Original Research Paper

# **Evaluation of Digestibility of Palm Fronds Shaving (PFS) Supplemented with Vitamin B Complex on the Quality of Fermentation by** *in vitro* **Method and Degradation Kinetics by in Sacco Method**

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Article history Received: 10-09-2023 Revised: 23-02-2024 Accepted: 29-02-2024

Corresponding Author: Armina Fariani Department of Animal Science, Faculty of Agriculture, Universitas Sriwijaya, Sumatera Selatan, Indonesia Email: arminafariani@unsri.ac.id Abstract: This study aimed to determine the digestibility value, fermentation quality and degradation rate of palm frond shaving supplemented with vitamin B complex. The design used in this study was a Completely Randomized Design (CRD) consisting of three treatments and three replications. The treatments were as follows: Shaving palm fronds (CON), palm fronds shaving + vitamin B complex 1% (P1) and palm fronds shaving + vitamin B complex 3% (P2). The results showed that the P2 treatment was significantly different from the other treatments (p<0.05) on all parameters observed, including digestibility of dry matter, crude fiber and fiber fraction. The results of the analysis of VFA and microbial protein and the highest acetic acid value were found in the P2 treatment. In contrast, butyrate and NH3 values were found in the (control) treatment and propionate was found in the P1 treatment. Analysis of the degradation rate showed that the value of dry matter, crude fiber, ADF and cellulose in treatment P2 was significantly different compared to other treatments (p<0.05). Based on the results of the study, it can be concluded that palm frond powder supplemented with 3% vitamin B complex has great potential as a feed additive for ruminants.

Keywords: Palm Fronds, Shaving, Vitamins B Complex

## Introduction

The development of plantation production waste has become an important factor in increasing the value of productivity in the livestock industry. This is because the results of plantation waste can be used as ruminant animal feed. Plantation waste is found in various types such as tea, coffee and oil palm plantations. Oil palm plantations themselves in the last two years have become a popular type of business for many business actors because of the high value or price of palm oil in the market (Nurrochmat et al., 2020). However, the high production will also be proportional to the high waste generated. Palm frond waste can be used as ruminant animal feed if adequately processed. The parts of palm oil waste that can be used as animal feed include palm oil sludge (solid), oil palm cake and palm fronds. Oil palm fronds have tremendous potential as animal feed because apart from having the highest amount/quantity, palm fronds also contain high hemicellulose and cellulose content. Mohd Yasin *et al.* (2013) reported that the by-product of palm oil waste in the form of fronds has a high sugar fraction content of 27.97%. Meanwhile, the results of OPF fermentation that have been processed into a finer form (extract/juice) are known to contain sugars in the form of glucose, sucrose, arabinose and fructose of 40 g/L (Tan *et al.*, 2016).

The high fraction of structural sugars in palm fronds means it could be used as an energy source for ruminants. This is also supported because ruminants have the ability to utilize carbohydrates/structural sugars better than other types of animals. In addition, there is a potential source of epiphytic microorganisms in the palm fronds in the form of fungi and bacteria that produce lactic acid, resulting in the waste of palm fronds being a source of probiotics for ruminants. Fadzilah *et al.* (2015) reported that the fermentation of palm frond waste without adding specific inoculants to the tested samples showed the development of the fungi *Aspergillus niger, Trichoderma* and *Absidia sp.* 



While, in other experiments, the results were the types of bacteria Bacillus cereus. Bacillus subtilis and Saccharomyces cerevisiae which are types of microorganisms that produce lactic acid during the fermentation/storage process (Che Maail et al., 2014). According to Damayanti et al. (2015), the high activity of lactic acid bacteria in palm frond silage can be used as a good potential inoculant for feed because it can inhibit fungal activity.

The considerable potential produced by palm frond waste has an excellent opportunity to develop as animal feed or as a growth medium for good microorganisms (probiotics) for ruminants. However, the many patterns that can be used do not result in palm frond waste being maximally utilized as a source of energy or probiotics for livestock. It was due to the high activity of other pathogenic bacteria that require supplementation or the addition of other organic compounds, which can also increase the nutrient value of the palm frond waste. The addition/supplementation that can be used is vitamin B complex. Vitamin B complex supplementation in palm frond waste is expected to inhibit the growth of spoilage microorganisms and assist in the process of nutrient metabolism during the fermentation process in the digestive tract of ruminants.

