

# *In vitro* Rumen Fermentability of the Pelleted Feed Containing Water Spinach (*Ipomoea aquatica*)

<sup>1</sup>Himmatul Hasanah, <sup>2</sup>Eko Pangestu, <sup>3</sup>Ali Agus and <sup>2</sup>Joelal Achmadi

<sup>1</sup>Department of Biology, Faculty of Mathematic and Natural Science, Yogyakarta State University, Yogyakarta, Indonesia

<sup>2</sup>Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia

<sup>3</sup>Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia

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## Corresponding Author:

Joelal Achmadi

Faculty of Agriculture and  
Animal Sciences, Diponegoro  
University, Semarang,  
Indonesia

Email: jachmadi@gmail.com

**Abstract:** An *in vitro* technique based on two stages of batch culture was used to clarify fermentability and digestibility of pellet feed containing water spinach (*Ipomoea aquatica*). The portion of *Pennisetum purpureum* in the pellet feed was substituted with *Ipomoea aquatica* at respective levels of 0% (T1), 20% (T2), 40% (T3) and 60% (T4). The treatments were allocated in a completely randomized design with 5 replicates of each. The results showed that the substitution of water spinach in pellet did not effect on pH, ammonia (NH<sub>3</sub>), enzyme protein and microbial protein. Volatile Fatty Acids (VFA), acetate (C2), propionate (C3), butyrate (C4), methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) were decreased ( $p < 0.05$ ) by water spinach substitution level in the treatments. The ratios of C2: C3 were increased ( $P < 0.05$ ) in both of T3 and T4. The increasing water spinach substitution level enhanced ( $p < 0.05$ ) dry matter digestibility and organic matter digestibility but crude fiber digestibility was unaffected. In conclusion, substitution of *Pennisetum purpureum* with *Ipomoea aquatica* at the level of 20% may be the best fiber source for ruminant diet.

**Keywords:** Digestibility, Fermentation, Water Spinach, *In vitro*

## Introduction

The quality of forage is one of the factors affecting feed consumption, nutrient utilization and ruminant products. In the tropical countries such as Indonesia, the low quality of forage (contains high level of indigestible fibre) has been a common problems. The latter condition may lead to less efficiency of nutrient utilization resulting in the decrease of ruminant productivity. Therefore, alternative forage with high nutrient content is needed in the hope that it can be maximally utilized by ruminants.

Water spinach (*Ipomoea aquatica*) is an herbaceous aquatic or semi-aquatic perennial plant of the tropics and subtropics that may be exploited as a fibre source. In Indonesia, the area of water spinach plantation reaches 47,805 ha, with production about 276,976 tons or 5.79 tons per ha (SI, 2017). Some study results suggest that water spinach has a potency as forage for ruminants based on its nutrient content. The study by Khamparn and Preston (2006), Opene *et al.* (2018) and Umar *et al.* (2007) showed that water

spinach contains crude protein ranging from 5.18-24.6%, fibre 13-17.67%, dry matter 10.3% and organic matter 87.6%. With regard to the crude protein content, water spinach has been noted to have greater solubility of crude protein and thereby improving the N utilization by animals (Silivong and Preston, 2016).

In general, forage with high fiber content is marginally utilized by ruminants so it may need a technology for degrading fiber such as pelleting technology. Bertipaglia *et al.* (2010) and Oyaniran *et al.* (2018) reported that pelleting break downs the cell wall of forage (cellulose, hemicellulose and lignin) so that it will be easier to be degraded by microbes in the rumen. In the present study, water spinach was mixed with other feedstuffs before being pelleted. To best of our knowledge, the study on the pelleted feed containing water spinach is still scarce. Therefore, the objective of this present study was to evaluate the *in vitro* rumen ferment ability and digestibility of pelleted feed containing water spinach.

## Materials and Methods

### Experimental Diet and *in vitro* Test

*Pennisetum purpureum* cv Mott grass was cultivated in Sleman Yogyakarta and harvested at 60 days. Concentrate feed was purchased from local feed supplier and water spinach was purchased from the local market in Yogyakarta, Indonesia. *Pennisetum purpureum* cv Mott grass and water spinach were sundried and ground before being mixed with concentrate. The mixture was then subsequently pelleted. The composition and nutrient contents of the feedstuffs used to formulate pelleted feed are presented in Table 1. The study consisted of four treatments, i.e., T1: Control or pelleted feed containing no water spinach, T2: Pelleted feed containing 20% of water spinach, T3: Pelleted feed containing 40% of water spinach and T4: Pelleted feed containing 60% of water spinach. Treatments were allocated according to a completely randomized design with 5 replicates in each treatment. The feed ingredient and nutrient composition of pelleted feed are shown in Table 2.

