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Research Article

Muscle Morphology and Meat Tenderness of the Swamp Buffalo (*Bubalus Bubalis*) Raised Under Two Herd Health Programs

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ABSTRACT

This study evaluated the effects of herd health programmes (HHPs) on muscle histomorphology and meat tenderness in swamp buffaloes (*Bubalus bubalis*). Eight male buffaloes, aged 18–24 months and weighing 290 ± 29 kg, were assigned into two management groups: proper herd health program and non-proper herd health program (NHHP), with four animals per group. Immediately after slaughter, samples of *longissimus dorsi* (LD), *supraspinatus* (SS), and *semitendinosus* (ST) muscles were collected for micromorphological assessment. Instrumental tenderness was determined using Warner–Bratzler shear force (WBSF), while muscle morphometric traits, including fibre size, distance between fibres (DBF), and cross-sectional area (CSA), were evaluated. Histologically, there are lipid droplets within connective tissue between muscle fibres; however, no intramuscular fat infiltration observed. Glycogen deposition was detected in all muscle samples. No significant differences ($p > 0.05$) were found between HHP and NHHP groups for fibre size, DBF, or CSA. Nevertheless, the SS muscle from the NHHP group was more tender than that from the Proper HHP group. CSA also showed a moderate correlation with meat tenderness. Overall, the results suggest that herd health management may influence tenderness in specific buffalo muscles, although larger studies are needed to confirm this relationship.

Keywords: Herd health program (HHP), histomorphology, swamp buffalo

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INTRODUCTION

Swamp buffalo (*Bubalus bubalis*) is a native large ruminant species of Asia. They are distributed across the Yangtze River of China, Southeast Asia, and Assam in northeastern India (Mishra et al., 2015; Yindee et al., 2010). They have long served as the basis for rural livelihoods (Siriburee et al., 2025) in the region for centuries for draft power in rice cultivation (Pineda et al., 2021), coconut (Prawitasari et al., 2023), and oil palm plantations (Grinnell et al., 2022). In addition, they are recognized for their role in meat production, contributing significantly to food security and rural economies (Naveena & Kiran, 2014; Wanapat & Kang, 2013). Given the significance of swamp buffalo to the national economy and food security, ensuring meat quality at a superior level remains a primary objective for producers.

Meat tenderness is considered the most critical quality trait affecting consumer acceptance (Elzalaky, 2024; Henchion et al., 2017; Santos et al., 2021). Meat is composed of muscle tissue, along with connective and adipose tissues (Listrat et al., 2016). Any physiological, biochemical, or mechanical modification to these components significantly impacts the final eating experience (Listrat et al., 2016; Sakowski et al., 2022; Sofyan et al., 2020). Muscle morphology and tissue composition are the result of a complex interaction of genetics, environment, nutrition, and physical activity (Faria et al., 2022; Joo et al., 2013; Velotto et al., 2002) within broader production systems and farming practices (De Brito et al., 2017; Ikusika et al., 2020; Webb & Erasmus, 2013).

Herd health program (HHP) is a part of the production system that represents an integrated management approach encompassing disease control, nutrition, and proper rearing practices (Svensson et al., 2018). This program is essential to optimize beef performance (Agus & Mastuti Widi, 2018), as well as to reduce disease incidence (Galyean et al., 1999), minimize carcass rejection (Gruber et al., 2006; Lee et al., 2017), lower antibiotic residues (Beyene, 2015), and improve marbling and tenderness (Bruns et al., 2004).

The investigation of muscle morphology and meat quality is dominated in cattle, poultry, pigs, sheep, and goats, with the least attention given to the swamp buffaloes. However, established links exist between health management and productivity in livestock (Kappes et al., 2023) and between muscle morphology and meat quality (Velotto et al., 2002). Therefore, the objective of this study was to investigate the effects of the HHP on the histomorphology of muscle and meat tenderness in the swamp buffaloes.

MATERIALS AND METHODS

Animal

Two groups of male swamp buffaloes (*B. bubalis*) weighing 290±29 kg at the age of 18-24 months, with four animals (n = 4) in each group. Animal ethics approval was not required, as the muscle samples were collected from animals slaughtered under routine commercial practices.

Management System and Herd Health Implementation Program

The Herd health program (HHP) for swamp buffaloes was developed by Saad et al. (2003) based on manuals and guidelines from the Department of Veterinary Services (DVS, 2010). The first group was raised under a Proper HHP, while the other group was raised under a non-proper herd health program (NHHP). The HHP group was established through rotational grazing with *Brachiaria decumbens* on a 300-acre grazing area owned by the DVS Sabah. The HHP paddock was fertilized with 200-300 kg of N, 40-60 kg of P, and 100-150 kg of K/ha/year. The HHP group was also supplemented with palm kernel cake (PKC) for 3 days/week at 1.5 kg/animal/day when animals returned to the holding yard (usually 7 pm to 7 am). The NHHP group, which had a similar number, sex, and age distribution, grazed freely 24 h/day on 449 acres of DVS land, managed by farmers without feed supplementation or fertilizer in the paddock (Engkias, J. personal communication, Dec 18, 2018).