Vitamin B complex is one type of vitamin needed by livestock and consists of vitamins B1(thiamine); B2 (riboflavin); B3 (niacin or niacinamide); B5(pantothenic acid); B6 (pyridoxine); B7 (biotin); B9 (folic acid); and B12 (cobalamins). Vitamin B complex is a vitamin that is easily soluble in water and has an important role in metabolic and reproductive activities. A deficiency of vitamin B complex will impact the performance and reproduction of livestock. On the other hand, excess vitamins (overdose) can also have a fatal impact on all activities. Shahzad et al. (2018) reported that vitamin B has a good synergy against the growth of MRSA (Methicillin-resistant Staphylococcus aureus) bacteria; and also has an important role in various processes of carbohydrate metabolism; including a breakdown of sterols, formation of essential amino acids and the activity various enzymes in protein metabolism of (Vijayalakshmy et al., 2018). Based on the problems, an experiment was carried out on the fermentation of palm frond waste with the addition of vitamin B complex on the quality of the fermentation in vitro and the digestibility of the fiber fraction in Sacco.

# **Materials and Methods**

## Sample Collection and Processing

The samples used in this research are a salt solution, palm frond waste and vitamin B complex. The first stage is preparing a 3% salt solution which is then dissolved in 20 L of water. The use of salt is intended to reduce contamination by microorganisms when processing palm frond waste based on the results of research by Kim et al. (2021). The second stage was followed by collecting palm frond waste obtained from palm fruit harvesting. The palm frond waste used is separated from the leaves first. Then after the leaves are separated, the remaining branches/stems (petioles) are peeled off so that the contents of the Palm Frond Shaving (PFS) are obtained. The contents of the palm midrib are then soaked using salt water and after that, it is grated using a coconut grater that has been modified previously. So that it will get palm frond shavings, every step of the process can be seen in Fig. 1. In the third stage, namely the preparation of supplementation materials in vitamin B complex tablets, obtained from PT. USFA VET FARMA. The vitamin B complex tablets used during the research process were ground until smooth and obtained in powder form.

After all, samples were successfully prepared, the process continued with mixing 1 kg of PFS and vitamin B complex powder based on the treatment dose. When the mixed sample has been obtained, put into a 1 mm thickness bag and stored for 14 days; during the storage/curing process, the gas produced will be expelled periodically using a syringe. The hole caused by using the syringe is closed again using an insulator so that anaerobic conditions in the bag can be maintained. The chemical composition of Palm Frond Shaving (PFS) can be seen in Table 1.



Fig. 1: Palm fronds Shavings (PPS) manufacturing work process

Table 1: Che	mical com	position of	Palm fr	ond shav	ving (PFS)
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Variable	PFS con
DM % as fed	81.07
CP, % of DM	7.41
CF, % of DM	52.45
NDF, % of DM	39,19
ADF, % of DM	8,91
Hemicellulose	30,28
Cellulose	7,47

Note: DM, dry matter; CP, crude protein; CF, crude fiber; NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber. Means with different superscript letters in the same line differ significantly (p<0.05)

#### Experimental Design, Livestock and Diet

The design used in this study was a completely randomized design consisting of three treatments and five replications. The treatments tested were as follows: Palm fronds shaving (CON), palm fronds shaving + vitamin B Complex 1% (P1) and palm fronds shaving + vitamin B Complex 3% (P2). The objects used in this study were three female fistula buffaloes aged two years with an average weight of 200 kg. Buffaloes were placed in closed outdoor cages (3 m<sup>2</sup>) and fed with ODOT grass previously harvested in the experimental cage laboratory of Sriwijaya University. The feeding was given *ad libitum* during the trial period. This phase was carried out for two weeks before the study was conducted.

## Fermentation Quality (in vitro Technique)

The *in vitro* method used in this study was based on Tilley and Terry (1963). Parameters of observations carried out included analysis of partial VFA, NH<sub>3</sub> and microbial protein. Measurement of VFA levels was carried out using a Gas Chromatography (GC) based method (Filípek and Dvořák, 2009). Meanwhile, NH<sub>3</sub> and microbial protein measurements were based on the Conway and O'Malley (1942); Lowry (1951).

