 240 µl of 0.5% phosphoric acid and 300 µl of butanol (Merck, Darmstadt, Germany) were added. The sample was vortexed for 30 s, shaken for 5 min, followed by sonication for 5 min. Then, the microtube was centrifuged at 3,000 ×*g* for 10 min. 180 µl of supernatant (organic layer) was transferred to a vial insert, and 20 µl of butanol was added. The samples in the vials were then randomly arranged and analyzed using a gas chromatography system according to the following specifications. GC system: Agilent 7000C Triple Quadrupole GC/MS System (Agilent, Santa Clara, USA); column: DB-WAXms (30 m×0.25 mm×0.25 µm); gas: He (2.25 ml/min); injection volume: 1 µl; delay: 4 min; inlet: splitless, 250°C, 14.7 psi; oven: 70°C, 170°C (10°C/min, 0 min), 240°C (25 °C/min, 15 min); detector: MS, source: 230°C, 40-550 m/z, scan time: 0.2 sec. Solvent blank, reagent (derivatization) blank, and method blank were selected as quality control for each batch analysis (Fiehn 2016; Broadhurst et al. 2018; Eurachem 2019).

Data analysis

Raw growth performance data were processed, and the significance of performance was determined by statistical analysis on Minitab® 16.1.1.1 (Minitab Ltd, Pennsylvania, USA). The statistical analysis stages performed were (1) outlier identification (NIQR box-plot), (2) assumption checking (data normality test: Shapiro-Wilk test, homogeneity or homoscedasticity of data: Bartlett test), (3) omnibus test (parametric test: ANOVA or non-parametric test: Kruskal-Wallis), and (4) post hoc test (parametric test: Tukey test or non-parametric test: Dunn test) (Granato et al. 2014). Non-parametric tests are performed for samples <15 data.

Chromatogram data from the metabolomic analysis was processed using Masshunter Qualitative Analysis B.07.00 software (Agilent, Santa Clara, USA). Chromatogram peaks with a minimum height of 10^5 mAU (mili Absorbance Unit) were identified from the TIC (Total Ion Chromatogram), and then the deconvolution process was performed. The detected peaks were then matched to the National Institute of Standards and Technology (NIST) database with a similarity score of at least 80 %. Raw data in peak intensity height, RT (Retention Time), and compound name were processed in MS Excel by adopting the procedure from Fiehn (2016). Curation data from the analysis of broad-spectrum and volatile compounds were combined into one, then outlier identification, compound name filtering and box-plot generation using MS Excel. Multivariate analysis and compound categorization were performed with MetaboAnalyst 5.0 (Wishart Research Group, Alberta, Canada). The compounds obtained were grouped by class using the 'Enrichment Analysis' feature. All compounds identified by NIST from the two metabolomic analyses were confirmed by (the Human Metabolome Database) HMDB library-based matching. Compounds identified but not matched and indicated not to be metabolites were excluded from further analysis. The proportion (p) of data was determined by calculating the number of metabolites that appeared (x) per number of samples (n). Identified metabolites that have a proportion >0.8 are then processed using the 'Statistical Analysis (one factor)' feature on MetaboAnalyst 5.0 with the stages of (1) data upload, (2) data integrity checking, (3) data filtering, (4) data normalization ($\log_{10}$) and (5) statistical processing. This statistical process is divided

into three: (5a) Principal Component Analysis (PCA) (score plot and loading plot), (5b) PLS-DA (Partial Least-Squares Discriminant Analysis) (Variable Importance in Projection (VIP) score) and (5c) heatmap.

RESULTS AND DISCUSSION

Growth performance

The chicken used in this study used the Ross 308 strain. This Ross 308 strain performs better than other strains in BW and FCR parameters (Martínez & Valdivié 2021). The total number of samples was 30 for each treatment except BCLP, which had 29 samples due to one bird being affected by the Runting Stunting Syndrome (RSS). RSS in broiler chickens is observed on 4-7 days with shorter shanks, lower body weight, pale, distention of the abdomen, poor feather development, listlessness, and diarrhea (Li et al. 2020; Aviagen 2018).

Assumption tests were conducted to ensure that the data followed a normal distribution pattern and that data variance was homogeneous (Kozak & Piepho 2018; Orcan 2020). The RSD of the avgBW parameter ranges from 7.08 to 11.07 %. Data uniformity is acceptable if the ±RSD value is <10 % (Aviagen 2018). The performance profile of the chickens showed that only avgBW was significantly different after ANOVA and Tukey tests with the highest to lowest weights in order: 0-10 days (LP^{a}, $BCLP^{ab}$, NC^{b}, BC^{b}), 0-21 days: (LP^{a}, $BCLP^{b}$, BC^{b}, NC^{b}), 0-30 days (LP^{a}, BC^{ab}, $BCLP^{ab}$, NC^{b}). The data indicate that treatment with *L. plantarum* yields the best avgBW performance across all rearing periods.

Table 3. Growth performance for parameters avgBW, cumFI, adjFCR, mortalityPEF with RSD values and letter notations from post hoc tests for data significance

Parameters	Treatment	0-10 d	0-21 d	0-30 d
avgBW (g/bird) ±RSD (%) (P≤0.05)	NC	300.57^{b}±10.77	940.67^{b}±10.85	1843.57^{b}±11.57
	LP	319.5^{a}±9.72	1057^{a}±8.72	1999.43^{a}±9.21
	BC	299.5^{b}±8.66	972.33^{b}±7.99	1895.87^{ab}±10.06
	BCLP	299.67^{ab}±10.86	994.69^{b}±9.43	1892.96^{ab}±7.08
cumFI (g/bird) ±RSD (%) (P≤0.05)	NC	313.57±8.49	1232.77±10.31	2553.53±7.21
	LP	330.30±6.14	1336.37±10.57	2748.00±7.77
	BC	316.43±4.93	1257.17±4.01	2605.63±3.73
	BCLP	311.17±2.86	1269.43±6.05	2619.88±4.76
adjFCR ±RSD (%) (P≤0.05)	NC	1.05±7.83	1.33±9.36	1.38±6.14
	LP	1.04±8.18	1.25±8.19	1.33±3.36
	BC	1.06±1.63	1.30±3.86	1.35±2.07
	BCLP	1.05±4.70	1.28±8.66	1.36±7.48
mortality (%)	NC	0	0	0
	LP	0	0	0
	BC	0	0	0
	BCLP	0	0	3.33
PEF ±RSD (%) (p≤0.05)	NC		444.62±9.75	
	LP		485.64±2.75	
	BC		459.88±4.53	
	BCLP		458.57±6.93	

NC= negative control, LP = *Lactobacillus plantarum,* BC= *Bacillus coagulans,* BCLP= *Bacillus coagulans* & *Lactobacillus plantarum,* avgBW= average body weight, cumFI= cumulative feed intake, adjFCR= adjusted feed conversion ratio, PEF= performance efficiency Factor on 0-30 day observation, RSD= relative standard deviation

The non-parametric significance difference test (Kruskal-Wallis) was conducted on several other performance parameters (cumFI, adjFCR, and PEF). These parameters stated that they were not significantly different, as indicated by a P>0.05. However, when viewed from the average data, the LP treatment still has the best value compared to other treatments. One bird in the BCLP treatment was excluded due to stunting, resulting in a mortality rate of 3.33%.

FCR measures feed utilization efficiency or production efficiency; the smaller the FCR value, the better or more efficient (Prakash et al., 2020). Meanwhile, PEF is used to measure overall growth performance, which indicates that the higher the PEF value, the better the growth performance (Aviagen 2018). Ross 308 broilers at 28 and 35 days will have FCR (1.5 and 1.56) and PEF (371.31 and 405.65), respectively (Petričević et al. 2024). The results of this study showed that all treatments had FCR and PEF values better than the performance in large-scale rearing. Comparisons can also be made with the parameter values of the Ross 308 growth standard (Aviagen 2022). Compared to this standard, avgBW values were higher in all treatments for 0-30 days. While cumFI was higher than the standard in all treatments and all rearing days. However, adjFCR had worse results for all treatments and rearing days. Other studies have found that *L. plantarum* can significantly improve chicken growth performance (Banu et al. 2019; Peng et al. 2016; Humam et al. 2019; Wang et al. 2023). Separately, *B. coagulans* has also been shown to improve chicken performance (Zhang et al. 2021; Zhen et al. 2018). A comparison between *L. plantarum* and another endospore-forming probiotic (*B. subtilis*) in broilers (22-98 days old) studied by Nam et al. (2022) showed that treatment with *L. plantarum* resulted in better BW compared to *B. subtilis* while the FCR parameters demonstrate no significant differences, with the best value is observed with *L. plantarum* treatment. At the same time, the study on the effects of *L. plantarum* and *B. coagulans* on broilers (1-42 days) shows that the treatment with *L. plantarum* yields the most favorable outcomes for the Average Daily Gain (ADG) and Average Daily Feed Intake (ADFI) but not FCR parameters (Yu et al. 2022).

