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Rumen Volatile Fatty Acids and Morphology of the Rumen Mucosa of Swamp Buffalo Raised under Semi-Intensive and Extensive System in Tropical Environment

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ABSTRACT

Swamp buffaloes are mostly raised under an extensive system because they can adapt to the harsh environment. However, exploring the rumen mucosa (RM) morphology and volatile fatty acids (VFA) of swamp buffalo associated with different production systems is still lacking. This study evaluated the rumen VFA and morphology of RM between two groups of buffalo raised under semi-intensive (SI) and an extensive system (EX). VFA was analysed using gas chromatography. The morphology of rumen mucosa was evaluated macro and microscopically for papillae length and width, surface area, density, and muscle thickness, and the microscopic evaluation for stratified squamous epithelium (SSE) and keratin thickness. SI has a greater VFA concentration than the EX. The SSE layer on the dorsal region of the rumen was thicker in the EX group than in the SI group ($p \leq 0.05$). Within the group, the SSE of the dorsal region of rumen was thicker than the ventral region ($p \leq 0.05$) in the EX group. However, the ventral region of the rumen was thicker than the dorsal region in the SI group. The thickness of the keratin layer in the EX group was significantly thicker than the SI group ($p \leq 0.05$) only on

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the dorsal region. In conclusion, swamp buffalo from the SI production system has a greater concentration of volatile fatty acid than the EX-group contributed by feeding management under a semi-intensive system. Nevertheless, the advantage in VFA concentration alone is not sufficient to conclude semi-intensive production system exerts a favourable effect on the morphology of the rumen mucosa.

Keywords: Morphology, production system, rumen mucosa, swamp buffalo, volatile fatty acids

INTRODUCTION

Swamp buffaloes (*Bubalus bubalis*) is an important ruminant animal in many parts of the world, especially in Eastern and Southeast Asia. They are excellent fibrous converters and well-adapted to low-quality feed under harsh environmental conditions. Although the different environments may result in different livestock productivity, Wanna et al. (2012) reported that swamp buffaloes grazing on the land produced yield better. However, they are raised under small hold-based agriculture by smallholders (Escarcha et al., 2020) for a longer period without proper forage production area (Suphachavalit et al., 2013) or fed based on agricultural residue (Savsani et al., 2017) in relatively difficult and poor feeding areas (Suhaimi et al., 2019).

Buffaloes, like other ruminants, generally have similar anatomy of the digestive system, but morphophysiological might be different in terms of digestion, absorption, and metabolism. The ratio of volatile fatty acid (VFA) composition in the rumen is affected by the type of feed, forage, species, and quantity of the microbes (L. Wang et al., 2020). This fermentation process is highly associated with ruminant species (Ferreira et al., 2017), diet, and feeding regime (Sutton et al., 2003). Ruminant productivity is generally associated with digestive efficiency, which converts the fibres into volatile fatty acids as a primary energy source by the fermentation process (Bergman, 1990). VFA is absorbed through rumen epithelium via passive transport (Steele et al., 2011) and associated with osmotic pressure, depending on the permeability of the epithelium and blood flow (Storm et al., 2012) within the mucosa (Kern et al., 2016). VFA production and rumen mucosa development are highly influenced by the diet type and composition (Celi et al., 2017; Henderson et al., 2015; Wanapat et al., 2009), the feeding regimes (Bergman, 1990), and the species of the animal (Mao et al., 2012), which might also be caused by feeding behaviour on forage utilisation (Lin et al., 2011). It was also subjected to geographical factors (Henderson et al., 2015) and seasonal influence (Ding et al., 2018), as well as the production systems (Kotresh Prasad et al., 2019), which influence the adaptive modification of the morphophysiological toward survival under extreme conditions, habitat, climate, season, and human intervention.

Many previous works agreed that the type and the quantity of VFA influence the growth of rumen papillae in various ruminant species such as in goats (Y.

H. Wang et al., 2009), sheep (Álvarez-Rodríguez et al., 2012; Baldwin, 1999), dairy cattle (Steele et al., 2011; Storm et al., 2012), beef (Kern et al., 2016), and wild ruminants (Mason et al., 2019). In addition, they also influence the growth of the earlier age of lamb (Y. H. Wang et al., 2009), kids (Kotresh Prasad et al., 2019) or calves (Consalvo et al., 2016; Suárez et al., 2007). However, there is a limited exploration of the rumen mucosa morphology and volatile fatty acids of swamp buffaloes associated with the production system in a tropical environment. Therefore, it was interesting to investigate the rumen volatile fatty acid and mucosae morphology of swamp buffaloes under different farming or production management systems. Since the animals reared under a semi-intensive system have better management and feeding system compared to the extensive system, it is hypothesised that buffalo under a semi-intensive farming system (SI) will result in a greater concentration of volatile fatty acids and improve rumen mucosal morphology. Thus, the objective of this study was to evaluate the rumen volatile fatty acid and mucosae (macro and microscopically) of swamp buffaloes raised under semi-intensive and extensive production systems in a tropical environment.