Both HHP and NHHP groups underwent disease screening, investigation, and prevention conducted by the DVS Sabah. While all animals in both groups were vaccinated against haemorrhagic septicemia (HS) twice a year, anthelmintics were administered only in the HHP group. No foot-and-mouth (FMD) vaccination was given to either group, as the area is an FMD-free zone.

Feed sampling process and determination of nutrient Content

The feed samples were taken at 3% dry matter (DM) by body weight and mixed well for nutrient analysis at 200-300 g. The grass samples from both paddocks (HHP and NHHP groups) at 3% DM of body weight were collected using 1 m^2 quadrats at five locations in each paddock. The DM content of the feeds was determined by

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heating at 105℃ for 3 h (method 930.15; Balthrop et al., 2011), and the ash content was subsequently determined after incineration at 550℃ for 2 h (method 942.05; Balthrop et al., 2011). The Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using a fiber analyzer (ANKOM Technology, USA) according to the procedure of Van Soest et al. (1991), without correction for residual ash. The ether extract (EE) and nitrogen (N) content were respectively determined by solvent extraction and by the Kjeldahl method (methods 954.02 and 976.05; Balthrop et al., 2011). The nutrient content of the feed consumed by both groups is summarized in Table 1.

Table 1. *Nutritive value of the diet given to the swamp buffaloes, HHP and NHHP groups*

Nutrient	[1]HHP group (n = 4)	[2]NHHP group (n = 4)
[3]DM (%)	99.49	99.50
Ash (% DM)	5.69	5.09
Crude fiber (%DM)	23.73	26.03
Ether extract (%DM)	2.92	2.03
Crude protein (%DM)	8.08	6.09
[4]NDF (%DM)	57.96	64.27
[5]ADF (% DM)	28.70	33.86
[6]ADL (%DM)	3.30	3.55
Carbohydrate (%DM)	61.53	59.45
Gross energy (MJ/kg)	12.10	11.07
Hemicellulose (% DM)	29.25	30.41
Cellulose (%DM)	25.38	30.32

Note. [1]HHP: grazing Brachiaria decumbens + 1.5kg PKC/animal/ day. [2]NHHP: grazing Brachiaria decumbens [3]DM: dry matter, [4]NDF: Neutral Detergent Fiber, [5]ADF: acid detergent fiber, [6]ADL: acid detergent lignin. Data retrieved with permission from Mohd Azmi et al. (2021)

Slaughter Protocol

Authorized professionals from DVS Sabah properly conducted the slaughter, exsanguination, and skinning processes in accordance with Malaysian Standards MS1500:2019 (Halal Slaughter) and MS2023-4:2016 (Halal Protocol for Slaughtering). Ante- and post-mortem examinations were performed by veterinary officers to ensure animal health status and safety for human consumption. The slaughtering, carcass processing, and sampling were conducted at the Sabah Meat Technology Center (SMTC), Kinarut, Kota Kinabalu, Sabah.

Sampling of the Muscles, Tissue Processing, and Morphological Evaluations

Immediately after 45-60 min post-slaughter, the muscles (*longissimus dorsi* [LD], *supraspinatus* [SS], and *semitendinosus* [ST]) from both groups of the swamp buffalo carcasses were collected for tenderness and morphological analysis. For tenderness analysis, approximately 30-72 g of muscle samples were collected from designated anatomical locations and cut into 2.54 cm-thick slices and 1.27 cm cylindrical cores (American Meat Science Association, 2016) before the muscles were chilled at 3-4°C and 75% humidity for 24 h (McSharry et al., 2021). These collected samples were then frozen at -80°C for later tenderness analysis.

For morphological analysis, the muscle samples were excised as 1.0 cm × 1.0 cm × 1.5 cm cubes from the same anatomical locations as for tenderness analysis. These cube-incised tissues were then snap-frozen in 2-methylbutane chilled with liquid nitrogen (Song et al., 2020) before preservation at -80°C for later histological analysis. The overall sampling process flow is illustrated in Figure 1.