Metabolite profile

Principal Component Analysis (PCA) is a method to reduce the dimensionality of specific datasets (Debik et al., 2022). It improves interpretability without losing much information (Hasan & Abdulazeez, 2021). The PCA score plot between the overlapping treatment groups in Figure 1 shows no significant difference between the treatments. If there is no clear separation between groups on the PCA graph, then there is no significant effect between treatments, and it can be considered indistinguishable (Fiehn 2016; Jiang et al. 2022). However, the LP and BC treatment groups visually provide a more oval cluster than the other treatments. PCA loading plot graph serves to visualize the loading contribution of each metabolite to the variance observed in the data between treatments (Withers et al. 2020). The further away from the center, the more influential the metabolite is to the treatment (Ren et al. 2015).

In summary, the loading plot illustrates the direction of projection of the metabolite features of the PCA score plot in space where it has the most extended vector for the highest variation in the data (Van Dyk, 2022). Metabolites that contribute strongly, as seen in the loading plot in Figure 1, are palmitic acid, 1-octadecanol, and 5-oxoproline. Metabolites that indicated a negative correlation were 1-octadecanol and palmitic acid. One comparative study examining the metabolites of the probiotics *B. coagulans* and *L. plantarum* through untargeted metabolomic analysis is reported by Cukkemane et al. (2020). This study utilized various probiotics to ferment different milk media, including four lactic acid bacteria (LAB) and one spore-forming bacterium (SFB), specifically *B. coagulans* ATCC 12425 and *L. plantarum* NRC 716. The PCA and heatmap analysis results of each class of detected compounds indicated that *B. coagulans* and *L. plantarum* metabolites differed significantly. However, while utilizing the same bacteria, this study does not detail the chicken host's metabolite conditions, as it employs milk for fermentation. Zhang et al. (2023) reported significant differences in the PCA analysis of cecum samples from chickens undergoing LAB probiotic treatment in response to heat stress.

The PLS-DA score plot in Figure 2 does not demonstrate a clear separation between treatments. However, the clustering observed indicates that the BC, LP, and NC treatments exhibit distinct patterns and directions. Worley and Powers (2016) state that PLS-DA aggressively enforces separations between experimental groups and is often employed as an alternative method when PCA fails to reveal group separation. However, this practice carries significant risks. Without proper validation, PLS-DA can quickly produce statistically unreliable group separations. Q^2 is the estimated value of a model's predictive ability, calculated through cross-validation. A strong prediction will yield a high Q^2 value; conversely, if Q^2 is negative, the model is deemed non-predictive (Szymańska et al., 2012). This study's PLS-DA model demonstrates positive Q^2 values for three principal components (PCs), precisely 0.10, 0.13, and 0.14. Therefore, it can be concluded that the model has good predictive capability.

VIP (Variable Importance in Projection) is a parameter used to calculate a cumulative measure of the

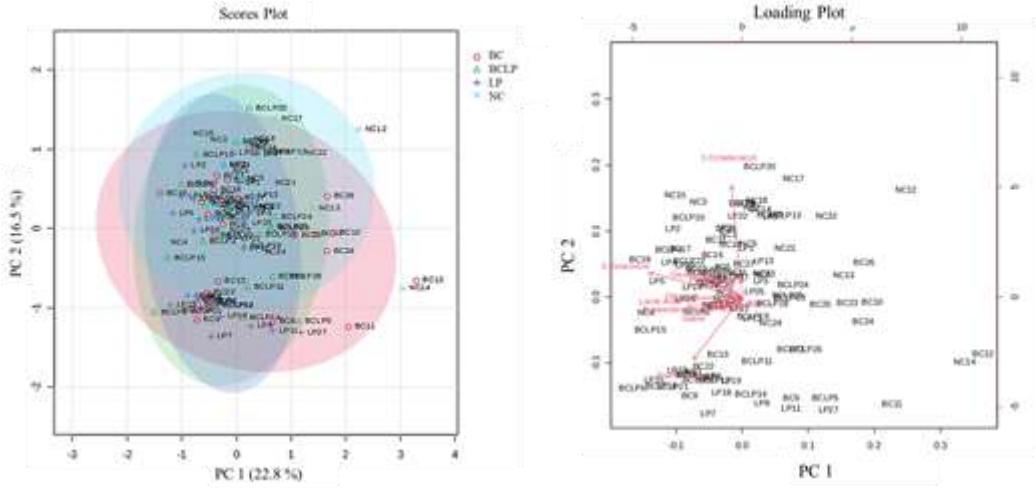

Figure 1. The PCA score plot (left) and loading plot (right) illustrate the differences between treatments and influential metabolite variance

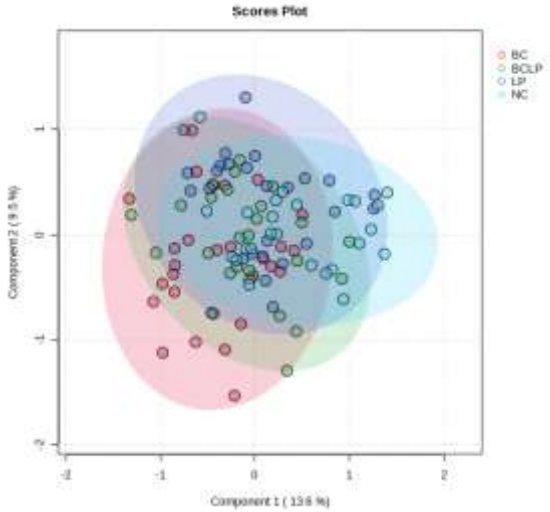

Figure 2. The PLS-DA score plot illustrates the enforced separation between treatments. Clustering indicates that the BC, LP, and NC treatments exhibit distinct patterns and directions but do not demonstrate clear separation

influence of individual variables on the model (Galindo-Prieto et al., 2014). This analysis reflects the loading weight for each component and the response variability explained by the PLS-DA components that can be used for feature selection (Thevenot 2016; Zheng et al. 2023). Metabolites (features) with VIP values >1 in PLS-DA models are identified as important differential metabolites (Deng et al. 2021; Gromski et al. 2015). Mapping metabolites between treatments on a heatmap provides an overview of the hierarchical clustering of metabolite profiles (Vacanti 2019). Heat maps allow users to easily visualize changes in metabolite concentration patterns across samples and treatments, displaying actual data values using color gradients (Chong & Xia 2020). The heatmap dendrogram in Figure 3 shows that LP treatments are grouped with BC and continue to be further grouped with NC. BC provided the most distinct profile compared to the other treatments. Separation of important metabolites is done using VIP analysis in PLS-DA. Metabolite screening based on VIP score >1 in Figure 3 resulted in palmitic acid, 1-octadecanol, acetic acid, lactic acid, and butanoic acid as important metabolites. Broiler chickens with poor performance are indicated by the increase of several metabolites in the cecum, namely D-mannose, hexadecanoic acid, cholesterol, L-valine, L-leucine, glutamic acid, glucopyranose, α-D-allopyranose and phosphoric acid (Chen et al. 2021). In this study, it was described as increasing 1-octadecanol and glycolic acid.