MATERIALS AND METHODS

Animals

Two groups of male swamp buffaloes (*Bubalus bubalis*) weighed 290 ± 2.90 kg at 24 months old from two different production systems were used. The first group was four animals raised under a semi-intensive system (SI), while the other group was six males raised under an extensive system (EX). SI group raised with rotation grazing in 300 acres of grazing area owned by Department Veterinary Services (DVS) (Mohd Azmi et al., 2021), disease screening conducted as prescribed by DVS, vaccinated against hemorrhagic septicemia (HS) twice/year, and anthelmintics was also given. The paddock of the SI group was fertilized with 200-300 kg of nitrogen (N), 40-60 kg of phosphorus (P), and 100-150 kg of potassium (K)/ha/year. The main grass available was *Bracharia decumbens*. The swamp buffaloes of the SI group were also supplemented with palm kernel cake (PKC) three days a week based on the calculated amount at 1.5 kg/animal/day basis when return to the holding yard (usually within 7 p.m. to 7 a.m.). However, in the EX group, the animals were on free grazing 24 hours/day on 449 acres of DVS land managed by local farmers without feed supplementation and with no vaccination or anthelmintics given, as well as no fertilization applied on the paddock. The main grass was also *Bracharia decumbens* (J. Engkias, 2018, personal communication, December 18, 2018).

Sampling Process and Determination of Nutrient Content

The feed samples taken for analysis was based on 3% dry matter bodyweight and mixed with 1.5 kg/animal/day. 200–300 gm of well-mixed feed of the SI group was used for nutrient analysis. The grass sample [3% dry matter (DM) of body weight (BW)] was sampled using 1 m^2 quadrat from five locations for both 300 acres (SI group) and 449 acres (EX group). The DM content of the feeds was determined by heating at 105 ℃ for 3 h (method 930.15; Balthrop et al., 2011), and the ash contents were subsequently determined after incinerated at 550 ℃ for 2 h (method 942.05; Balthrop et al., 2011). The neutral and acids detergent fibres [neutral detergent fibre (NDF) and acid detergent fibre (ADF), respectively] was analysed using a fibre analyser (ANKOM Technology, USA) following the procedure suggested by Van Soest et al. (1991) without correction for residual ash. The ether extract (EE) and N content were determined by the solvent extraction and the Kjeldahl method (method 954.02 and 976.05; Balthrop et al., 2011). The detail of the nutrient contents of feed consumed by both groups is summarized in Table 1.

Table 1
Nutritive value of the diet given to the swamp buffaloes under two type of production system

Nutrient	[1]SI group (n = 4)	[1]EX group (n = 6)
[3]DM (%)	99.49	99.50
Ash (% DM)	5.69	5.09
Crude fibre (%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]SI: Semi-intensive group grazing *Brachiaria decumbens*, [2]EX: Extensive group grazing *Brachiaria decumbens* + 1.5 kg PKC/animal/ day. [3]DM: Dry matter, [4]NDF: Neutral detergent fibre, [5]ADF: Acid detergent fibre, [6]ADL: Acid detergent lignin. Data retrieved with permission from Mohd Azmi et al. (2021)

Environmental Condition

The temperature ranges between 21 ℃ - 32 ℃ at dry (throughout the year) and southwest monsoon season (September to March) (Masud et al., 2014), while the rainfall ranges from 1500 mm – 4600 mm (Malaysian Meteorological Department [MetMalaysia], 2018).

Collection of Rumen Fluid and Volatile Fatty Acid Determination

All the sampling procedures were conducted after animals were slaughtered. Before rumen fluid was collected, an incision at 10 – 20 cm was made to insert hand to mix the rumen fluid before the collection. A 10 ml of rumen fluid was collected and immediately filtered with cheesecloth, and 2 ml of 25% of metaphosphoric Acid was added to prevent fermentation. The fluid was then centrifuged for 10 min, 4 °C at 15,000 x *g*. The supernatant was taken and directly injected into gas chromatography (GC) for volatile fatty acid determination using GC with HP-INNOWAX (19091N-133) polyethylene glycol (PEG) stationary phase column (30 m, 0.25 mm ID, 0.25 µm film thickness) in an Agilent 7890B gas-liquid chromatography system (Agilent Technologies, USA) equipped with a flame ionization detector (FID). The injector/detector temperature was programmed at 260 °C (Ebrahimi et al., 2017). The column temperature was set at the range of 80 - 205 °C, at the rate of 10 °C/min increments, to achieve the best separation. The peaks were identified by comparing each VFA (Sigma-Aldrich, USA). Pivalic acid was used as an internal standard for quantification. The values obtained from the peaks were calculated and shown according to their density and molecular weight. Each sample was calculated proportionate to the volatile fatty acid of the ruminal fluid.

Collection of Rumen Mucosae for Morphometrical Evaluation

Immediately after the ruminal fluid was collected (after slaughtered), the rumen was taken out from the abdominal cavity. The dorsal and ventral region of the rumen was identified. Rumen tissue samples were taken as suggested by (Álvarez-Rodríguez et al., 2012), where five incisions of 5 cm x 5cm square from the dorsal and ventral region were taken. Tap water was used to wash this ruminal wall to remove digesta before rinsing it with phosphate buffer saline (PBS) with pH 7.4 at 37 ºC. The macro-morphometry of rumen mucosa was conducted, where ten papillae were gently extracted from 5 cm x 5 cm square of each rumen mucosa of the swamp buffaloes using forceps and scalpel blade No. 11, 4% buffered formalin was a drop on the papillae to fix the samples to ease measurements. The readings of rumen papillae were taken from well-oriented papillae of each rumen site. In contrast, the length measurement was taken from the tip to the base of the papillae along the long axis. The width of the papillae was measured at halfway perpendicular to the papillae length. In contrast, the papillae surface area was measured by circling each papilla using a stereo microscope with an image analyser (Leica M80, Leica Application suite ver. 4.3.0, Switzerland) (Figure 1). The thickness of the tunica muscularis was taken perpendicular to the muscles layer (Figure 2). The papillae density was manually counted on each incision with the help of magnifying glass, and the readings were taken as average and expressed in papillae/cm^2. The colour score was assessed by three evaluators simultaneously as suggested by Álvarez-Rodríguez et al. (2012) (1: yellow, 2: light brown, 3: dark brown-grey).