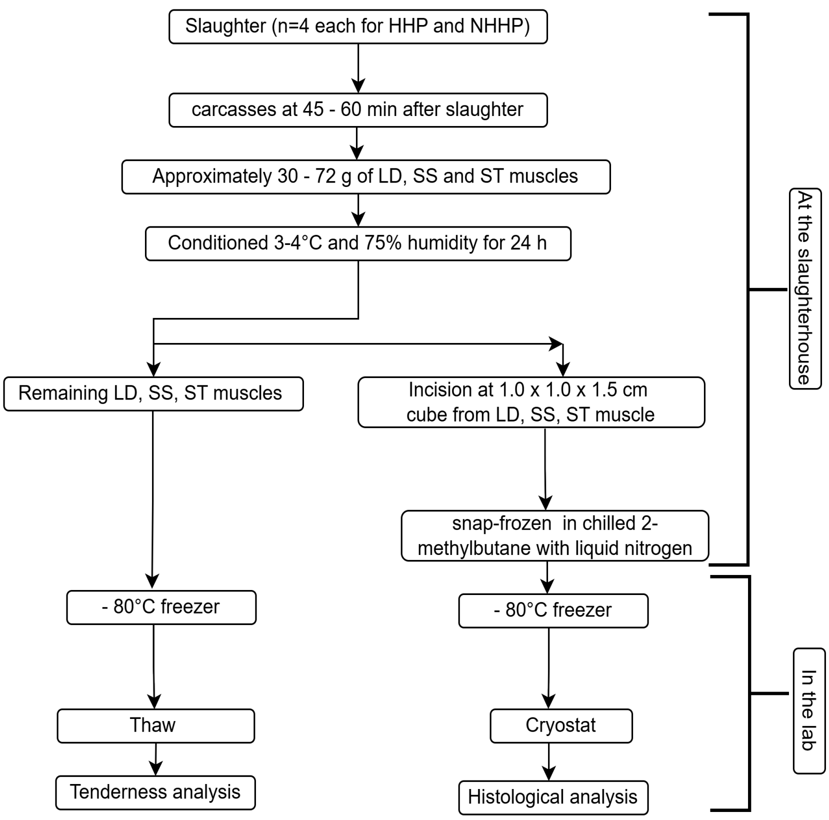

Figure 1*. Schematic diagram of sampling process flow for morphology and tenderness analysis of the swamp buffalo muscle and meat*

Note: LD = longissimus dorsi; SS = supraspinatus; ST = semitendinosus; HHP = proper herd health program; NHHP = non-proper herd health program.

Muscle tissue was prepared for histological evaluation using a cryostat. The cryostat blade was pre-cooled to -20 ± 2°C for at least 20 min. Approximately 10-16 µm (±12 µm) thick sections were prepared and brought to room temperature. The sections were stained with Hematoxylin and Eosin (H&E), Periodic Acid-Schiff (PAS), and Oil Red O (ORO) stain. The diameter of the muscle fibers was determined by measuring the transverse sections of three fasciculi from each muscle sample. The measurements were obtained from randomly selected slides to minimize errors and biases. Muscle tissue diameters were recorded longitudinally and perpendicular to each other. The measurements taken were Distance Between Fiber (DBF), muscle fiber width, and cross-sectional area (CSA), as displayed in Figure 2.