Relationship between chicken performance and metabolite profile

Rinttilä & Apajalahti (2013) summarize that metabolites derived from microbiota composition can influence growth performance and suggest that the

cecal microbial profile may reflect the efficiency of feed digestion and nutrient absorption in the intestine. The relationship between chicken performance and metabolite profiles can be summarized in Table 4. LP and BC profiles have significant metabolite differences and significant avgBW performance differences, particularly for 0-21 rearing days. The primary metabolites differentiating between LP and BC quite far from the white mid-spectrum were acetic acid, lactic acid, and butanoic acid. The BC treatment also had a significantly lower concentration than NC. Differentiating metabolites that are indicators of unsupplemented by probiotics are the decrease of palmitic acid and the increase of 1-octadecanol. Metabolites expressed as differentiating metabolites (biomarker candidates) are lactic acid as a good avgBW performance distinguisher and 1-octadecanol and palmitic acid as distinguishers without probiotic supplementation. Xing et al. (2020) reported changes in unique compounds that could serve as biomarkers in the digestive tract of laying hens supplemented with *B. coagulans* and exposed to lead (Pb). These changes included the presence of antioxidant and antibacterial compounds, such as 4-acetamido butanoic acid, dodecanoic acid, L-3-phenylacetic acid, apigenin, and daidzein. Zhang et al. (2021) found an increase in SCFA compounds such as acetic acid, propionic acid, butyrate, isobutyric acid, and valeric acid in the digestive tract of broiler chickens administered *B.* coagulans. Additionally, Ito et al. (2022) noted that while the concentrations of certain SCFAs, such as propionate and butyrate, would increase, other types, including acetate and lactate, would decrease.

Analysis of differences in growth performance characteristics in the administration of probiotics *L. plantarum* and *B. coagulans* resulted in only avgBW parameters significantly different with the highest to lowest weights in order LP, BCLP, BC, NC.

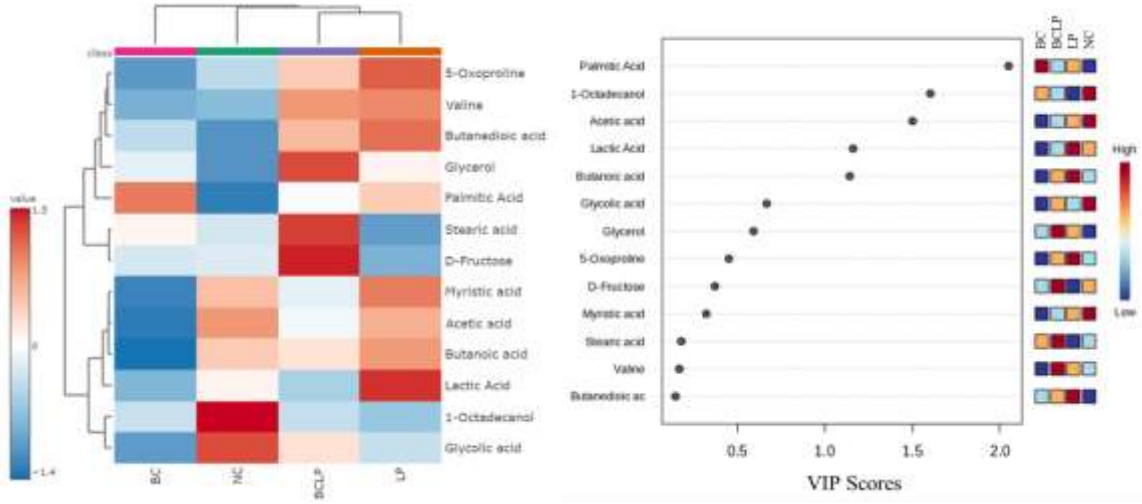

Figure 3. The heatmap (left) and VIP score (right) of identified metabolites with a proportion >0.8 illustrate the hierarchical grouping of treatments and metabolites based on their relative levels.

Table 4. Relationship mapping between significant performance parameters (avgBW) and important metabolites with a VIP score >1

				avgBW				
0-10 day	LP^{a}	>	BCLP^{ab}	>	BC^{b}	>	NC^{b}	
0-21 day	LP^{a}	>	BCLP^{b}	>	BC^{b}	>	NC^{b}	Metabolite
0-30 day	LP^{a}	>	BCLP^{ab}	>	BC^{ab}	>	NC^{b}	
		a		*b*		*a*		*b*	Palmitic acid
		b		*b*		*b*		*a*	1-Octadecanol
		a		*a*		*b*		*a*	Acetic acid
		a		*b*		*ab*		*ab*	Lactic acid
		-		-		-		-	Butanoic acid

NC= negative control, LP= *Lactobacillus plantarum*, BC= *Bacillus coagulans*, BCLP= *Bacillus coagulans* & *Lactobacillus plantarum*. Diferent superscript letters mean significant different. Treatments that do not have the same letter notation are significantly different (α= 0.05).

Other performance parameters (adjFCR, cumFI, and PEF) did not differ significantly. Metabolite profile analysis on the administration of probiotics *L. plantarum* and *B. coagulans* in the digestive tract of broiler chickens with NC, LP, BC, and BCLP treatments gave results that were not significantly different after PCA analysis. Important metabolites with VIP score >1 are acetic acid, lactic acid, butanoic acid, 1-octadecanol and palmitic acid. Metabolites expressed as distinguishing metabolites between LP and BC are acetic acid, lactic acid, and butanoic acid. At the same time, metabolites expressed as distinguishing metabolites of biomarker candidates are lactic acid as a good avrBW performance distinguisher and 1-octadecanoic and palmitic acid as a distinguisher without probiotic supplementation.

CONCLUSION

The administration of probiotics *Lactobacillus plantarum* (LP) and *Bacillus coagulans* (BC) significantly influenced broiler growth performance, with average body weight (avgBW) being the only parameter showing significant differences. The highest to lowest avgBW values were observed in the order of LP, BCLP, BC, and NC treatments. Other performance parameters, including adjFCR, cumFI, and PEF, showed no significant differences. Metabolite profile analysis indicated no significant differences between treatments based on PCA and PLS-DA results. However, important metabolites with a VIP score >1 were identified, including acetic acid, lactic acid, butanoic acid, 1-octadecanol, and palmitic acid. Acetic acid, lactic acid, and butanoic acid were key distinguishing metabolites between LP and BC. Additionally, lactic acid was identified as a potential biomarker for good avgBW performance, while 1-octadecanol and palmitic acid were differentiating metabolites in treatments without probiotic supplementation. These findings suggest that probiotic supplementation can selectively influence broiler growth performance and metabolite profiles, providing valuable insights for optimizing broiler nutrition strategies.

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REFERENCES

Aviagen. 2018. Ross broiler: management handbook. Huntsville (USA): Aviagen.

Aviagen. 2018. Ross broiler: pocket guide. Huntsville (USA): Aviagen.

Aviagen. 2022. Ross 308 Ross 308 FF broiler: performance objective. Huntsville (USA): Aviagen.

Banu LA, Mustari A, Ahmad N. 2019. Efficacy of probiotics on growth performance and hemato-biochemical parameters in broiler. Res in Agri Livest and Fish, 6: 91–100. DOI: 10.3329/ralf.v6i1.41390

Broadhurst D, Goodacre R, Reinke SN, Kuligowski J, Wilson ID, Lewis MR, Dunn WB. 2018. Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. Metabol. 14: 1-17. DOI: 10.1016/j.jpba.2017.07.044

Chen X, Zhao W, Liu YZ, Aschalew ND, Sun Z, Zhang XF, Zhen YG. 2021. Caecal microbiome and metabolites associated with different growth performances of broilers. Indian J Anim Res. 55: 109-114. DOI: 10.18805/ijar.B-1062

Chong J, Xia J. 2020. Using MetaboAnalyst 4.0 for metabolomics data analysis, interpretation, and integration with other omics data. Comput Met Data Anal Metabol. 337-360.

Cukkemane A, Kumar P, Sathyamoorthy B. 2020. A metabolomics footprint approach to understanding the benefits of synbiotics in functional foods and dietary therapeutics for health, communicable, and non-communicable diseases. Food Res Int. 128:108679. DOI: 10.1016/j.foodres.2019.108679.

Debik J, Sangermani M, Wang F, Madssen TS, Giskeødegård GF. 2022. Multivariate analysis of NMR-based metabolomic data. NMR Biomed. 35:e4638. DOI: 10.1002/nbm.4638

Elshaghabee FM, Rokana N, Gulhane RD, Sharma C, Panwar H. 2017. Bacillus as potential probiotics: status, concerns, and future perspectives. Front Microb. 8: 1490. DOI: 10.3389/fmicb.2017.01490

Eurachem. 2019. Blanks in Method Validation Supplement to Eurachem Guide The Fitness for Analytical Methods. Bucharest (Romania): Eurachem.