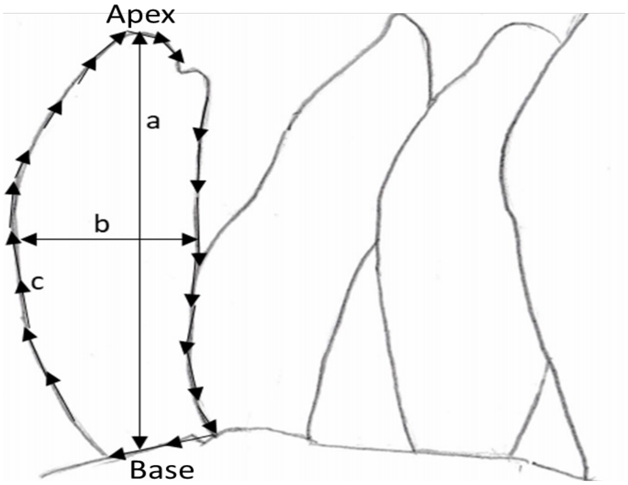

Figure 1. The illustration shows how the morphometrical measurement on the rumen papillae was conducted. The reading of the length was taken longitudinally between base and apex (a), while the width of the papillae was taken perpendicular across the papillae (b), and the surface area was measured by encircling the papillae boundaries (c). These processes were repeated on the five well-organised and prominent papillae. Less developed papillae were avoided for measurements

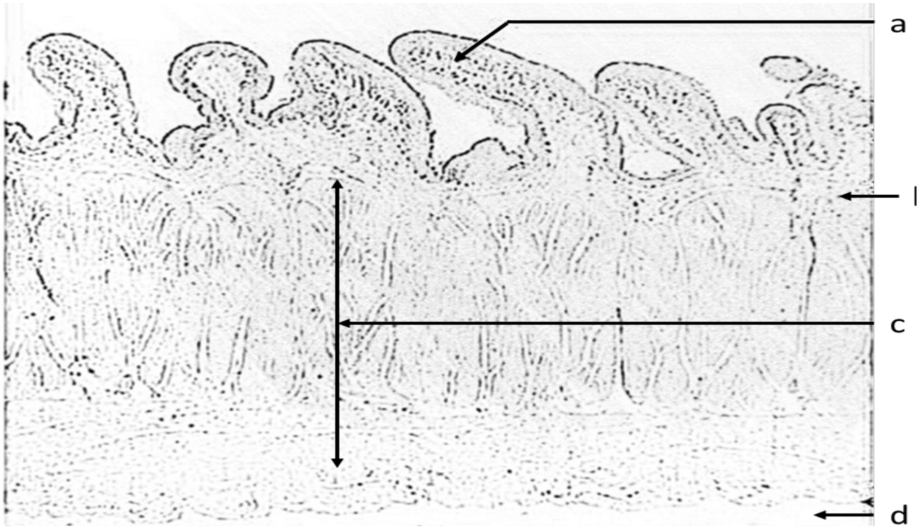

Figure 2. The illustration of the rumen wall of the ruminants; a: papillae, b: submucosa, c: tunica muscularis/ muscle layer, d: serosa layer. The morphometrical measurements of the tunica muscularis of the rumen wall should cover the inner circular and the outer longitudinal muscle (c)

Microscopic Evaluation of the Rumen Mucosae

Five pieces of the rumen mucosae at 1 cm^2 from every swamp buffalo's ventral and dorsal regions (Almeida et al., 2018) were fixed in Bouin's solution for 24 – 36 hours, dehydrated, cleared, and embedded in paraffin. Five paraffin blocks from each animal were randomly selected and cut (Feathers, Japan) using a rotary microtome (HM315R, Artisan, USA) at 5 µm, then stained with hematoxylin and eosin (H&E) for general histological and morphometrical analysis using a microscope (Leica DM2500, Leica Biosystem, Germany). Three readings of SSE and keratin layer thickness from five slides were calculated into mean.

Statistical Analysis

Data were analysed using the Statistical Product and Service Solutions (SPSS) statistical software (IBM SPSS Statistics 23, USA). A normality test was performed, and a non-parametric *t*-test (Mann-Whitney U test) was conducted on volatile fatty acid concentration, macro-morphometry (papillae length, width, surface area, density, and muscle thickness), and micro-morphometry (SSE and keratin layer thickness) to exhibit differences between the two groups. The results were reported as least square means and their associated standard errors (SE). The *p*-values lower than 0.05 ($p<0.05$) were considered significant.

RESULTS

Volatile Fatty Acids

There was no significant different ($p\geq0.05$) in pH value in rumen fluid between SI and EX groups. The acetic acid, propionic acid, butyric acid, isobutyric acid, propionic acid %, butyric acid %, and total VFA were significantly higher ($p<0.05$) in the SI group than EX group. The acetic acid was present in the highest concentration in VFA, with 77.17% in SI and 84.43% in the EX group. The acetic:propionic and acetic:butyric acid ratio was higher ($p<0.05$) in the EX group than in the SI group (Table 2). The valeric and isovaleric acid was not significantly different ($p\geq0.05$) between SI and EX groups. Isobutyric, valeric, and isovaleric acid were present in low concentrations in the rumen fluid. However, 2.11% VFA from the SI group and 0.15% from the EX group was undetected in this study.