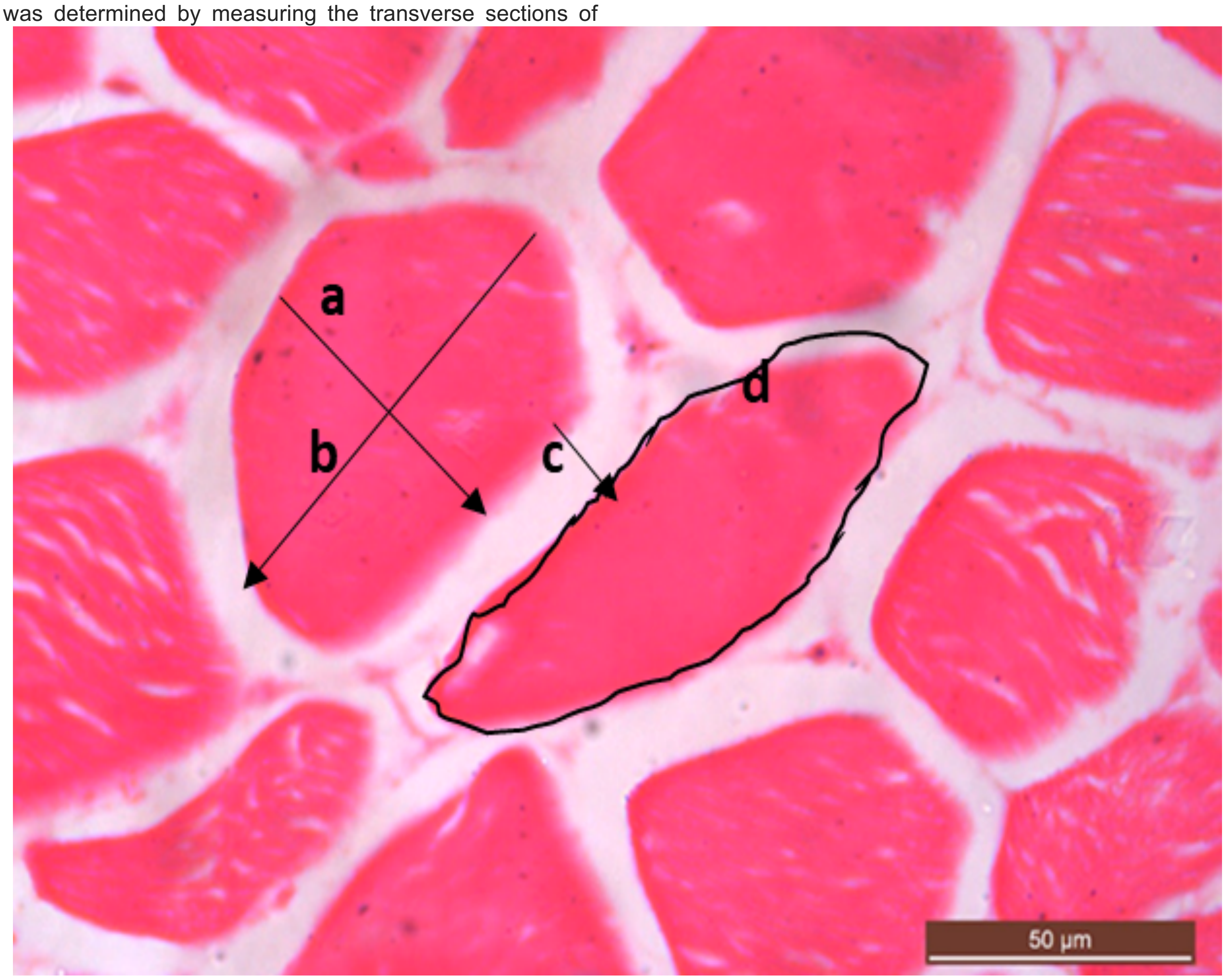

Figure 2. Illustration of how the measurements are taken from the muscle section. The letters a, b, c, and d represent the width, length, DBFs, and CSA of the muscle section, respectively

Meat Tenderness Analysis

Muscle samples (LD, SS, and ST) weighing 30±1.24 g to 72±2.26 g from both the HHP and NHHP groups were excised from carcasses within 45-60 min post-slaughter for tenderness analysis using the Warner-Bratzler shear force (WBSF) method. The meat was thawed in the chiller overnight at 4°C, then cooked to an internal temperature of 72°C, maintained at this temperature for 10 min, and cooled overnight at 4°C (Luz et al., 2017). Each meat sample was cored using an 11.1 mm-diameter puncture probe, with at least six cores prepared parallel to the muscle fiber orientation as much as possible. The samples were then placed under a V-blade and cut at 200 mm/min. The fibers were then oriented perpendicularly to the blade using a texture analyzer (Instron Technology, Norwood, MA, USA) (Novaković & Tomašević, 2017), which features a 3 mm-thick steel blade with a 73° cut. The mean peak WBSF was determined for each steak by averaging six cores (Bloomberg et al., 2013). The lowest value was considered to indicate the tenderest meat. In contrast, the highest value was considered to indicate the toughest meat.

Data Analysis

An independent t-test was performed to determine the differences in meat morphometry and tenderness between the two groups. Pearson's correlation analysis was conducted to evaluate the relationship between muscle morphological characteristics and meat tenderness. One-way Analysis of Variance (ANOVA) was conducted to assess the effects of muscle type and WBSF. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0, with $p < 0.05$ as the significance level.

RESULTS

Muscle Morphology

ORO staining was used to detect lipid droplets and fatty tissues. Lipid droplets were exclusively observed in the connective tissue, with no fat or adipose tissue detected within the muscle fibers. Meanwhile, PAS staining revealed small glycogen deposits, appearing as purple dots, scattered throughout some muscle tissues. In the LD muscle of the NHHP group, ORO staining revealed the presence of neutral triglycerides and lipid droplets (Figure 3) within adipocytes. This finding indicates that this fat was located within the connective tissues outside the muscle fibers. The cross-section of the muscle fibers stained with PAS demonstrates glycogen and lipid droplets in adipocytes in the LD, SS, and ST muscles of the HHP group, where glycogen is widely distributed within the muscle fibers (Figures 4A and 4B).