### *In vitro* Fermentation

A two stages of culture batch was used in the *in vitro* technique. In first stage, sample of diet was incubated in rumen liquid anaerobically for 48 h (Menke and Steinngas, 1988). A buffer solution was also added in a ratio of 2:1 (v/v) with rumen liquid. The sample residue from first stage was then incubated in pepsin + HCl mixture for 72 h in the second stage (Tilley and Terry, 1963). The rumen liquid was obtained from fistulated cows fed on a diet containing 60% grass and 40% concentrate feed. The first and second stages were subjected for studying feed rumen

fermentability and nutrient digestibility, respectively. Each dietary treatment was tested with 5 replicates.

### Sampling and Analysis

After 48 h of fermentation process on Menke and Steinngas method, pH of the samples were immediately measured and then centrifuged (3,000 ×g, 15 min 4°C). The filtrate was divided into 2 parts, one part was stored at -20°C for Volatile Fatty Acids (VFA) and ammonia (NH<sub>3</sub>) analysis. The other part was continued by centrifugation (10,000 ×g, 15 min, 4°C) and stored at -20°C for determination of enzyme and microbial protein content. On the Tilley and Terry method, both of 48 h and 72 h of fermentation samples were centrifuged (3,000 ×g, 15 min, 4°C) and then both residues were used to determine nutrient digestibility including Dry Matter Digestibility (DMD), Organic Matter Digestibility (OMD) and crude fiber digestibility.

Analysis of DM, OM and crude fiber content in residue were determined according to AOAC (2005). Cellulose, hemicellulose and lignin content of feed were conducted according to Van Soest (1963). Enzyme and microbial proteins content were measured by Lowry method (Plummer, 1987). Rumen NH<sub>3</sub> concentration was measured using spectrophotometer as described by Chaney and Marbach (1962). Volatile fatty acids concentrations were determined by using Gas Chromatography (GC-2010, Shimadzu, Tokyo, Japan) as described by Flipek and Dvorak (2009). The CH<sub>4</sub> and CO<sub>2</sub> were estimated according to Church (1988).

### Statistical Analysis

The data were statistically assessed using one way ANOVA. To determine the difference among means, Duncan's multiple range test was subsequently used.

**Table 1:** Nutrient content of feedstuffs used in the study

Feedstuffs (%)	<i>Pennisetum purpureum</i> cv Mott grass	Concentrate <sup>1</sup>	Water spinach
Dry matter	92.93	88.87	89.42
Organic matter	83.43	89.77	84.60
Crude fiber	31.49	17.59	21.62
Crude protein	06.70	12.60	10.65
Crude fat	01.35	02.66	01.86
Ash	16.57	10.23	15.40
Nitrogen free extract	43.89	56.92	50.47
Total digestible nutrient	47.16	61.85	54.28
Hemicellulose	21.18	28.48	27.24
Cellulose	30.70	25.35	26.88
Lignin	12.67	11.47	12.22

<sup>1</sup>Concentrate consisted of corn meal 17.5, rice by product 5%, corn gluten fat 6.5%, copra 7.5%, palm kernel meal 10%, bran 5%, cassava by product 5%, tapioca by product 15%, rice bran 7.5%, peanut hulls 5%, cassava hulls 5%, soybean hulls 7%, molasses 2.5%, mineral mixture 1%

**Table 2:** Feed ingredient and nutrient composition of dietary treatments

Feedstuffs	T1	T2	T3	T4
Formulation (%)				
<i>Pennisetum purpureum</i> cv Mott grass	60.00	40.00	20.00	0.00
Water spinach	0.00	20.00	40.00	60.00
Concentrate	40.00	40.00	40.00	40.00
Total	100.00	100.00	100.00	100.00
Nutrient composition (%)				
Dry matter	90.74	90.39	90.13	88.08
Organic matter	86.27	87.91	89.94	89.23
Crude protein	13.72	14.36	15.06	15.04
Crude fat	2.14	2.02	1.87	2.18
Crude fibre	21.43	22.03	21.29	20.97
Ash	13.73	12.09	10.06	10.77
Hemicellulose	19.77	22.86	24.04	26.02
Cellulose	18.60	23.83	28.15	31.23
Lignin	12.48	10.55	11.10	11.77

T1: Control or pelleted feed containing no water spinach, T2: Pelleted feed containing 20% of water spinach, T3: Pelleted feed containing 40% of water spinach, T4: Pelleted feed containing 60% of water spinach.