Table 2

The concentration of the volatile fatty acids (VFA) of the rumen of the swamp buffaloes raised under semi-intensive (SI) and extensive system (EX) (mmol/ml)

Parameters	Buffalo group		
	SI group (n = 4)	EX group (n = 6)	*P*-value
pH value	6.28 ± 0.07	6.29 ± 0.09	0.557
Acetic acid (mmol/ml)	66.31 ± 3.77^{x}	65.23 ± 1.29^{y}	0.034

Table 2 *(Continue)*

Parameters	Buffalo group		
	SI group (n = 4)	EX group (n = 6)	*P*-value
Propionic acid (mmol/ml)	6.17 ± 2.14^x	2.910 ± 0.51^y	0.001
Butyric acid (mmol/ml)	14.08 ± 3.54^x	9.01 ± 0.88^y	0.008
Isobutyric acid (mmol/ml)	0.26 ± 0.15^x	0.11 ± 0.01^y	0.007
Valeric acid (mmol/ml)	0.13 ± 0.044	0.11 ± 0.01	0.609
Isovaleric acid (mmol/ml)	0.28 ± 0.173	0.23 ± 0.11	0.569
Acetic: propionic ratio	4.71 ± 1.06^x	7.24 ± 1.47^y	0.045
Acetic: Butyric ratio	10.58 ± 0.60^x	22.42 ± 2.50^y	0.007
Acetic acid (%)[1]	77.17 ± 3.44^x	84.43 ± 0.72^y	0.045
Propionic acid (%)[1]	5.95 ± 0.43^x	3.77 ± 0.68^y	0.037
Butyric acid (%)[1]	14.77 ± 0.65^x	11.65 ± 1.00^y	0.016
[2]Total VFA (mmol/ml)	85.92 ± 4.34^x	77.26 ± 1.47^y	0.033

Note. $^{x, y}$ the value in the row differed significantly at $p<0.05$
[1]The concentration of acetic, propionic, and butyric acid to the total VFA presented in percentage
[2]2.11% of VFA from the total VFA in the SI group and 0.15% in the EX group was undetected

Morphology and Morphometry of Rumen Mucosae

Figure 3 shows the morphology of the rumen mucosa, and Table 3 shows the morphometry of the rumen mucosa of both swamp buffaloes of the current study. Generally, the rumen walls are composed of papillae in the innermost layer, followed by submucosa, tunica muscularis, and serosa (Figure 3). The tunica muscularis comprises inner circular and outer longitudinal, which are not very obvious compared to the inner circular muscle layer (Figure 3). The papillae in the dorsal region are flat, smaller, and shorter than in the ventral region. As the size of the papillae of the ventral region was prominent and longer, the comparison was not statistically conducted.

Table 3 shows the measurements of the dorsal and ventral region of the rumen papillae in the swamp buffaloes raised under semi-intensive (SI) and extensive (EX) production systems. There are no significant differences ($p>0.05$) in the absorptive capacity (papillae length, width, and surface area) and motility (tunica muscularis thickness) between SI and EX groups. However, SI is showing superiority in papillae while EX is in muscle thickness in both regions.

The morphology of the rumen papillae comprises keratinized stratified squamous epithelium (SSE) as epithelium at the outermost layer and interstitium (Figure 4). The cellular proliferation at the stratum basale, where the cell mitotic occurred, is very active compared to other regions of the epithelium.