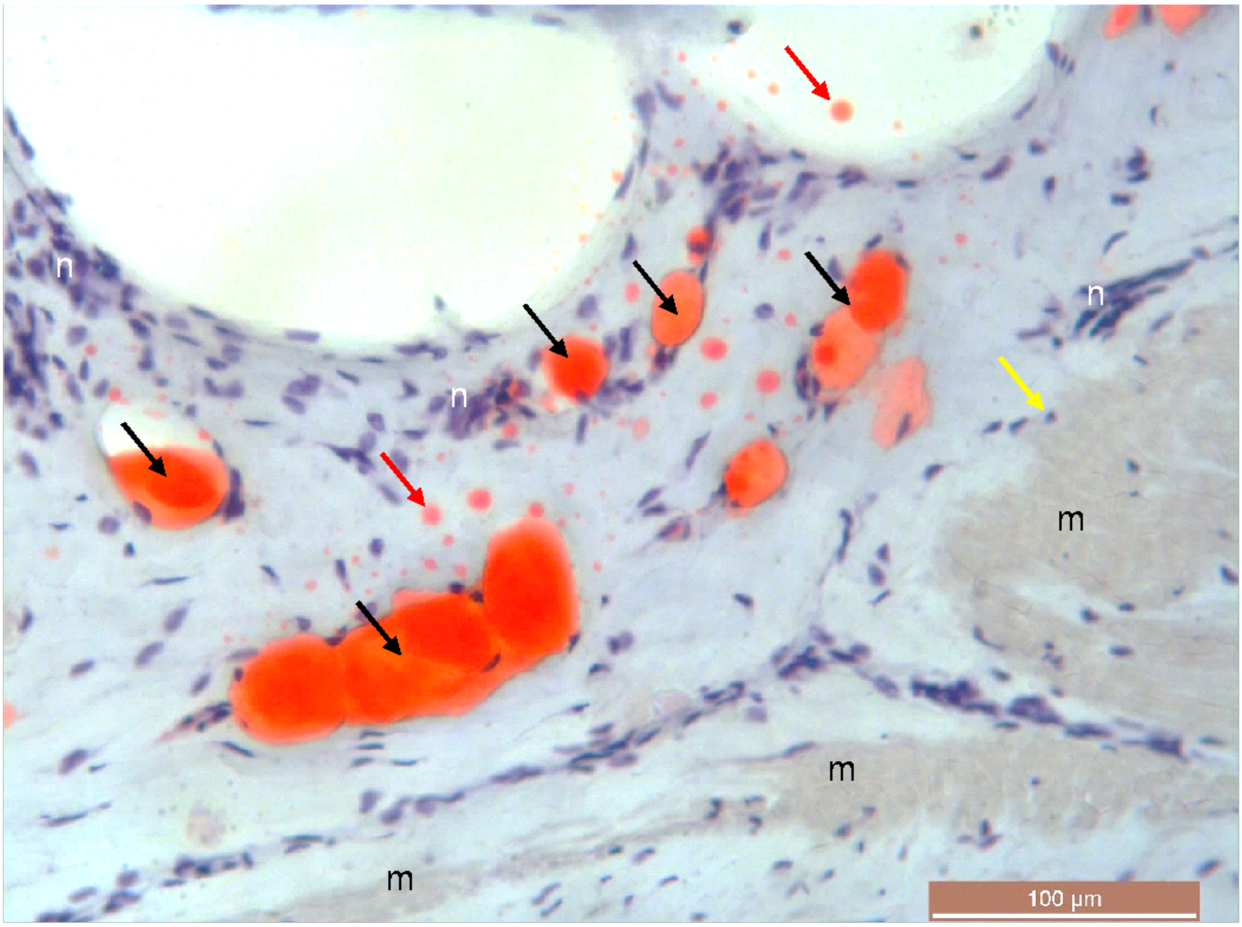

Figure 3. Histological section of the LD muscle of the NHHP group stained with ORO. Black arrows indicate adipocytes, and red arrows indicate lipid droplets. The fat is present within the connective tissues outside the muscle fiber (m). n = nucleus of a cell. Scale = 100 μm.

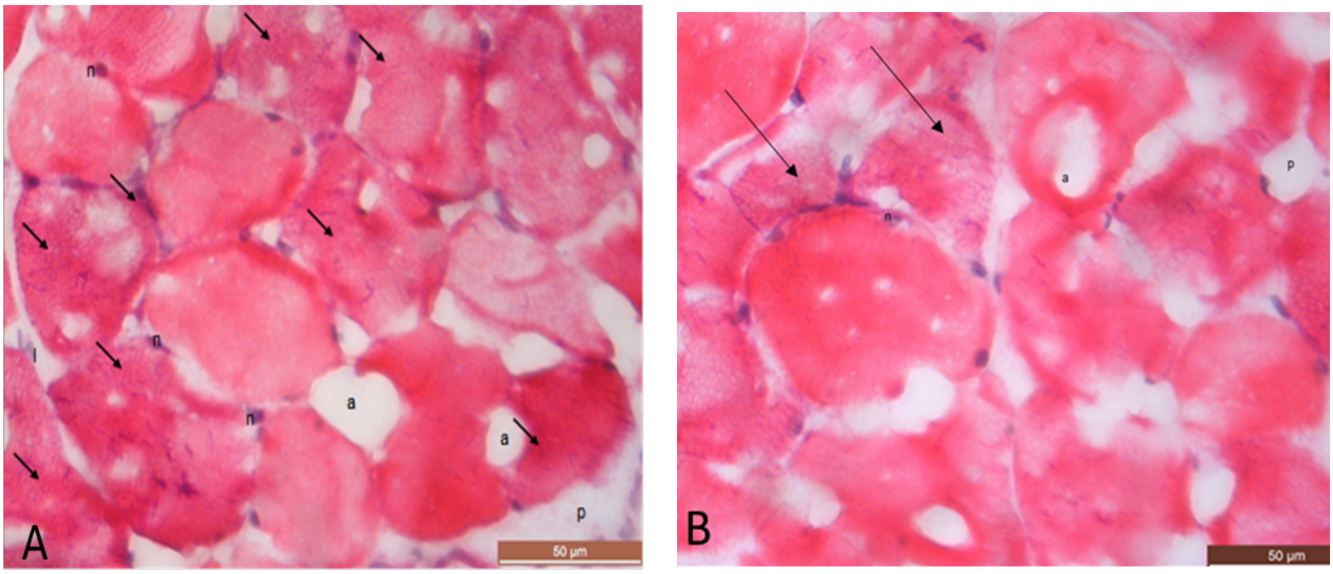

Figure 4. Cross-section of the muscle fibers stained with PAS demonstrating glycogen (arrows) and lipid droplets (adipocytes) in the muscle fibers of LD (A), SS (B) muscles of swamp buffaloes raised under HHP. The nuclei (n) of muscle fibers are pushed to the periphery. a = adipocyte, p = perimysium. Scale = 50 μm.