Fiehn O. 2016. Metabolomics by gas chromatography-mass spectrometry: Combined targeted and untargeted profiling. Cur Prot Mol Bio. 114: 30.4.1–30.4.32. DOI:10.1002/0471142727.mb3004s114.

Frainay C, Jourdan F. 2017. Computational methods to identify metabolic sub-networks based on metabolomic profiles. Briefings in Bioinformatics, 18: 43-56. DOI: 10.1093/bib/bbv115

Galindo-Prieto B, Eriksson L, Trygg J. 2014. Variable influence on projection (VIP) for orthogonal projections to latent structures (OPLS). J Chemometrics. 28: 623-632. DOI: 10.1002/ cem.2627

Granato D, de Araújo Calado VM, Jarvis B. 2014. Observations on the use of statistical methods in food science and technology. Food Res Int. 55: 137-149. DOI: 10.1016/j.foodres.2013.10.024

Gromski PS, Muhamadali H, Ellis DI, Xu Y, Correa E, Turner ML, Goodacre R. 2015. A tutorial review:

Metabolomics and partial least squares-discriminant analysis–a marriage of convenience or a shotgun wedding. Analytica Chimica Acta. 879: 10-23. DOI: 10.1016/j.aca.2015.02.012

Gu SB, Zhao LN, Wu Y, Li SC, Sun JR, Huang JE, Li DD. 2015. Potential probiotic attributes of a new strain of Bacillus coagulans CGMCC 9951 isolated from healthy piglet feces. World J Microb and Biotech. 31: 851-863.

Hasan BMS, Abdulazeez AM. 2021. A review of principal component analysis algorithm for dimensionality reduction. J Soft Comput Data Mining. 2: 20-30. DOI: 10.30880/jscdm.2021.02.01.003

Hsu YL, Chen CC, Lin YT, Wu WK, Chang LC, Lai CH, Kuo CK. 2019. Evaluation and optimization of sample handling methods for quantification of short-chain fatty acids in human fecal samples by GC–MS. J Proteome Res. 18: 1948-1957. DOI: 10.1021/acs.jproteome.8b00536

Humam AM, Loh TC, Foo HL, Samsudin AA, Mustapha NM, Zulkifli I, Izuddin WI. 2019. Effects of feeding different postbiotics produced by Lactobacillus plantarum on growth performance, carcass yield, intestinal morphology, gut microbiota composition, immune status, and growth gene expression in broilers under heat stress. Animals, 9: 644. DOI: 10.3390/ani9090644

Ito K, Miyamoto H, Matsuura M, Ishii C, Tsuboi A, Tsuji N, Nakaguma T, Nakanishi Y, Kato T, Suda W, et al. 2022. Noninvasive fecal metabolic profiling for the evaluation of characteristics of thermostable lactic acid bacteria, *Weizmannia coagulans* SANK70258, for broiler chickens. J Biosci Bioeng. 134(2): 105-115. DOI: 10.1016/j.jbiosc.2022.05.006

Jiang L, Sullivan H, Wang B. 2022. Principal component analysis (PCA) loading and statistical tests for nuclear magnetic resonance (NMR) metabolomics involving multiple study groups. Analytical Letters. 55: 1648-1662. DOI: 10.1080/00032719.2021.2019758

Khajeh Bami M, Afsharmanesh M, Ebrahimnejad H. 2020. Effect of dietary Bacillus coagulans and different forms of zinc on performance, intestinal microbiota, carcass and meat quality of broiler chickens. Probiotics and Antimicrobial Proteins. 12: 461-472. DOI: 10.1007/s12602-019-09558-1

Kozak M, Piepho HP. 2018. What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. J Agro Crop Sci. 204: 86-98. DOI: 10.1111/jac.12220

Li H, Hu B, Luo Q, Hu S, Luo Y, Zhao B, Gan Y, Li Y, Shi M, Nie Q, Zhang D, Zhang X. 2020. Runting and stunting syndrome is associated with mitochondrial dysfunction in sex-linked dwarf chicken. Frontiers in Genetics. 10, 1337. DOI: 10.3389/fgene.2019.01337

Liu Q, Yu Z, Tian F, Zhao J, Zhang H, Zhai Q, Chen W. 2020. Surface components and metabolites of probiotics for regulation of intestinal epithelial barrier. Microb Cell Fact. 19: 1-11. DOI: 10.1186/s12934-020-1289-4

Martínez Y, Valdivié M. 2021. Efficiency of Ross 308 broilers under different nutritional requirements. Journal of Applied Poultry Research. 30: 100140. DOI: 10.1016/j.japr.2021.100140

Orcan F. 2020. Parametric or non-parametric: Skewness to test normality for mean comparison. International Journal of Assessment Tools in Education. 7: 255-265. DOI: 10.21449/ijate.656077

Peng Q, Zeng XF, Zhu JL, Wang S, Liu XT, Hou CL, Tacker PA, Qiao SY. 2016. Effects of dietary Lactobacillus plantarum B1 on growth performance, intestinal microbiota, and short chain fatty acid profiles in broiler chickens. Poultry Science. 95: 893-900. DOI: 10.3382/ps/pev435

Petričević V, Lukić M, Škrbić Z, Dosković V, Rakonjac S, Petričević M, Milenković D. 2024. Comparative analysis of production and slaughter parameters of fast-growing broiler hybrids. 2nd International Symposium on Biotechnology. DOI: 10.46793/SBT29.24VP

Prakash A, Saxena VK, Singh MK. 2020. Genetic analysis of residual feed intake, feed conversion ratio and related growth parameters in broiler chicken: a review. World Poul Sci J. 76: 304-317. DOI: 10.1080/00439339.2020.1735978

Ren S, Hinzman AA, Kang EL, Szczesniak RD, Lu LJ. 2015. Computational and statistical analysis of metabolomics data. Metabolomics. 11: 1492-1513. DOI: 10.1007/s11306-015-0823-6

Rinttilä T, Apajalahti J. 2013. Intestinal microbiota and metabolites—Implications for broiler chicken health and performance. J App Poul Res: 22:647-658. DOI: 10.3382/japr.2013-00742

Szymanska E, Saccenti E, Smilde AK, Double-check JW. 2012. Validation of diagnostic statistics for PLS-DA models in metabolomics studies. Metabol. 8: 3-16. DOI: 10.1007/s11306-011-0330-3

Thevenot EA. 2016. ropes: PCA, PLS (-DA), and OPLS (-DA) for multivariate analysis and feature selection of omics data. R package version, 1. DOI: 10.1021/acs.jproteome.5b00354

Tran C, Cock IE, Chen X, Feng Y. 2022. Antimicrobial Bacillus: metabolites and their mode of action. Antibiotics. 11:88. DOI: 10.3390/antibiotics11010088

Vacanti NM. 2019. The fundamentals of constructing and interpreting heat maps. In: Fendt SM, Lunt S. (ed) Metabolic signaling. Met Mol Biol. 1862. New York UK): Humana Press. DOI: 10.1007/978-1-4939-8769-6_20

Van Dyk W. 2022. Visualizing data through biplots using Categorical PCA and clustering [Dissertation]. Stellenbosch (ZA): Stellenbosch University.

Wang J, Yao L, Su J, Fan R, Zheng J, Han Y. 2023. Effects of Lactobacillus plantarum and its fermentation products on growth performance, immune function, intestinal pH, and cecal microorganisms of Lingnan yellow chicken.

Poultry Science, 102: 102610. DOI: 10.1016/j.psj.2023.102610

Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, Ba X, Xie J, Wang Y, Geng W. 2021. Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. Front Bioeng Biotech. 9, 612285: 1-19. DOI: 10.3389/fbioe.2021.612285

Worley B, Powers R. 2016. PCA as a practical indicator of OPLS-DA model reliability. Current Metabolomics, 4:97-103. DOI: 10.2174/2213235X04666160613122429

Withers E, Hill PW, Chadwick DR, Jones DL. 2020. Use of untargeted metabolomics for assessing soil quality and microbial function. Soil Biol Biochem, 143: 107758. DOI: 10.1016/j.soilbio.2020.107758

Xing SC, Chen JY, Chen YX, Wu RT, Huang CB, Zhang Y, Mi JD, Liao XD. 2020. The combination of lead and Bacillus coagulans R11 increased the concentration of alpha-Solanine in the cecum of laying hens and the pathogens abundance decreased. Front Microbiol. 11, 585197. DOI: 10.3389/fmicb.2020.585197

Zhang L, Wang Y, Jia H, Liu X, Zhang R, Guan J. 2023. Transcriptome and metabolome analyses reveal the regulatory effects of compound probiotics on cecal metabolism in heat-stressed broilers. Poul Sci, 102(1): 102323. DOI: 10.1016/j.psj.2022.102323

Zhang B, Zhang H, Yu Y, Zhang R, Wu Y, Yue M, Yang C. 2021. Effects of Bacillus coagulans on growth performance, antioxidant capacity, immunity function, and gut health in broilers. Poultry Science. 100(6):101168. DOI: 10.1016/j.psj.2021.101168.