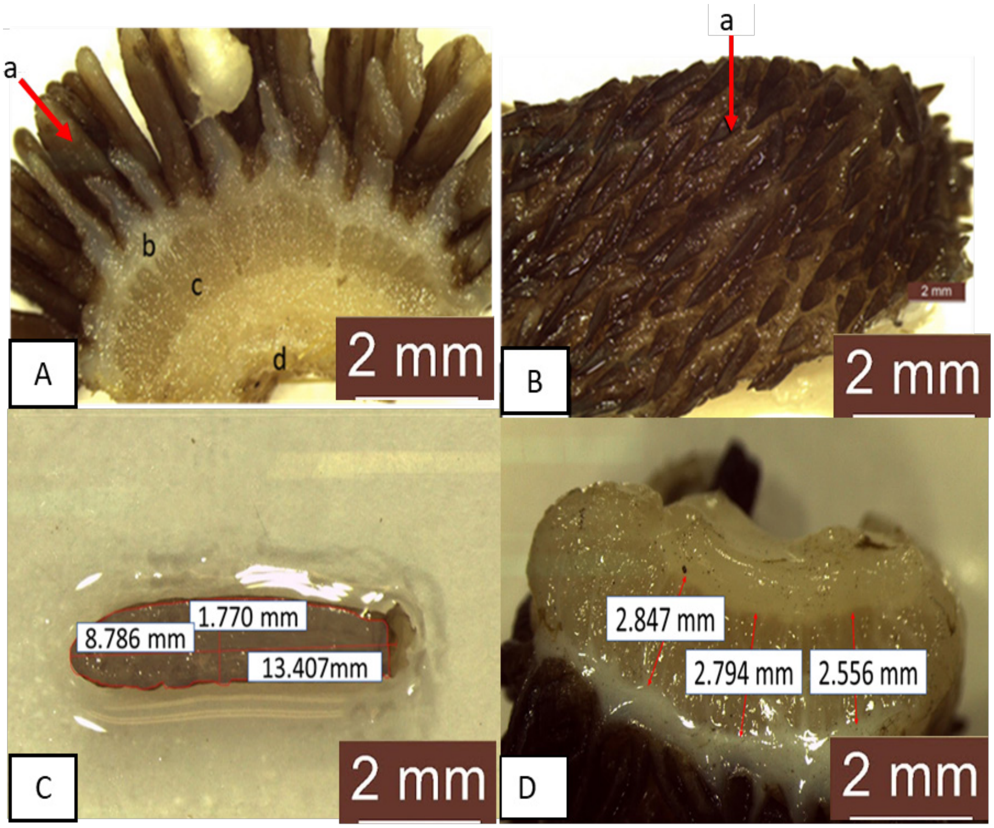

Figure 3. The morphology and morphometrical evaluation of the rumen papillae and rumen mucosae. (A) The gross morphology of the ventral region's rumen papillae and mucosal layer comprises of the papillae, muscle, and connective tissue, which comprises of a: papillae, b: submucosa layer, c: tunica muscularis, and d: serosa; (B) The surface of the rumen at the dorsal region, the papillae (e) of this region are less pronounced compared to the ventral region; (C) The measurement of the papillae (length, width, and surface area); (D) The measurement of the rumen muscle. Leica M80 equipped with Leica Application suite ver. 4.3.0, Switzerland. Scale: 2 mm

Table 3

Macro-morphometrical measurements of the dorsal and ventral region of the rumen papillae in the swamp buffaloes under semi-intensive (SI) and extensive (EX) system

Variables	Buffalo group		
	SI group (n = 4)	EX group (n = 6)	*P*-value
[2]Dorsal region			
Papillae length (mm)	1.932 ± 0.09	1.71 ± 0.12	0.171
Papillae width (mm)	11.69 ± 0.78	9.95 ± 1.39	0.300

Table 3 *(Continue)*

Variables	Buffalo group		
	SI group (n = 4)	EX group (n = 6)	*P*-value
[2]Dorsal region			
Papillae surface area (mm^2)	18.76 ± 0.84	14.66 ± 2.08	0.079
Papillae density (per cm^2)	73.22 ± 1.98	72.56 ± 2.60	0.952
Muscle layer thickness (mm)	3.81 ± 0.36	4.15 ± 0.53	0.7302
[1]Papillae colour Score	3.00	3.00	-
[2]Ventral region			
Papillae length (mm)	13.19 ± 1.05	12.06 ± 1.48	0.548
Papillae width (mm)	1.91 ± 0.059	1.74 ± 0.098	0.164
Papillae surface area (mm^2)	20.24 ± 1.818	17.61 ± 2.00	0.354
Papillae density (per cm^2)	275.44 ± 4.92	256.22 ± 5.60	0.746
Muscle layer thickness (mm)	4.41 ± 0.15	4.42 ± 0.68	0.932
[1]Papillae colour Score	3.00	3.00	-

Note. [1]The evaluation of papillae colour was based on the visual characteristics based on three individuals evaluated at the same time

There are no significant differences ($p \geq 0.05$) in papillae of the dorsal and ventral region of the rumen in the SI and EX group of swamp buffaloes

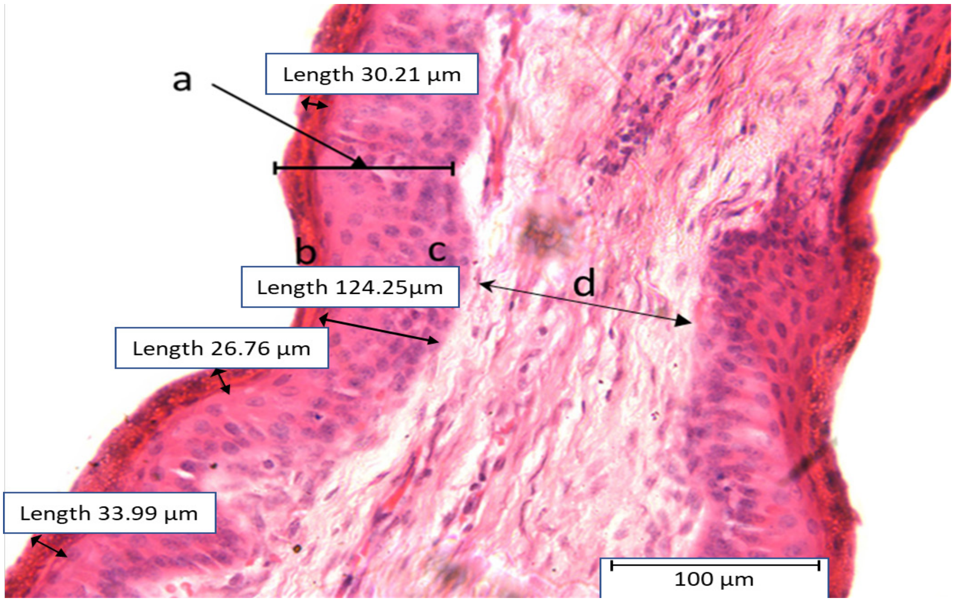

Figure 4. The morphology and measurement of the rumen papillae of the swamp buffaloes: a: SSE layer, b: keratin layer, c: stratum basale, d: interstitium of the papillae. H&E stain. Scale: 100 µm

The thickness of the stratified squamous epithelium (SSE) and keratin layer of the swamp buffaloes raised under semi-intensive (SI) and extensive (EX) production systems are shown in Tables 4 and 5, respectively. In dorsal papillae, the SSE thickness of the EX group is thicker ($p \leq 0.05$) than the SI group, however in ventral papillae, the SSE thickness between SI and EX does not differ significantly ($p \geq 0.05$). In the SI group, the thickness of SSE in the ventral is thicker ($p \leq 0.05$) than dorsal region, while in the EX group, the SSE thickness of the papillae in the dorsal region is thicker than ($p \leq 0.05$) in the ventral region (Table 4).