Muscle Morphometry

The values of the fiber diameter (μm), DBFs (μm), and CSA (μm) of LD, SS, and ST muscles from HHP and NHHP implementation groups are presented in Table 2. Although all muscle histomorphometry variables were greater in the NHHP group than in the HHP group, the differences were not significant. While CSA was significantly affected by muscle type ($p < 0.05$), this was only in the NHHP group.

Table 2. The morphometry of the LD, SS, and ST muscles of the swamp buffalo raised under HHP and NHHP

Variables	Muscle	Swamp Buffalo Group		
		HHP (n = 4)	NHHP (n = 4)	p-value
Diameter (μm)	LD	49.83±14.58	52.33±5.57	0.790
Diameter (μm)	SS	50.33±15.65	52.83±14.58	0.869

Variables	Muscle	Swamp Buffalo Group		
		HHP (n = 4)	NHHP (n = 4)	p-value
Diameter (µm)	ST	44.83±8.10	47.33±14.50	0.809
Diameter (µm)	Muscle effect	ns	ns	
DBF (µm)	LD	18.32±4.76	18.98±4.29	0.862
DBF (µm)	SS	17.79±2.08	19.45±1.26	0.386
DBF (µm)	ST	18.18±4.20	19.85±1.41	0.627
DBF (µm)	Muscle effect	ns	ns	
CSA (µm^2)	LD	2338.03±201.07	2346.94±213.55^a	0.923
CSA (µm^2)	SS	2424.30±224.93	2515.22±274.83^b	0.742
CSA (µm^2)	ST	2417.73±178.78	2524.09±224.09^c	0.694
CSA (µm^2)	Muscle effect	ns	0.010*	

*Note. *The significance at $p < 0.05$ using One-Way ANOVA and Tukey's HSD test to identify the differences between rows (effect between LD, SS, and ST muscles).*
$^{a, b, c.}$ The values with superscripts in the columns were significantly different at $p < 0.05$.

LD= Longissimus dorsi

SS= Supraspinatus

ST= Semitendinosus

Meat Tenderness

Table 3 summarizes the WBSF values used to evaluate the meat tenderness of the swamp buffalo (*B. bubalis*) muscles raised under HHP and NHHP conditions. Furthermore, the SS muscle of HHP buffaloes had a significantly higher WBSF (p = 0.021) than that of the NHHP group. At the same time, tenderness for the LD-ST and LD-SS muscle pairs differed significantly (p < 0.001), though this was not between the SS-ST muscles.

Table 3. *The tenderness (WBSF) of the swamp buffaloes (Bubalus bubalis) muscles raised under HHP and NHHP*

Muscle	Buffalo groups		*p*-value
	HHP (n = 4)	NHHP (n = 4)	
Longissimus dorsi (LD)	59.76±6.28	54.44±4.29	0.930
Supraspinatus (SS)	76.30±7.86^a	67.88±8.33^b	0.021
Semitendinosus (ST)	78.61±4.49	75.62±3.85	0.630
Effect between muscle[1]			
LD*ST	***	***	
LD*SS	***	***	
SS*ST	ns	ns	

Note. $^{a, b}$ the values superscripted were significantly different at $p < 0.05$

****p-value < 0.001*

[1]The differences between muscles were assessed using the post hoc Tukey's HSD test.

Table 4 presents the association between muscle morphometric traits and meat tenderness. Among the parameters evaluated, only CSA (µm^2) demonstrated a significant association with tenderness (p = 0.0321), whereas other muscle traits remained non-significant.

Table 4. *The association of muscle morphometry with meat tenderness*

Trait of the muscle fiber	Correlation Coefficient	p-value
Fiber diameter (µm)	-0.032	0.9166
DBF (µm)	-0.359	0.2281
CSA (µm^2)	0.595	0.0321

Note. The association between muscle morphological traits and meat tenderness was assessed using Pearson's correlation at the $p < 0.05$ significance level.

DISCUSSION

Cattle are the primary source of beef production. Nevertheless, buffalo meat is also widely consumed worldwide. In India, for instance, cattle slaughter is prohibited in several states due to legal protections for cows under the Indian Constitution (Khan, 2016). However, this ban was not imposed on buffalo, which can serve as an alternative protein source (Fischer, 2019).