## Rumen Digestibility (in Sacco Technique)

The in-sacco method used in this study is based on the ILCA manual book feed evaluation. It is using  $5\times10$  cm nylon bags with a porosity of  $50\pm15$  µm. A total of 3 g of forage samples were weighed and placed into a nylon bag that had previously been considered. Each type of grass had three replications and was incubated for 0, 6, 12, 24, 36 and 48 h in the buffalo rumen. So in each cannula, there are five pockets. The nylon bags have been removed according to a predetermined time, sorted according to grass type, washed in running water and stored at -20°C until all grass species have been incubated. After all the grass has been successfully incubated, the stored samples are thawed at  $65^{\circ}$ C until dry and then weighed.

## Chemical Analysis and Calculations

Analysis of fiber fraction content in each grass includes NDF, ADF, Hemicellulose, Cellulose and Lignin based on the Van Soest (1987). The analysis results are divided into two parts, namely before and after the incubation period. The fiber fraction difference is assumed to be part of the digested/lost fraction value during the incubation process. As for the calculation of the loss in fiber fraction value based on the ILCA method with formula 1:

$$Disappearance = \frac{(SWa-BW) \times DMa - (SWb-BW) \times DMb}{(SWa-BW) \times DMa}$$
(1)

where:

SWa = Weight of the original sample + nylon bag

BW = Weight of empty nylon bag

SWb = Weight of the sample + nylon bag after incubation

DMa = Dry matter of feed sample

DMb = Dry matter of residue sample

The value in the formula used in dry matter is then adjusted to the value of the fiber fraction used as the research parameter. Furthermore, to calculate the fraction degradation rate (kd) of NDF (kd-NDF), ADF (kd-ADF), Hemicellulose (kd-H) and Cellulose (kd-C) were calculated according to the calculation model Ørskov and McDonald (1979) (formula 2):

$$Y = a + b(1 - e^{-ct})$$
(2)

where,

- Y = Degradability at time (*t*)
- a =Intercept/dissolved fraction,
- b = Potentially degradable fraction
- c = Rate of degradation of b. The Analysis was performed using the curve fit program excel solver

Fraction a is used to describe the fraction that dissolves easily/fastly. In contrast, fraction b describes the number of fractions that dissolve slowly and Fraction c describes the rate/speed of degradation resulting from the long incubation period in the rumen fistula (Ørskov *et al.*, 1980).

#### Data Analysis

Data were analyzed using a randomized block design; if there is a difference between treatments, the Duncan Multi Range Test (DNMRT) further test is carried out (Steel and Torrie 1980).

#### **Results and Discussion**

## Results

## Total Digestibility

The study's digestibility analysis on nutrient composition and fiber fraction showed a significant

difference to palm midrib powder supplemented with vitamin B complex (p<0.05). Overall, the data showed that 3% vitamin B complex supplementation significantly impacted dry matter digestibility, crude fiber and fiber fraction (NDF, ADF, hemicellulose and cellulose). Meanwhile, the digestibility of organic matter was not significantly different compared to the vitamin B complex supplementation of 1%.

## Quality of Fermentation by in vitro Method

The analysis of feed fermentation quality parameters, including acetic acid, propionic acid, butyric acid,  $NH_3$  and microbial protein, showed significant differences to palm frond powder supplemented with vitamin B complex (p<0.05). In the P2 treatment, it was shown that acetic acid production was not significantly different from that in the P1 treatment but it was higher than that in the control treatment (Table 3). Meanwhile, the highest value was found in the P1 treatment in Propionic acid and Butyric acid, in the control treatment. Furthermore, the highest  $NH_3$  concentration was found in the control treatment of microbial protein, but the observation of microbial protein was not significantly different compared to the P2 treatment.

 Table 2: Total nutrient and fiber fraction digestibility of palm

 fronds shaving supplemented with vitamin B complex

	Treatment			
Item	 P0	P1	P2	
Dry matter	45,25 <sup>a</sup>	48,14 <sup>b</sup>	50,98°	
Orgnanic matter	51,90 <sup>a</sup>	52,91 <sup>b</sup>	54,37 <sup>b</sup>	
Crude fiber	46,02 <sup>a</sup>	53,93 <sup>b</sup>	62,63°	
NDF	75,59ª	75,56 <sup>a</sup>	81,05 <sup>b</sup>	
ADF	46,84 <sup>a</sup>	50,61 <sup>b</sup>	56,27°	
Hemicellulose	84,04 <sup>a</sup>	85,64 <sup>a</sup>	89,95 <sup>b</sup>	
Cellulose	63,08 <sup>a</sup>	75,80 <sup>b</sup>	81,04 <sup>c</sup>	