Table 4

The values (mean ± SE) of stratified squamous epithelium thicknesses of the rumen papillae in ventral and dorsal regions of the swamp buffaloes in semi-intensive (SI) and in extensive (EX) production system

Ruminal region	Buffalo group		
	SI group (n = 4)	EX group (n = 6)	*P*-value
Dorsal (µm)	$67.45 \pm 2.93^{a, x}$	$128.50 \pm 13.43^{b, x}$	0.002
Ventral (µm)	82.62 ± 5.38^{y}	93.03 ± 9.63^{y}	0.200
p-value	0.024	0.019	

Note. $^{a, b}$ the mean with a superscript letter in the row differs significantly at $p \leq 0.05$
$^{x, y}$ the mean with superscript a letter in the column differs significantly at $p \leq 0.05$
The analysis of data using Mann-Whitney U Test to compare two independent datasets

The thicknesses of the keratin layers in the SI and EX group are shown in Table 5. The keratin thickness of the EX group is significantly greater ($p<0.05$) than the SI group at the dorsal region of the rumen. However, in the ventral region, the thickness of keratin is greater ($p<0.05$) in the SI than in the EX (Table 5).

Table 5

The values (mean ± SE) of the keratin layer thicknesses of the rumen papillae of the swamp buffaloes under a semi-intensive (SI) and extensive (EX) production system

Ruminal Region	Buffalo group		
	SI group (n = 4)	EX group (n = 6)	*P*-value
Dorsal (µm)	$18.11 \pm 1.186^{a, x}$	$25.53 \pm 2.425^{b, x}$	0.011
Ventral (µm)	19.97 ± 0.571^{y}	$16.32 \pm 0.623^{b, y}$	0.003
p-value	0.103	0.003	

Note: $^{a, b}$ the mean with a superscript letter in the row differs significantly at $p \leq 0.05$
$^{x, y}$ the with superscript letter in the column differs significantly at $p \leq 0.05$
The analysis of data using Mann-Whitney U Test to compare two independent datasets

DISCUSSION

The present study evaluated the VFA and rumen mucosa morphology of the swamp buffaloes raised under two different production systems. Livestock production systems ensure the continuity of production to achieve adequate protein supplies by considering the rearing method, genetics, feeding management, and environmental factors. Generally, in the ruminant sector, there are three primary systems practised: extensive, semi-intensive, and intensive, which are synchronized with animal behaviour, economics, welfare, feed resources, and environment.

A ruminant diet predominantly composed (90%) of roughage (Mottet et al., 2017) is freely accessible through grazing on rangeland or pastures. It has a positive association between plant growth and rainfall and climatic condition (Marshal et al., 2005; McGrath et al., 2018), and finally may affect the production of VFA and rumen morphology. Globally, there are three different tropical climates identified according to the annual precipitation levels throughout the year. Many studies stated that tropical rangeland generally has a shorter wet but longer dry season. However, a country like Malaysia is influenced by monsoon and contains high humidity and the sunlight almost constant throughout the year. This high rainfall and sunlight in tropical monsoon (unlike other tropical regions) promote rapid growth and maturity of the grass to be low in digestibility and crude protein but higher in lignin as compared to other tropical areas (Moore & Jung, 2001). It may also cause undernutrition in livestock production (Duarte et al., 2018) to a certain extend. In tropical monsoon, an animal usually grazed at greater amount and composition during higher rainfall or wet season. (Andrew & John, 1998).

This study can be considered as the first data showing the effect of different production systems and feeding practices and their effect on the rumen VFA composition and mucosal morphology of the swamp buffaloes in the tropical region. Production systems (feeding regimes, housing, and rearing practices) are generally used to synchronize between livestock requirements, farming input, and environmental constraints. Therefore, differences in the production system may change the feeding behaviour, nutritive values, and animal-environment interactions. However, the current study only focused on the rumen VFA composition and morphology of rumen mucosae. Nevertheless, our findings revealed the difference in VFA concentration and microscopic level of the rumen papillae in different production systems, and thus this had proven our earlier hypothesis.

In digesting fibre in the rumen, the main products are VFA, ammonia, and gases. At the same time, the pH also changed accordingly. Thus, feed consumed in the rearing practices significantly influences rumen physiology (Diao et al., 2019; Ferreira et al., 2017; Kay et al., 1980; Steele et al., 2011) and the digestive morphology of the ruminants (Mason et al., 2019).

The pH range of ruminants fed on roughage should range from 6.2 to 7.0

(Wanapat & Pimpa, 1999). A pH higher than 6.5 promotes protease activity and growth rate of rumen microbes (Bach et al., 2005), while a value lesser than 6.2 will depress the growth of rumen microbes (Franzolin & Alves, 2010). However, this acidic environment was not conducive to rumen fermentation (Van Kessel & Russell, 1996) and limited the optimal VFA production. Therefore, the rumen pH has to be controlled through feeding management to prevent adverse effects on the rumen wall.