Zhen W, Shao Y, Gong X, Wu Y, Geng Y, Wang Z, Guo Y. 2018. Effect of dietary Bacillus coagulans supplementation on growth performance and immune responses of broiler chickens challenged by Salmonella enteritidis. Poul Sci. 97(8): 2654-2666. DOI: 10.3382/ps/pey119

Zheng R, Chen Z, Guan Z, Zhao C, Cui H, Shang H. 2023. Variable importance for projection (VIP) scores for analyzing the contribution of risk factors in severe adverse events to Xiyanping injection. Chinese Medicine. 18: 15. DOI: 10.1186/s13020-023-00718-8

Zhou L, Yu D, Zheng S, Ouyang R, Wang Y, Xu G. 2021. Gut microbiota-related metabolome analysis based on chromatography-mass spectrometry. Trends Anal Chem. 143: 116375. DOI: 10.1016/j.trac.2021.116375.

Effect of Liquid Smoke on the Characteristics of Sensory Quality of Chicken Nugget

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ABSTRAK

Hafid H, Herniati S, Nasiu F, Pancar FM, Ananda SH. 2025. Pengaruk asam cair terhadap karakteristik kualitas sensori nuget ayam. JITV 30 (2):125-131. DOI:http://dx.doi.org/10.14334/jitv.v30i2.3514.

Berbagai bahan digunakan untuk meningkatkan kualitas makanan olahan termasuk nugget ayam yang merupakan salah satu produk olahan daging unggas yang banyak dikonsumsi masyarakat. Salah satu bahan yang digunakan diindustri pangan adalah penggunaan asap cair yang dapat berfungsi sebagai bahan antioksidan dan antimikroba. Penelitian ini bertujuan untuk mengetahui pengaruh penggunaan asap cair terhadap pH dan kualitas sensoris nugget ayam broiler. Penelitian ini dilaksanakan di Laboratorium Teknologi Pengolahan Hasil Ternak, Fakultas Peternakan Universitas Halu Oleo, Kendari. Perlakuan dalam penelitian ini terdiri dari empat kelompok yaitu nugget ayam tanpa asap cair 0% (P0), nugget ayam dengan asap cair 2% (P1), nugget ayam dengan asap cair 4% (P2), dan nugget ayam dengan asap cair 6% (P3). Penelitian ini dirancang dengan menggunakan Rancangan Acak Lengkap (RAL) dengan 4 perlakuan dan 4 kali ulangan. Data yang diperoleh dianalisis menggunakan analisis sidik ragam (ANOVA) dan jika terdapat pengaruh yang signifikan maka dilanjutkan dengan uji Duncan multiple range tests (DMRT) untuk mengetahui perbedaan antar perlakuan. Hasil penelitian menunjukkan bahwa penambahan asap cair pada pembuatan nugget ayam tidak memberikan pengaruh nyata terhadap warna, aroma, tekstur, dan keempukan nugget ayam, tetapi berpengaruh nyata terhadap penurunan nilai rasa nugget yang dihasilkan. Hal ini menunjukkan bahwa asap cair berpotensi digunakan sebagai salah satu bahan dalam proses pembuatan nugget ayam, meskipun perlu dilakukan perbaikan formula untuk meningkatkan cita rasa produk akhir.

Kata Kunci : Nugget Ayam, Asap Cair, Kualitas Sensori

ABSTRACT

Hafid H, Herniati S, Nasiu F, Pancar FM, Ananda SH. 2025. Effect of liquid smoke on the characteristics of sensory quality of chicken nugget. JITV 30 (2): 125-131. DOI:http://dx.doi.org/10.14334/jitv.v30i2.3514.

Various ingredients are used to enhance the quality of processed foods, including chicken nuggets, one of the widely consumed processed poultry meat products. One of the ingredients used in the food industry is liquid smoke, which can serve as an antioxidant and antimicrobial agent, providing a color effect and a distinctive smoke flavor to the product. This study aims to determine the Effect of using liquid smoke on pH and the sensory quality of broiler chicken nuggets. This research was conducted at the Laboratory of Animal Product Processing Technology, Faculty of Animal Husbandry, Halu Oleo University, Kendari. The treatments in this study consisted of four groups: chicken nuggets without 0% liquid smoke (P0), chicken nuggets with 2% liquid smoke (P1), chicken nuggets with 4% liquid smoke (P2), and chicken nuggets with 6% liquid smoke (P3). This study employed a completely randomized design (CRD) with four treatments and four replications. The data obtained were analyzed using analysis of variance (ANOVA). If a significant effect was found, it was followed up with Duncan's multiple range tests (DMRT) to determine differences between treatments. The results showed that the addition of liquid smoke in making chicken nuggets had no significant effect on the color, aroma, texture, or tenderness of the chicken nuggets, but significantly reduced the taste value of the chicken nuggets. This suggests that liquid smoke can improve the sensory quality of chicken nuggets.

Key Words: Chicken Nugget, Liquid Smoke, Sensory Quality

INTRODUCTION

It has been realized that the culinary industry in Indonesia, particularly in Kendari City, is progressing. This situation requires the guarantee of culinary quality to meet the needs of both the market and consumers, ensuring that the product is safe for consumption. One of the raw materials in the culinary industry that should be guaranteed to maintain its quality is chicken meat. Chicken meat is a protein food source that can be easily found in a traditional market, is cheaper, and easily processed, digested, and has a delicious taste that makes

it desirable (Hafid et al. 2017a; Hafid et al. 2017b; Mir et al. 2017; Ismail & Joo 2017). However, this product is easily spoiled due to the existence of endogenous cathepsin enzyme and bacterial contamination (Patriani et al. 2020; Saenz-García et al. 2020). Therefore, meat spoiling should be prevented by either processing or preservation action (Patriani et al. 2020). Processing will have a positive effect on the formation of product diversity, increase the storage period, and enhance the economic value of the product. One alternative to chicken meat processing is making nuggets, a type of fast food made from livestock products that Indonesians love to consume and is widely available in society (Tasse et al. 2015).

Nuggets are one of the meat-processed products made from ground meat, formed into a rectangular shape, and coated with seasoned flour (Sumina et al. 2018). Nugget is defined as a chicken processed product that is formed, cooked, and made from a mixing of ground chicken meat added with coating ingredients, with or without the addition of other ingredients and other allowed ingredients (BSN 2002). Moreover, the nutritional content per 100 g of chicken nuggets was as follows: moisture 60 g, protein 12 g, fat 20 g, carbohydrate 25 g, calcium 30 mg, and energy 1364 kilojoules or 326 kcal.

In making chicken nuggets, liquid smoke, which can be produced from coconut shell smoke, is an important ingredient (Kailaku et al. 2017; Rizal et al. 2020). Liquid smoke from coconut shells is obtained by condensing the smoke from coconut shells through a pyrolysis process at a temperature of 400°C. This liquid smoke contains various chemical compounds, including phenol, ketone, organic acid, alcohol, and ester (Budaraga et al. 2016a; Budaraga et al. 2016b). Those chemical compounds can serve as antioxidants and antimicrobials, imparting a color effect and a special smoke flavor in agricultural products (Sorour et al. 2022; Suryani et al. 2022; Wibowo et al. 2023; Abustam et al. 2018). Nowadays, liquid smoke is generally used in fish preservation. It can be hypothesized that liquid smoke can bind water in meat, thereby enhancing the sensory quality of chicken nuggets.