The current study focuses only on males, as they perform better and are preferred for beef production compared to females. Notably, females are typically retained on farms for breeding. Although some researchers do not specify sex in meat quality studies (Joo et al., 2013; Lee et al., 2016), using both sexes without stratification is expected to introduce extraneous variability into the study (Luger et al., 2020). Sex may cause differences in muscle composition, morphology, and activity (Natsis et al., 2016). The effect of sex on muscle fiber size has also recently been reported by Nuzzo (2024), who observed larger muscle fibers in males than in females. Interestingly, the work of Nuraini et al. (2013) on the swamp buffalo revealed that age is the most impactful factor on muscle fiber diameter, while the effects of WBSF and pH between male and female were less pronounced than in cattle.

Two main issues that may introduce bias and compromise the reproducibility of this study were the small sample size of four animals per group and the utilization of histological staining methods such as H&E and PAS. Nonetheless, the limited sample size in the histological evaluation is not considered a major issue, since histological evaluation often emphasizes details at the cellular and tissue levels (Odenkirk et al., 2022). In addition, histological evaluation involves intensive sampling and replication within specimens, which are adequate to detect treatment effects (Bastiaenen et al., 2020).

Meat quality may be derived from various factors. The implemented HHP may be one of the extrinsic factors contributing to the muscle's morphology and tenderness. An HHP reduced carcass rejection (Gardner et al., 1999) and improved muscle-to-meat conversion (Ouali et al., 2006), while also reducing welfare-related issues (del Campo et al., 2025). The current study revealed that the CSA was affected by muscle type, particularly in the NHHP group. This finding is consistent with Sarker et al. (2024), who highlighted that smaller muscle fibers with lower CSA were associated with improved meat tenderness. These findings are consistent with those of Rivero et al. (1996), Nakatani et al. (2000), and Hene et al. (2023), but contrast with the findings of Verdiglione and Cassandro (2013), who reported no significant variation in the pectoralis major muscle of broilers. These differences suggest that CSA is a muscle- and species-specific trait. The present study further examined the influence of muscle on LD meat tenderness compared to SS and ST. Differences in meat tenderness may be associated with the functional role of specific muscles. The LD muscle serves as a body-supportive muscle, whereas SS and ST are locomotive muscles. This is supported by a previous finding by Vestergaard et al. (2000) that grazing animals under an extensive system demonstrated a significantly greater percentage of slow-twitch oxidative (Type I) muscle fibers in the LD and SS muscles than intensively raised animals. This finding contrasts with that reported by Su et al. (2024), who underscore that the ST muscle had tougher meat due to movement and load-bearing activities. The SS muscle is generally less exposed to intense movement than the ST muscle, which may explain the differences in muscle characteristics and the greater meat tenderness observed in the forelimb rather than in the hindlimb (Schmidt & Fischer, 2009).

The sampling process in this study was designed to consider the relationship between muscle morphology and tenderness. This approach helps standardize the muscle structure at similar points for tenderness and muscle histology. However, interpreting these differences should be done cautiously, as this study was conducted under field conditions rather than a controlled experimental design. In the current study, the HHP and NHHP groups represent actual farming practices, where HHP are not clearly separated from other single management-related factors, as in a controlled experiment. Rotational grazing and PKC supplementation may independently affect meat quality and muscle morphology. Previous studies by Krusinski et al. (2022), Maltin et al. (2003), Mwangi et al. (2019), and Wicks et al. (2019) have suggested that nutritional, environmental, muscle physiology, and pasture grazing, which may act as an integrated approach (Gagaoua et al., 2017), influence muscle growth, development, and muscle morphology. Therefore, the differences observed in CSA, muscle morphology, and meat tenderness across different muscle types can be interpreted as an association with overall herd health management (HHP vs NHHP) rather than an individual management package.

This physical activity may have altered the muscle fiber composition (Adala & Campos, 2017), potentially shifting the muscle toward more oxidative, fatigue-resistant fibers, thereby improving meat tenderness. Additionally, animal movement associated with the rearing practices of HHP and NHHP, which differ in grazing patterns and management, walking distances, and supplementation, may also promote muscle hypertrophy. During the growth stage, hypertrophy eventually changes the shape, tenderness, sarcomere length, myofibrillar protein, and calpain proteolytic activity of the muscle (Koohmaraie et al., 2002), all of which are known to affect meat quality.

In addition to CSA, DBF also plays an important role in determining meat tenderness. Although it was not observed in this study, Listrat et al. (2020) reported that fiber arrangement and fiber spacing strongly affect tenderness across all muscles. Post-slaughter practices, such as post-mortem aging, improve the separation of muscle fibers and reduce WBSF scores (Soji, 2021). At least 24 h of aging is sufficient for this separation to occur (Soji & Chulayo, 2020). In this study, no tenderness-related effects attributable to DBF were observed in both groups. This was possibly due to the sampling process being performed immediately after slaughter, before the post-mortem effect on the muscle had occurred, allowing fiber separation.