Our findings agreed with Wanapat et al. (2009), who suggested that the pH value of swamp buffalo was not influenced by diet. However, Franzolin et al. (2010) demonstrated differently where the ruminal pH might differ among diets, energy, and nitrogen source between cattle and buffaloes. This higher buffering capacity in buffaloes than in cattle, resulting in a higher pH value, as reported by Franzolin and Alves (2010), it was 6.28 in cattle and 6.29 in buffaloes, which was lower than what was suggested by Chanthakhoun et al. (2012), but in similar trend (6.51 in cattle and 6.78 in buffaloes). However, Rostini et al. (2018) reported differently where the pH was 5.62 (swamp buffalo), 5.58 (river buffalo), and 6.46 in Bali cattle.

A gap of sampling time after feeding might also affect the pH of the rumen. L. Wang et al. (2020) suggested that the lowest pH value was at four hours post-feeding, different from Goularte et al. (2011), who stated that it might take six to nine hours post-feeding. However, the feeding gap to the sample collection time was not monitored in the current study as the sample was collected immediately after slaughter. Therefore, the relationship between diet, VFA concentration, and pH value was unable to be determined accurately. Therefore, further investigation on the feeding, sampling time, and management system and their effects on the VFA in swamp buffaloes are required.

In the SI group, supplementation of PKC was given three days/week at a calculated ratio of 1.5 kg/animal/day but was not practised in the EX group. Therefore, it may affect the concentration of VFA in both groups. Dieho et al. (2016) suggested that different energy levels and nutritive value of diet cause different VFA production, which was shown in the SI group compared to the EX group. Furthermore, the tendency of different dry matter intake (DMI) may differ individually as each animal possess different feeding behaviour.

In this study, the total VFA, acetic, propionic, and butyric acid were more significant in SI than EX group indicated that the diet offered under different production systems affects the VFA concentration. Our finding agreed with all the previous studies where acetic acid was the most prominent VFA (Aluwong et al., 2013), while propionic and butyric acid were also prominent in both swamp buffalo groups. Still, it may fluctuate according to the type of diet given. High energy diet (Ma & Zhao, 2010) promotes higher production of total VFA, acetic, propionic, and butyric acid in the rumen than forage (Bergman, 1990; Kristensen, 2005; L. Wang et al., 2020; Mao et al., 2012). Nevertheless, using different forages also caused different proportions of VFA. The total VFA produced from alfalfa was greater than bromegrass (Khorasani et al., 2001), although their digestible energy seems similar (Moyer & Hironaka, 1993). The present study was based on *Brachiaria decumbens* and PKC supplementation, which was considered very marginal to support the findings of Palmieri et al. (2012). Additional PKC as an energy source in the basal diet of less than 45% did not result in total VFA, acetic, butyric, and propionic acid changes. A higher proportion of concentrate as energy diet increased up to 70% (Suárez et al., 2007) under controlled feeding management did not observe increment of VFA production in some studies (Khorasani et al., 2001; Penner et al., 2009), which differed from the present findings. The butyric and propionic acid production seemed to be ambiguous under almost similar pH values in this study. However, according to L. Wang et al. (2006), in the micro-aerobic circumstances, the accumulation of VFA in acidogenic conditions can occur at any pH value; more investigation is required to elucidate the rumen fermentation of the swamp buffaloes.

The VFA concentration may differ between cattle and buffaloes, with the buffaloes producing less acetic acid but more propionic acid than cattle but not in butyric acid between them (Franzolin et al., 2010). Bergman (1990) suggested that acetic: propionic: butyric acid ratio was 75:15:10 to 40:20:20, which was not observed in the present study. The acetic: propionic acid and acetic: butyric acid ratio was higher in the EX group than in the SI group. It was contributed by the diet of EX, which only depended on grass and no substitution of concentrates given.

Species has a significant role in influencing VFA production. (Candyrine et al., 2019). VFA concentration was reported to be higher in buffaloes (Parmar et al., 2014) but found to be lower by Franzolin et al. (2010). Increment or reduction of VFA between cattle and buffaloes were suggested due to the supplementation introduced (Boniface et al., 1992). However, Rostini et al. (2018) showed that swamp buffaloes have the highest acetic and propionic acid compared to river buffaloes and Bali cattle. However, it has the lowest butyric acid among the three breeds studied.

Stress may also affect fermentation and VFA absorption. The authors agree with the suggestion by Lam et al. (2018) that the difference in VFA between these two groups is due to stress. Stress may disrupt VFA absorption. Generally, in an extensive farming system, human-animal contact is at the minimum where the stress factor is generated only during animal handling. Heat stress was reported to reduce the fermentation and VFA absorption process (Bernabucci et al., 2010; Silanikove, 2000). However, it may depend on the location of the tropical region (Wanapat et al., 2013). Animals raised extensively were more prone to be exposed to climatic, human-animal interaction, and feed stress (Temple & Manteca, 2020) than other production systems. Handling stress is expected to be lesser in the SI group than the EX group as they are being exposed to the workers during feed supplementation in the holding yard. In the current study, the effect of heat stress was not obvious in both groups as the ambient temperature was almost constant. In the natural environment, wallowing behaviour is used to control body heat.

Human handling involved in the herd health program, such as disease screening, vaccination, deworming, may not impact the extensive group. However, the stress due to handling transport and slaughter cannot be avoided in both groups and maybe worsen an extensive group. Searching for food might also cause stress in the EX group because there is no food, supplementation and they have to walk a long distance. The effect of handling and rearing stress to distinguish effect on VFA and rumen mucosal morphology was not conducted in this study. Therefore, it was not discussed in detail. The study of stress factors caused by rearing practice was extensively conducted in beef cattle. However, further investigation on swamp buffaloes is still required as the buffaloes have behaviour, morphology, and genes different from the beef cattle.