MATERIALS AND METHODS

This study was conducted in the Laboratory of Processing Technology of Animal Products, Faculty of Animal Science, University of Halu Oleo, Kendari, Indonesia. Ingredients used were chicken meat, liquid smoke, ice cube, tapioca flour, sea salt, seasonings, bread flour, fried oil, egg, and aquadest. In contrast, the tools used were a blender, plastic washbowl, plate, grounding machine, binder, knife, analytical balance, cutting board, frying pan, stove, and pH meter.

Chicken nugget-making procedures

There are many processes in making chicken nuggets. The first step is to clean the broiler chicken meat separately and then balance all the ingredients, including broiler chicken meat, garlic, pepper, tapioca flour, ice cube, sea salt, and liquid smoke, according to the determined formula. The next step is to grind the meat in a grinding machine for six minutes. Then, add an ice cube to the grinding container to prevent the container from becoming too hot and to prevent the meat protein from decaying. Additionally, mix all the ingredients according to a predetermined formula. All the ingredients, including a grounded protein source, filler, garlic, pepper, sea salt, and liquid smoke, are then mixed in the batter. All the treatment ingredients are then stirred into the plate of each treatment using a spoon to produce a homogeneous mixture of ingredients and liquid smoke.

A further step is to form the chicken nugget batter on the cutting board, which has been previously oiled with frying oil to prevent the batter from sticking when the nuggets are cooked. Nugget batter is formed as thick as possible and then steamed for 20 minutes to make it cooked solid and easy to cut. The cutting process is used to produce uniform chicken nuggets that are easy to fry and cook. There will be two further steps to produce fried chicken nuggets. First of all, the chicken nugget batter is then coated with egg yolk for a better taste and increased nutritional content, and then coated with bread flour to coat the outer part of the nugget. Additionally, bread flour is used to achieve the golden yellow color after the frying process. The final step is to fry the nuggets for one minute, which strengthens the taste of the cooked nuggets and enhances their flavor, creating a crunchy texture and making them ready to be served.

Nugget formula

The formula of ingredients in the nugget-making process was modified to analyze the sensory quality characteristics of nuggets, using chicken meat with the addition of liquid smoke, as presented in Table 1.

pH measurement

pH was measured according to Soeparno (2015) by weighing 10 g of the ground sample and mixing it with 10 mL of aquadest, then stirring to form a homogeneous mixture. A clean pH meter was then put into a pH 7 buffer to calibrate the pH. The next step was to measure the pH of each homogeneous mixture solution three times, and the results were then averaged to obtain the average pH value.

Testing of sensory quality characteristics of nugget

A sensory quality test is a test of ingredients or products based on the level of fondness (Hedonic test)

Table 1. Chicken nugget batter ingredients per unit sample

Ingredients	Treatments			
	P0	P1	P2	P3
Broiler chicken meat (grams)	100	100	100	100
Liquid smoke (%)	0	2	4	6
Tapioca flour (%)	10	10	10	10
Garlic (%)	2	2	2	2
Pepper (%)	2	2	2	2
Sea salt (%)	1	1	1	1
Ice cube (%)	1	1	1	1
Egg yolk (%)	1	1	1	1

P0 = liquid smoke 0 % per 100 g of broiler chicken meat, P1 = liquid smoke 2 % per 100 g of broiler chicken meat, P2 = liquid smoke 4 % per 100 g of broiler chicken meat, P3 = liquid smoke 6 % per 100 g of broiler chicken meat

Table 2. Hedonic scale for sensory quality test

Parameters	Hedonic Scale	Criteria
Color	1	Dark brown
	2	Brown
	3	Yellow-brown
	4	Golden yellow
	5	Yellow
Aroma	1	Very dislike
	2	Dislike
	3	Like enough
	4	Like
	5	Very like
Texture	1	Very rough
	2	Rough
	3	Medium
	4	Soft
	5	Very soft
Tenderness	1	Very not tender
	2	Not tender
	3	Tender enough
	4	Tender
	5	Very tender
Flavor	1	Very dislike
	2	Dislike
	3	Dislike enough
	4	Like
	5	Very like

(Julianto et al. 2021). Testing is conducted by using the panelist' responses to describe the level of their fondness for the result of the experiment. The number of panelists is twenty-five students, categorized as untrained panelists. The hedonic scale includes color, aroma, texture, tenderness, and flavor. The hedonic scale is presented in Table 2 (Sari et al. 2023).

Research design

The design used was a completely randomized design consisting of 4 treatments and 25 untrained panelists as replication. The model of the experiments was categorized as P0= liquid smoke 0 % per 100 g of broiler chicken meat, P1= liquid smoke 2 % per 100 g of broiler chicken meat, P2= liquid smoke 4 % per 100 g of broiler chicken meat, P3= liquid smoke 6 % per 100 g of broiler chicken meat. The observation value of treatment at-i on replication at-j (Y_{ij}) calculated used formula as follow:

$$Y_{ij} = \mu + \alpha i + \varepsilon ij$$

where μ is middle value; α_i is effect of treatment at-i; ε_{ij} is trial error of treatment at-i on replication of-j; i is treatment of 0,1,2,3, and so on and j is replication at 1,2,3, and so on.

Data analysis

The data were analyzed using analysis of variance (ANOVA). If the result of the ANOVA shows a significant effect, it will be further analyzed using the Duncan Multiple Range Test (Paiman, 2015).

RESULTS AND DISCUSSION

pH and sensory quality characteristics of chicken nuggets, consisting of color, texture, flavor, and tenderness, are presented in Table 3.

pH

pH value can affect the texture and juiciness of the nugget since pH affects the interaction between protein, water, and fat, whereas at the low pH, protein electricity becomes neutral, and protein clamps and solidifies, creating a harder and drier texture (Torun et al. 2023; Cornet et al. 2021; Barbut 2024). Since the liquid smoke contains organic acids, it was assumed that a higher level of liquid smoke may decrease the pH. However, there was no significant effect of liquid smoke on pH in this study, indicating that the use of liquid smoke up to a level of 6% has an insufficient organic acid content to decrease the pH.

Color

An interesting color will increase the acceptance of processed chicken nuggets with liquid smoke. Color is also a quality characteristic that becomes a consumer consideration because of the first sight of the consumer on a product in color. Based on the ANOVA result in Table 3, it shows that using liquid smoke in the making process of chicken nuggets has no significant effect (P>0.05) on nugget color. The measurement score is 3.88-3.90, indicating that the color of the chicken nuggets is yellow-brown to yellow. Treatment of liquid smoke at levels up to 6% could not significantly affect the color because liquid smoke does not affect the concentration of the myoglobin pigment in meat. The color of meat is mainly affected by myoglobin pigment concentration in meat (Soeparno 2005; Han et al. 2024; Gupta et al. 2018; Bekhit et al. 2019; Yu et al. 2017; Tushar et al. 2023).

Aroma

Aroma is one of the key indicators used to determine the level of consumer acceptance of a product. The addition of liquid smoke to the chicken nugget-making process has no significant effect (P>0.05) on the aroma of the chicken nuggets. Measurement scores ranged from 3.82 to 3.96, indicating that the aroma of chicken nuggets with liquid smoke was generally liked and liked enough. The highest score for aroma was achieved by the treatment without liquid smoke addition, at 3.96, indicating that the aroma of chicken nuggets without liquid smoke is more appealing. In many ways, food deliciousness is determined by the aroma and smell of that dish because aroma, which can stimulate the appetite, will be a suitable parameter for consumers to choose the product (de Araújo et al. 2022; Berčík et al. 2021; Flores 2018; Sherina et al. 2023).

Texture

Texture is a primary characteristic of food quality, as each food product has distinct characteristics and a unique structure. Based on Table 3, it was shown that the addition of liquid smoke in the chicken nugget-making process has no significant effect (P>0.05) on the texture of the product. Measurement scores ranged from 3.67 to 3.87, indicating that the chicken nugget texture was middle soft to soft. The addition of liquid smoke up to a level of 6% had no significant effect on the improvement of the water-binding capacity of nugget protein; therefore, it cannot significantly affect the texture. The factor affecting the density, compactness, and elasticity of meat-processed products is the water-binding capacity of meat protein (Xu et al. 2024; Bao & Ertbjerg 2019; Zhang et al. 2020; Shen et al. 2020).

Table 3. pH, score of color, aroma, texture, flavor, and tenderness of broiler meat chicken nuggets with liquid smoke.