Note: Con, untreated PFS; P1, vitamins B complex 1%; P2, vitamins B complex 3%. Means with different superscript letters in the same line differ significantly (p<0.05)

 Table 3: Fermentation quality of palm frond powder supplemented with vitamin B complex

	Treatment				
Item	 P0	P1	P2		
Acetic acid	27,33ª	57,33 <sup>b</sup>	64,69 <sup>b</sup>		
Propionic acid	24,44 <sup>b</sup>	32,00 <sup>c</sup>	12,66 <sup>a</sup>		
Butyric acid	33,00°	20,00 <sup>b</sup>	14,00 <sup>a</sup>		
NH <sub>3</sub>	4,13 <sup>b</sup>	2,30 <sup>a</sup>	2,25ª		
Microbial protein	129,5 <sup>b</sup>	115,2ª	128,8 <sup>b</sup>		

Note: Con, untreated PFS; P1, vitamins B complex 1%; P2, vitamins B complex 3%; N-NH<sub>3</sub>, ammonia. Means with different superscript letters in the same line differ significantly (p<0.05)

## **Degradation Pattern**

The results of the analysis showed that the palm frond powder supplemented with vitamin B complex at 3% (P2) had a significantly different value compared to other treatments (p<0.05). Furthermore, the fraction values used to describe the degradation kinetics of all components of the research variables include dry matter, organic matter, crude fiber content and fiber fractions, including NDF, ADF, Hemicellulose and Cellulose, show high values for each fraction.

Based on the analysis, results showed a significant increase in the P2 treatment, especially on the variables of dry matter, crude Fiber, ADF and cellulose. Meanwhile, the observed variables for organic matter, NDF and hemicellulose did not show a significant difference, except for the value of fraction c, which observed the degradation kinetics of NDF and Hemicellulose.

## Discussion

## Total Digestibility

The high digestibility value produced in each observation variable is thought to be due to the role of the vitamin B complex in helping the digestion process of fiber fractions. Crude fiber or fiber fraction is a limiting factor in feed chemical composition. If the digestibility of fiber or fiber fraction in a feed ingredient increases, the digestibility of the nutrient composition in the feed will be impacted (McDonald, 2010).

Vitamin B complex has become an important key in increasing the activity of carbohydrate metabolism and enzymatic activity during the fermentation process in the rumen. The study results stated that the supplementation of vitamin B complex had a significant impact on the performance of ruminants because of its ability in the mechanism of metabolic action; and involvement with enzymatic reactions in the digestive system of livestock. Such as biotin, which contributes to carbohydrate metabolism, folic acid to enzymatic reactions and vitamin B12, which is involved in amino acid synthesis (Girard and Matte, 2006). In addition, there are many other roles reported regarding types of B complex vitamins such as Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin), Vitamin B3 (Niacin), Vitamin B5 (Pantothenic acid) and Vitamin B6 (Pyridoxine) in increasing metabolic processes. Carbohydrates in the digestive system of livestock (Vijayalakshmy et al., 2018). According to Watanabe and Bito (2017), essential vitamin B12 synthesized by ruminants has a symbiotic relationship with microorganisms in the body.

Table -	<b>1:</b> I	Degradation <sup>•</sup>	pattern/rate o	of nutrient	degradation of	palm frond	powder supplemented	with vitamin B complex
					and Arthurstone of the			