Previous findings suggested that the presence of VFA as the result of carbohydrate digestion in the rumen (Dieho et al., 2016; Shen et al., 2005) and the physical structure of the feed (Suarez-Mena et al., 2016; Xu et al., 2009) were the cause to induce morphological changes of rumen mucosae. Among all the VFAs, this role was mainly played by butyric acid (Liu et al., 2019). All the above statements agree that starch altered the papillae development and rumen wall thickness at the different thresholds (Álvarez-Rodríguez et al., 2012; Y. H. Wang et al., 2009).

A better diet offered will create a better rumen mucosa morphology, as suggested in several reports (Cui et al., 2019; Diao et al., 2019; Penner et al., 2009). However, it was not observed in this study. Cui et al. (2019) demonstrated that the growth of rumen papillae positively correlated to protein and energy diet, which can be found in a well-managed farming system. As a general agreement, the papillary size is associated with the absorption capacity, which occurs in a large amount of rich in carbohydrate diet, resulting in excessive VFA concentration and low pH (de Resende-Junior et al., 2006).

Mason et al. (2019) reported that farmed deer have lesser papillae characteristics than wild deer, slightly different from the current study. The evaluation of the absorptive and motility capacities is required for macro and micro levels to support our findings as suggested by Consalvo et al. (2016) that microscopic evaluation gave a better description of rumen mucosae morphology. Our finding disagreed with the earlier suggestion that a greater concentration of VFA in the SI group can still induce the papillae's induced growth significantly. The papillae colour was also evaluated as it was initial indicator for ruminal acidosis (Consalvo et al., 2016) and keratinization (Álvarez-Rodríguez et al., 2012). The colour of the papillae started to darken as mild acidosis. Still, Nurliani et al.

(2015) suggested that the darken colour of the rumen mucosa of swamp buffaloes was unique to themselves due to blood circulation. Our study agreed with Álvarez-Rodríguez et al. (2012), where the colour was only subjected to a type of diet.

Many reports stated that the energy diet was the leading cause of papillae growth (Diao et al., 2019; Penner et al., 2009); this was supported by Cui et al. (2019) as the protein and energy decrease the growth of rumen papillae also reduced. The researchers assumed, in the beginning, this was probably due to the higher digestibility, protein, and energy of the diet offered to the SI group, which had enhanced the better growth of rumen papillae. However, this was not observed, but they hypothesized the macroscopic differences might have occurred at a younger age, as suggested by Diao et al. (2019) and Gupta et al. (2016). Still, this effect was slowly reduced as animals grew older based on Penner et al. (2009) that the response of papillae growth was subjected to time.

The macro-morphometrical comparison between the ventral and dorsal region was not performed as there is a prominent difference between the size of the rumen papillae (Clauss et al., 2009). Therefore, the effect of type and nature of the diet on muscular and mucosal development was not observed in this study at a macroscopic level. This finding agreed with Beharka et al. (1998) and Suarez-Mena et al. (2016), who proposed that the rumen muscle thicknesses were hardly influenced by the type, physical form (fine or coarse) and level of roughage.

In the current study, the stratified squamous epithelium (SSE) was thinner in SI than EX. It was probably due to the cellular response to higher VFA production to promote absorption, where at the thinner epithelium layer, VFA can be absorbed rapidly. At the same time, the thickness of keratin may be associated with the digesta mass in the rumen, as suggested by Beharka et al. (1998). Thicker in keratin layer, slowing the absorption rate of VFA. Therefore, we can hypothesize that the stratified squamous epithelium and keratin was associated with the level of VFA in the rumen. It was agreed that the thickness of the SSE layer indicates papillae growth, but it was also reflected in the thickness of the keratin layer of EX in the present study. The rumen papillae keratinization was less common in animals raised under pasture than concentrates (Barros et al., 2015), supported by Melo et al. (2013) that the grazing cattle may reduce papillae absorptive surface area and basal cell mitotic index but increase in epithelium layer and keratin thickness.

Digesta volumes in the animal raised extensively could not be expected to be full as in other systems applied. This condition seems to have a digesta attachment to the rumen wall where the mechanism of 'wear and tear' or abrasion occurred actively (Greenwood et al., 1997). This effect was also probably associated with PKC supplementation in the SI group caused by the abrasion mechanism. In Malaysia, supplementing ruminants with PKC was very common, but it can be in various forms of particle size that farmers did not consider (Saw et al., 2012).

CONCLUSION

Swamp buffalo from the semi-intensive group (SI) has a greater concentration of volatile fatty acid (VFA), such as acetic, propionic, and butyric, and also a greater percentage of propionic, butyric, and total VFA than the extensive group (EX) due to a proper feeding and management system. Nevertheless, the advantage of greater VFA concentration alone is not sufficient to conclude that the semi-intensive production system exerts a favourable effect on the morphology of the rumen mucosa. Despite the discrepancy in the feeding and management system between the semi-intensive and extensive systems, the development of rumen mucosae of the swamp buffaloes was equivalent except for the morphological alteration found only at the microscopic level on the thickness of keratin and stratified squamous epithelium. Further investigation on the rumen mucosa of the swamp buffaloes related to the production and management system in tropical environments is required to parse the mechanism of rumen mucosa alteration in swamp buffaloes.

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