Parameters	Treatment of liquid smoke addition			
	P0(0% of LS)	P1 (2% of LS)	P2 (4 % of LS)	P3 (6 % of LS)
pH	6.01±0.06	5.97±0.11	6.58±0.32	5.84±0.45
Color	3.85±0.15	3.88±0.18	3.69±0.10	3.90±0.20
Aroma	3,97±0.08	3.92±0.08	3.92±0.14	3.82±0.01
Texture	3.87±0.09	3.80±0.05	3.69±0.15	3.67±0.09
Flavor	4.05±0.06[a]	3.80±0.14[b]	3.78±0.13[b]	3.69±0.08[b]
Tenderness	3.78±0.035	3.95±0.06	3.85±0.015	3.77±0.06

P0 = liquid smoke 0 % per 100 g of broiler chicken meat, P1 = liquid smoke 2 % per 100 g of broiler chicken meat, P2 = liquid smoke 4 % per 100 g of broiler chicken meat, P3 = liquid smoke 6 % per 100 g of broiler chicken meat

Flavor

Flavor is the most affecting factor in determining the level of consumer fondness. The flavor of the nugget is directly proportional to panelist fondness (Dashdorj et al. 2015; Fiorentini et al. 2020; Lee et al. 2018; Miyaki et al. 2016). The more delicious the nugget product served, the higher the fondness level of the panelist for the nugget product served. Flavor also affects the acceptance of consumers of the agricultural product. In flavor measurement, the sense of taste is more commonly used, which is divided into several factors, including salty, sour, sweet, and bitter (Taylor et al. 2024).

The addition of liquid smoke in the chicken nugget-making process has a very real effect ($P<0.01$) on nugget flavor. The highest measurement score is achieved without liquid smoke treatment, reaching a value of 4.05, indicating that chicken nuggets without liquid smoke are more appealing. It is likely because the liquid smoke produces a flavor that is less appealing to panelists. The flavor of chicken nuggets is influenced by the addition of seasonings during the manufacturing process, which includes sea salt, garlic, pepper, and tapioca flour. Those seasonings play a primary role in giving taste to chicken nuggets (Hafid et al. 2017b), while the flavor of the product is more significantly affected by the addition of seasonings (Feng et al. 2018; Oktafa et al. 2022; Neves et al. 2021). Moreover, the flavor of the product can be enhanced by the addition of seasonings that are appealing to consumers. The addition of seasonings and flavoring ingredients is primarily intended to enhance or intensify the flavor.

Tenderness

Tenderness is the sensation of the ease with which the meat or food can be cut into smaller pieces by teeth. Meat is considered tender if it can be cut easily with incisors and is easy to chew and swallow after being chewed by molars. Meat could be considered not tender enough if it should be bitten with a canine tooth, and it is hard if bitten with molars (Patriani et al. 2020). Results of ANOVA indicated that the addition of liquid smoke in the chicken nugget-making process has no real effect ($P>0.05$) on nugget tenderness. The measurement score ranged from 3.77 to 3.95, indicating that it was tender enough. It was expected that the addition of liquid smoke could elevate the water binding capacity of the nugget because the smoke's ability to bind the free half water to and free half water, and water is free to fill the space between cells, causing an increase in water binding capacity, which automatically will decrease the shearing force of meat. Moreover, the lower the shearing force of meat, the higher its tenderness (Choe et al. 2015).

CONCLUSION

The addition of liquid smoke, up to 6% of the batter, in making chicken nuggets does not affect the color, aroma, texture, and tenderness parameters. However, it significantly decreases the flavor score of the nugget. The use of liquid smoke to enhance the flavor of the nuggets may not be effective for chicken meat.

CONFLICT OF INTEREST

Statement declaring that there is no conflict of interest with any party related to the materials discussed in the paper.

REFERENCES

Abustam E, Said MI, Yusuf M. 2018. The Effect of antioxidant activity of liquid smoke in feed supplement block on meat functional of muscle longissimus dorsi. In IOP Conf Ser.: Earth Environ Sci. 119:012046. IOP Publishing. DOI:10.1088/1755-1315/119/1/012046.

Bao Y, Ertbjerg P. 2019. Effects of protein oxidation on the texture and water-holding of meat: A review. Crit Rev Food Sci Nutr. 59:3564-3578. DOI:10.1080/10408398.2018.1498444.

Barbut S. 2024. Measuring water holding capacity in poultry meat. Poul Sci. 103:103577. DOI:10.1016/j.psj.2024.103577.

Bekhit AEDA, Morton JD, Bhat ZF, Kong L. 2019. Meat color: Factors affecting color stability. Encyclopedia Food Chem. 2:202-210. DOI:10.1016/B978-0-12-814026-0.21665-X.

Berčík J, Neomániová K, Gálová J, Mravcová A. 2021. Consumer neuroscience as a tool to monitor the impact of aromas on consumer emotions when buying food. Appl Sci. 11:6692. DOI:10.3390/app11156692.

[BSN] Badan Standardisasi Nasional. 2024. Nuget Ayam. SNI 01–6638–2024. Jakarta (Indones): Badan Standardisasi Nasional.

Budaraga IK, Arnim A, Marlinda Y, Bulain U. 2016a. Analysis of liquid smoke chemical components with GC MS from different raw materials variation production and pyrolysis temperature level. Int J ChemTech Res. 9:694-708.

Budaraga IK, Arnim A, Marlinda Y, Bulain U. 2016b. Liquid smoke production quality from raw materials variation and different pyrolysis temperatures. Int J Adv Sci Eng Informat Technol. 6:306-315. DOI:10.18517/ijaseit.6.3.737.

Choe JH, Choi MH, Rhee MS, Kim BC. 2015. Estimation of sensory pork loin tenderness using Warner-Bratzler shear force and texture profile analysis measurements. AJAS. 29:1029. DOI:10.5713/ajas.15.0482.

Cornet SH, Snel SJ, Lesschen J, van der Goot AJ, van der Sman RG. 2021. Enhancing the water holding capacity of model meat analogues through marinade composition. J Food Eng. 290:110283. DOI:10.1016/j.jfoodeng.2020.110283.

Dashdorj D, Amna T, Hwang I. 2015. Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: An overview. Eur Food Res Technol. 241:157-171. DOI:10.1007/s00217-015-2449-3.

de Araújo PD, Araújo WMC, Patarata L, Fraqueza MJ. 2022. Understanding the main factors that influence consumer quality perception and attitude towards meat and processed meat products. Meat Sci. 193:108952. DOI:10.1016/j.meatsci.2022.108952.

Feng Y, Tapia MA, Okada K, Lazo NBC, Chapman-Novakofski K, Phillips C, Lee SY. 2018. Consumer acceptance comparison between seasoned and unseasoned vegetables. J Food Sci. 83:446-453. DOI:10.1111/1750-3841.14027.

Fiorentini M, Kinchla AJ, Nolden AA. 2020. Role of sensory evaluation in consumer acceptance of plant-based meat analogs and meat extenders: A scoping review. Foods. 9:1334. DOI:10.3390/foods9091334.

Flores M. 2018. Understanding the implications of current health trends on the aroma of wet and dry cured meat products. Meat Sci. 144:53-61. DOI:10.1016/j.meatsci.2018.04.016.

Gupta J, Bower CG, Cavender GA, Sullivan GA. 2018. Effectiveness of different myoglobin states to minimize high-pressure induced discoloration in raw ground beef. LWT. 93:32-35. DOI:10.1016/j.lwt.2018.03.008.

Hafid H, Nuraini, Agustina D, Fitrianingsih, Inderawati. 2017a. Sensory quality of nuggets substituted with different levels of chicken intestine. Proc Nat Sem Livest Indust. Bogor (Indones): Bogor Agricultural University. p.72-77.

Hafid H, Nuraini, AgustinaD, Fitrianingsih, Inderawati. 2017b. Effect of chicken intestine substitution on the chemical quality of nuggets. Anim Prod. 19: 207-213. DOI:10.20884/1.jap.2017.19.3.615.

Han J, Wang Y, Wang Y, Hao S, Zhang K, Tian J, Jin Y. 2024. Effect of changes in the structure of myoglobin on the color of meat products. FMR. 4:e011. DOI:10.48130/fmr-0024-0003.

Ismail I, Joo ST. 2017. Poultry meat quality in relation to muscle growth and muscle fiber characteristics. Korean J Food Sci Anim Resour. 37:873. DOI:10.5851/kosfa.2017.37.6.87.