	Fraction			
Treatment	a (%)	b (%)	c (%/hour)	
kd-Dry matter				
P0 (control)	24,56 <sup>a</sup>	23,90ª	0,04 <sup>b</sup>	
P1 (palm fronds shaving + vitamin B complex 1%)	23,78 <sup>a</sup>	23,15ª	0,05 <sup>b</sup>	
P2 (palm fronds shaving + vitamin B complex 3%)	36,52 <sup>b</sup>	35,37 <sup>b</sup>	0,02 <sup>a</sup>	
kd-Organic matter				
P0 (control)	25,33 <sup>ns</sup>	23,60 <sup>ns</sup>	0,07 <sup>ns</sup>	
P1 (palm fronds shaving + vitamin B complex 1%)	26,31 <sup>ns</sup>	23,73 <sup>ns</sup>	0,07 <sup>ns</sup>	
P2 (palm fronds shaving + vitamin B complex 3%)	31,53 <sup>ns</sup>	27,91 <sup>ns</sup>	0,03 <sup>ns</sup>	
kd-Crude fiber				
P0 (control)	26,50 <sup>a</sup>	24,54ª	0,04	
P1 (palm fronds shaving + vitamin B complex 1%)	28,30 <sup>a</sup>	25,73ª	0,05	
P2 (palm fronds shaving + vitamin B complex 3%)	39,22 <sup>b</sup>	35,80 <sup>b</sup>	0,03	
kd-NDF				
P0 (control)	47,71 <sup>ns</sup>	33,50 <sup>ns</sup>	0,12 <sup>b</sup>	
P1 (palm fronds shaving + vitamin B complex 1%)	53,28 <sup>ns</sup>	36,36 <sup>ns</sup>	0,08 <sup>a</sup>	
P2 (palm fronds shaving + vitamin B complex 3%)	54,16 <sup>ns</sup>	36,80 <sup>ns</sup>	0,10 <sup>a</sup>	
kd-ADF				
P0 (control)	43,41ª	24,95ª	0,05 <sup>b</sup>	
P1 (palm fronds shaving + vitamin B complex 1%)	122,70 <sup>c</sup>	38,82 <sup>b</sup>	0,01ª	
P2 (palm fronds shaving + vitamin B complex 3%)	78,76 <sup>b</sup>	34,54 <sup>b</sup>	0,02ª	
kd-Hemicellulose				
P0 (control)	51,51 <sup>ns</sup>	33,72 <sup>ns</sup>	0,20 <sup>b</sup>	
P1 (palm fronds shaving + vitamin B complex 1%)	57,53 <sup>ns</sup>	35,96 <sup>ns</sup>	0,12 <sup>a</sup>	
P2 (palm fronds shaving + vitamin B complex 3%)	56,42 <sup>ns</sup>	35,61 <sup>ns</sup>	0,20 <sup>b</sup>	
kd-Cellulose				
P0 (control)	55,77ª	35,64 <sup>ns</sup>	0,05 <sup>b</sup>	
P1 (palm fronds shaving + vitamin B complex 1%)	67,12 <sup>b</sup>	40,02 <sup>ns</sup>	0,04 <sup>a</sup>	
P2 (palm fronds shaving + vitamin B complex 3%)	59,95 <sup>ab</sup>	37,56 <sup>ns</sup>	0,07 <sup>b</sup>	

Note: Degradation rate/kinetic degradation (kd) of NDF (kd-NDF); ADF (kd-ADF), Hemicellulose (kd-Hemicellulose); and Cellulose (kd-Cellulose). <sup>ns</sup>(non-significant); <sup>abc</sup>Means with different superscript letters in the same line differ significantly (p<0.05)

The digestibility of hemicellulose and cellulose content dramatically affects the NDF and ADF. This correlation can occur because NDF and ADF's main components are structural carbohydrate compounds, where NDF compounds are positively correlated with hemicellulose compounds and ADF with cellulose (Fariani et al., 2021). The high digestibility resulting from the activity of the vitamin B complex will indirectly affect the high activity of bacteria in the rumen. In this study, it can be seen that high digestibility has a strong correlation with the quality of the fermentation produced, which can be seen in Table 4. Furthermore, external factors such as high cellulolytic bacteria activity in the buffalo rumen resulted in increased digestion of fiber components during the fermentation process (Dadheech et al., 2018; Iqbal et al., 2018; Jaglan et al., 2019).

## Quality of Fermentation by in vitro Method

The high value of acetic acid in this study is thought to be caused by the high cellulose and hemicellulose (structural carbohydrates) content in the palm frond powder. Furthermore, the high digestibility of structural carbohydrates during the fermentation process in the rumen resulted in an increase in the value of acetic acid as a derivative resulting from the fermentation of structural carbohydrates. The research on high-fiber and low-fiber content showed a significant difference in the acetic acid value produced because fiber is the primary precursor in producing acetic acid (Emerson and Weimer, 2017; Weimer, 2019).