This observation was supported by the presence of muscle glycogen, indicated by PAS stain. PAS staining indicates the presence of glycogen deposits in muscle tissue but provides only a qualitative evaluation (Fairchild et al., 2004; Prats et al.,2018), since glycogen concentration and post-mortem pH were not quantified in the current study using biochemical glycogen quantification. Therefore, the interpretation of glycolytic activity in swamp buffalo muscle is still considered preliminary. The muscle energy, which is reserved in the form of glycogen, which had not been fully utilized before death, was converted into lactic acid through the glycolytic conversion of muscle into meat 45-60 min post-slaughter. Although previous literature has stated that glycogen conversion may begin rapidly after slaughter (Kiyimba et al., 2024; Qiu et al., 2025), this depends on the animal species and glycogen reserves (Stempa & Bradley, 2020; Terlouw et al., 2021). The complete glycolysis in pork generally occurs within 24 h post-slaughter. In contrast, in deer and cattle, it may take approximately 18 to 24 h (Strobel et al., 2021), while the glycolysis in water buffalo has been reported to last up to 48 h (Neath et al., 2007). This prolonged glycolytic period likely leads to glycogen deposits in buffalo tissue, as demonstrated in this study.

Animal age may contribute to muscle morphological variation and tenderness, as suggested by a previous study (Listrat et al., 2016). Nuraini et al. (2013) reported morphological differences in fiber diameter, although tenderness was not significantly different in some age groups. In this study, the age group and sex were standardized to explore HHP.

There was an established association between instrumental (objective) meat tenderness and sensory (subjective) meat tenderness evaluation (Adam, 2021; Warner et al., 2021). Nevertheless, using both methods depends on the objectives and purposes of the study. Instrumental tenderness measurements provide objectivity, accuracy, reproducibility, and standardization at a larger scale. Nonetheless, they fail to capture human perception and the complexity of texture and sensory attributes, such as juiciness and mouthfeel, which also contribute to tenderness. Despite this, the measurements remain widely used to facilitate standard comparisons across samples in industry and research, as these methods minimize human bias and provide precision in evaluation (Tornberg, 1996).

CONCLUSION

In conclusion, this study partially confirmed and validated the hypothesis that the HHP is associated with muscle morphology and meat tenderness in swamp buffalo. Overall, the muscle fibers remained unchanged in both buffalo groups. A significant observation was made in the SS muscle, which presented higher tenderness, suggesting muscle and practice effects; the group's physical activity was one of the contributing factors. This study also provides evidence that, in swamp buffalo, tenderness, as a meat quality attribute, may be affected by muscle type and management-related factors, including movement and rearing practices. The positive relationship observed between CSA and meat tenderness further indicated that muscle fiber morphology may contribute to the variation in tenderness of swamp buffalo meat in this study. However, this study was conducted under field observation rather than a controlled experiment. Therefore, it was interpreted as an integration of all interrelated factors rather than a singular factor. Hence, further studies using larger sample sizes, cohort designs, or controlled experimental designs may be required to validate these findings.

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ETHICS APPROVAL

The study did not involve live animal experimentation or treatment or invasive sampling. The sample obtained from a normal slaughterhouse operation under permission of Department of Veterinary Services and Sabah Meat Technology Centre.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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AUTHOR CONTRIBUTIONS

Fhaisol Mat Amin: Data curation, data analysis, preparation for the manuscript. Mardhati Mohammad: Data analysis, reviewing, editing. Rudy Priyanto: Manuscript reviewing, commenting and validation. Lokman Hakim Idris: Methodology, validation, Hasliza Abu Hassim: Project management and resource management. Mohd Zamri Saad: Project management, project proposal. Md Zuki Abu Bakar: Supervision, conceptualization, reviewing and editing and corresponding author.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon request.

AI-ASSISTED WRITING DISCLOSURE

AI were used at very limited capacity such as for grammar and data searching; however, the final citation and source of information were verified with credible databases such as Scopus, WoS, Google Scholar, PLOS, Pubmed, Wiley and others. Halucination of input were removed and discarded. The AI usage did not influence the results, conclusion and scientific integrity of the manuscript.

ABBREVIATIONS

Term	Definition
ADF	Acid Detergent Fiber
CSA	Cross-Sectional Area
DBF	Distance Between Fibers
DM	Dry Matter
DVS	Department of Veterinary Services
EE	Ether Extract
FMD	Foot-and-Mouth Disease
HiCoE	Higher Institution Centre of Excellence
H&E	Haematoxylin and Eosin
HS	Haemorrhagic Septicaemia
HHP	Proper Herd Health Program
LD	Longissimus dorsi
MARDI	Malaysian Agricultural Research and Development Institute
MS	Malaysian Standard
N	Nitrogen
NDF	Neutral Detergent Fiber
NHHP	Non-Proper Herd Health Program
ORO	Oil Red O Stain
PAS	Periodic Acid–Schiff Stain
PKC	Palm Kernel Cake
SMTC	Sabah Meat Technology Centre
SS	Supraspinatus
ST	Semitendinosus

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