Julianto, M. A. R., Sandiah, N., & Hafid, H. 2021. The chemical composition of free-range chicken nuggets soaked in liquid smoke is different.. J Ilmiah Peternak Halu Oleo. 3:400-406.

Kailaku SI, Syakir M, Mulyawanti I, Syah ANA. 2017. Antimicrobial activity of coconut shell liquid smoke. In IOP Conf Ser: Mater Sci Eng. 206:012050. IOP Publishing. DOI:10.1088/1757-899X/206/1/012050.

Lee N, Kwak HS, Joo JY, Kang J, Lee Y. 2018. Effects of partial replacement of pork meat with chicken or duck meat on the texture, flavor, and consumer acceptance of sausage. J Food Quality. 2018:6972848. DOI:10.1155/2018/6972848.

Mir NA, Rafiq A, Kumar F, Singh V, Shukla V. 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. J Food Sci Technol. 54:2997-3009. DOI:10.1007/s13197-017-2789-z.

Miyaki T, Retiveau-Krogmann A, Byrnes E, Takehana S. 2016. Umami increases consumer acceptability and perception of sensory and emotional benefits without compromising health benefit perception. J Food Sci. 81:S483-S493. DOI:10.1111/1750-3841.13195.

Neves TDM, da Cunha DT, de Rosso VV, Domene SMÁ. 2021. Effects of seasoning on the formation of heterocyclic amines and polycyclic aromatic hydrocarbons in meats: A meta-analysis. *Compr Rev Food Sci Food Saf. 20:* 526-541. DOI:10.1111/1541-4337.12650.

Oktafa H, Prayitno AH, Handayani HT, Rukmi DL. 2022. The effect of marinade concentrations of different local herbs and spices on the hedonic test of super native chicken breast. In IOP Conf Ser.: Earth Environ Sci. 980: 012015. IOP Publishing. DOI:10.1088/1755-1315/980/1/012015.

Paiman, P. 2015. Experimental Design for Agriculture. UPY Press. Yogyakarta.

Patriani, P., H. Hafid, R.E. Mirwandhono, T.H. Wahyuni. 2020. Technology. Meat Processing: Anugrah Pangeran Jaya Press.

Rizal WA, Maryana R, Prasetyo DJ, Pratiwi D, Jatmiko TH, Ariani D, Suwanto A. 2020. Chemical composition of liquid smoke from coconut shell waste produced by SME in Rongkop, Gunungkidul. In IOP Conf Ser.: Earth Environ Sci. 462:012057. IOP Publishing. DOI:10.1088 /1755-1315/462/1/012057.

Saenz-García CE, Castañeda-Serrano P, Mercado Silva EM, Alvarado CZ, Nava GM. 2020. Insights into the identification of the specific spoilage organisms in chicken meat. *Foods*. *9*:225. DOI:10.3390/foods9020 225.

Sari, A.S, Hafid, H, Fitrianingsih. 2023. The effect of adding tendons on the organoleptic quality of beef meatballs.Jurnal Ilmiah Peternakan Halu Oleo, 5(3), 216. https://doi.org/10.56625/jipho.v5i3.38434.

Shen H, Elmore JS, Zhao M, Sun W. 2020. Effect of oxidation on the gel properties of porcine myofibrillar proteins and their binding abilities with selected flavour compounds. Food Chem. *329*:127032. DOI:10.1016/j.f oodchem.2020.127032.

Sherina, Fitianingsih, H. Hafid. 2023. The effect of different muscle locations on the physical properties of horse meat floss. Jurnal Ilmiah Peternakan Halu Oleo. 5(3) : 211-215.

Soeparno. 2015. Ilmu dan teknologi daging edisi revisi. Yogyakarta (Indonesia): Gajah Mada University Press.

Sorour MA, El-Hamied A, Adel A, Mohamoud ESA, Mahmoud AR. 2022. Anti-oxidative impact of liquid smoke and thyme essential oil on the quality characteristics of chicken and turkey meatball products during frozen storage. Eur J Nut Food Saf. 14:14-26. DOI:10.9734/ejnfs/2022/v14i330482.

Sumina, Hafid H, Napirah A. 2018 Kadar asam, rendemen dan susut masak nugget dengan substitusi usus ayam yang berbeda. Proc Semin Nat. Kendari (Indones): University of Halu Oleo. p.352-358.

Suryani R, Daradwinta R, Az-Zahra S, Rahmah SA, Rizal WA, Jatmiko TH, Apriyana W, Purwestri YA. 2022. Antioxidant activity, total phenolic content, and chemical composition of liquid smoke derived from wood sawdust. In 7th Int Conf Biol Sci. p. 634-639. Atlantis Press International B.V. DOI:10.2991/absr.k.220406.091.

Tasse AM, Nurhinayah I, Hafid, H. 2015. Nugget of rejected chicken meat substituted with cow brain, chemical composition, and sensory quality. Proceedings of the National Seminar on Food Self-sufficiency. Kendari (Inodones): Unhalu Press. p.183-186.

Taylor, S, Colonna A, Jung J, Gutierrez J, Zhao Y. 2024. Consumer Acceptability and Sensory Profile of Sustainable Paper-Based Packaging. *J Food Sci.*, 89(4):2423-2437.

Torun MMR, Khan MMH, Rahman MM, Sadakuzzaman M, Hashem MA. 2023. Influence of degree of doneness temperature on the sensory, physicochemical, nutritional, and microbial properties of beef. Meat Res. 3. DOI:10.55002/mr.3.5.69.

Tushar ZH, Rahman MM, Hashem MA. 2023. Metmyoglobin reducing activity and meat color: A review. Meat Res. 3. DOI:10.55002/mr.3.5.67.

Wibowo S, Syafii W, Pari G, Herliyana EN. 2023. Utilization of lignocellulosic waste as a source of liquid smoke: A literature. J Environ Health. *15:*196-216. DOI:10.20473 /jkl.v15i3.2023.196-216.

Yu QP, Feng DY, Xiao J, Wu F, He XJ, Xia MH, Dong T, Liu, YH, Tan HZ, Zou SG, Zheng T, Ou XH, Zuo JJ. 2017. Studies on meat color, myoglobin content, enzyme activities, and genes associated with the oxidative potential of pigs slaughtered at different growth stages. AJAS. 30:1739. DOI:10.5713/ajas.17.0005.

Zhang T, Fan L, Yu Y. 2020. The influence of myoglobin on the color of beef during storage. Acad. J Agri. Life Sci. 1:9-19. DOI:10.25236/AJALS.2020.010102.

Zhang M, Yan W, Wang D, Xu W. 2020. Effect of myoglobin, hemin, and ferric iron on the quality of chicken breast meat. Anim Biosci. 34:1382-1392. DOI:10.5713/ajas. 20.0529.

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Book:
a. Alshelmani M, Abdalla E, Kaka U, Basit M. 2021. Advances in poultry nutrition research. In: Kumar Patra A, editor. Adv Poult Nutr Res. London (UK): IntechOpen; p. 19–32. DOI: 10.5772/intechopen.91547.
b. Reece W. 2015. Respiration in mammals. New Jersey (USA): Willey-Blackwell.
c. Van Soest P. 2018. Nutritional ecology of the ruminant. 2nd ed. New York (USA): Cornell University Press.

Proceeding:
Damayanti R, Wiyono A, Dharmayanti N. 2021. Pathogenicity study of ducks infected with a local isolate of highly pathogenic avian influenza-H5N1-clade 2.3. . In: Inounu I, Priyanti A, Burrow H, Morris S, Min R, Suhubdy, Sutaryono Y, editors. Proc 4th Int Semin Livest Prod Vet Technol. Bogor (Indones): Indonesian Center for Animal Research and Development; p. 277–288.

Thesis:
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economic contribution of donkey ownership and use to houshold livelihoods in Kiambu country, Kenya (Thesis). Nairobi (KE). University of Nairobi

Electronic magazines:

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Institution:

a. [PSA] Philippine Statistics Authority. 2016. Dairy Industry Performance Report, January – December 2015. Quezon City (Philiphine): Philippine Statistics Authority. P. 1-11
b. [FAO] Food and Agriculture Organization. 2021. Gateway to dairy production and products. Food Agric Organ United Nations. [accessed August 10, 2021]. https://www.fao.org/dairy-production-products/production/feed-resources/en/.

Patent:

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