On the other hand, the presence of vitamin B complex supplementation causes different values of acetic acid produced because Vitamin B12 is an essential part of the enzyme system involved in various metabolic reactions, especially in the formation of energy from rumen fermentation (González-Montaña *et al.*, 2020). In contrast to the obtained results for propionic acid measurement, the highest value was found with 1% vitamin B supplementation. The phenomena that occur in this study cannot be explained with certainty. However, there is a strong suspicion of an increase in Propionate because it has a relationship with the activity of the bacteria *Fibrobacter succinogenes* and *Selenomas ruminantium* and its interaction with the vitamin B complex. It can happen because the rumen fluid sampling used is not validly known for the number and type of bacteria. According to Clemmons *et al.* (2020), two types of bacteria play a role in producing Propionate in the rumen, namely *Fibrobacter succinogenes*, which plays a role in producing succinate, which will then be converted into Propionate with the help of Selenomas ruminant bacteria.

Meanwhile, from vitamin B itself, there is a "crossfeeding vitamin" phenomenon between rumen microbes where B vitamins produced by certain microbes are used to grow other microbes and vice versa become other potentials. However, the increase in propionic acid is also thought to occur due to vitamin B12 in the fermentation process. According to Parnian-Khaiehdizai and Taghizadeh (2018), the increase in Propionate during the fermentation process could occur due to vitamin B12 being needed by some Bacteroides species to produce Propionate. The amount of Propionate produced by bacteria occurs through randomization of the reaction sequence, which includes the conversion of succinyl-CoA to methylmalonyl-CoA; Furthermore, carbon is generated by rearrangement transfer. catalyzed bv vitamin B12-dependent methylmalonyl mutase. This result was proved in an experiment conducted by Strobel (1992) when P. ruminicola was grown on a medium without vitamin B12, acetic acid and succinate were the main fermentation products and almost no propionate was detected.

# Degradation Pattern

The increase in the digestibility value of fractions a and b in the treatment of 3% vitamin B supplementation was thought to be because vitamin B was able to significantly affect the development of rumen microbes, which would have an impact on increasing the digestibility value. The increase in the rumen microbial population may be due to the role of thiamine, riboflavin, niacin, biotin and pyridoxine contained in the vitamin B complex.

Kandathil and Bandla (2019) reported oral vitamin supplementation in ruminants and found that thiamine, riboflavin, niacin, biotin and pyridoxine were essential factors in the development of microbes in the rumen. Such as thiamine, which is needed for the development of cellulolytic bacteria such as Ruminococcus albus and Ruminococcus flavefaciens; riboflavin for the development of rumen bacteria in general; the hypothesized role of niacin may produce a significant impact on microbial fermentation activity as it forms an integral component of the coenzymes nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate which are involved in energy metabolism; and the role of biotin and pyridoxine which helps in creating an optimal environment/medium for the activity of major cellulolytic bacteria such as Butyrivibrio Fibrisolven, Bacteroides succinogenes, Ruminococcus albus and Ruminococcus flavefaciens.

Furthermore, based on the results of research by (Wang et al., 2016), reported several bacteria, such as **Butvrivibrio** fibrisolvens, **Ruminococcus** albus. Ruminococcus flavefaciens and Fibrobacter succigenes and also the activity of several enzymes such as xylanase, pectinase,  $\alpha$ -amylase and cellobiase increased squarely with folic acid supplementation protected in the rumen. Nonetheless, the results of studies regarding the evaluation of dietary supplementation of B vitamins and Hmbi on the kinetics of fermentation, ruminal diet, or post-ruminal digestion using modified in vitro techniques show that supplementation of vitamin B may adversely affect the kinetics of degradation due to the strong interaction between vitamins B9 and B12 (Parnian-Khajehdizaj and Taghizadeh, 2018).

# Conclusion

Based on the study results, it can be concluded that palm frond powder supplemented with 3% vitamin B complex can be used as a good source of energy for livestock and can increase metabolic and enzymatic reactions in the rumen.

# Acknowledgment

We would like to thank the 2017 batch of Sriwijaya University Animal Husbandry students (Mamas, Elfan, Zizan, Anggun, Wahyu, Faisal and Ilham) who helped with the research process, starting from the sampling process to the laboratory process.

# **Funding Information**

The authors have not received any financial support or funding to report.

# **Author's Contributions**

**Armina Fariani:** Conception, designed, analysis and interpretation of data.

Gatot Muslim: Acquisition of data.

**Anggriawan Naidilah Tetra Pratama:** Contribute in drafted the article.

**Lili Warly:** Give final approval of the version to be submitted and any revised version.

# Ethics

An animal feeding experiment was conducted at the experimental station, Department of Animal Science, Faculty of Agriculture, Universitas Sriwijaya. The Buffalos were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. The approval of the experiment was granted from Universitas Sriwijaya.

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