## Results

The results of *in vitro* fermentability of pelleted feed with water spinach are shown in Table 3. The inclusion of pelleted water spinach into pelleted diets did not affect ( $P>0.05$ ) pH, ammonia, protein enzyme and microbial protein. However, the incorporation of water spinach resulted in significant effects on the decrease in total production of VFA, acetic acid, propionate and butyrate, with the lowest levels were at T4 group. The ratio of acetate to propionate increased ( $P<0.05$ ) with the increased levels of water spinach in the pelleted feed. Methane and CO<sub>2</sub> production decreased ( $P<0.05$ ) with water spinach incorporation in the pelleted feed.

The inclusion of 60% (T4) water spinach reduced ( $P<0.05$ ) methane and CO<sub>2</sub> productions until 34% and 36%, respectively.

The results of *in vitro* digestibility of pelleted water spinach utilization as a fiber source in diets are shown in Table 4. Organic matter digestibility increased ( $P<0.05$ ) with the increased levels of water spinach in the pelleted feed at 48 h. On the 72 h, the increasing levels of water spinach increased the digestibility of dry matter ( $P<0.05$ ) and organic matter ( $P = 0.05$ ) of the pelleted feed.

## Discussion

The incorporation of water spinach as the source of fiber in the pelleted feed did not impact the pH, ammonia, enzyme protein and microbial protein. This finding was in accordance with a study conducted by Al-Arif *et al.* (2017); Li *et al.* (2003); Xu *et al.* (2014). Zhang *et al.* (2019) reported that rumen pH is highly dependent on feed fermentation and its

physical form. Pelleted feed was proved to be able to maintain acid base status without causing acidosis in the rumen. Parish and Rhinehart (2018) explained that feed with high fiber would stimulate rumination process and saliva production playing a role for maintaining rumen pH. Owens and Zinn (1988); Karsli and Russel (2001) found that protein contained in feed was related to ammonia level in rumen. Protein would be deaminated in rumen to produce ammonia, which is an important compound for the synthesis of microbial protein.

It was shown in this study that the increased levels of water spinach were attributed to the decreased levels of VFA production. The latter condition seemed to be associated with the increased levels of cellulose, hemicellulose and lignin in the pelleted feed containing water spinach (Table 2).

Previously, Nathani *et al.* (2015) and Li *et al.* (2003) reported that high fiber content in the diets resulted in low concentration of VFA. Cellulose and hemicellulose were structural carbohydrate which are difficult to degrade and take longer time for being used as VFA production substrate. McDonald *et al.* (2011) stated that the high cellulose and hemicellulose are commonly found in mature forage, the older the forage, the higher the cellulose and hemicellulose content. Jayanegara *et al.* (2019) and Aderao *et al.* (2018) suggested the high fiber content (neutral detergent fiber and acid detergent fiber) will reduce VFA production. However, VFA production, as an indicator of energy availability for ruminant, was not different between pelleted feed without water spinach and pelleted feed containing 20% water spinach. Therefore, this can be inferred that fermentation activity in control feed and pelleted feed containing 20% water spinach were higher than other treatment (Table 3).

**Table 3:** *In vitro* fermentability of pelleted feed containing water spinach

Parameter	T1	T2	T3	T4	SEM	P value
Ph	6.72	6.71	6.73	6.71	0.01	0.68
NH <sub>3</sub> , mg/dl	18.33	17.77	18.18	17.99	0.35	0.96
Protein enzyme, mg/mL	0.83	0.83	0.72	0.70	0.02	0.07
Protein microbial, mg/mL	160.72	169.12	166.91	154.00	2.63	0.17
VFA, Mm	32.13 <sup>a</sup>	29.63 <sup>ab</sup>	25.78 <sup>b</sup>	20.25 <sup>c</sup>	1.24	<0.01
Acetate, Mm	22.04 <sup>a</sup>	20.23 <sup>ab</sup>	18.06 <sup>b</sup>	14.09 <sup>c</sup>	0.83	<0.01
Propionate, Mm	8.07 <sup>a</sup>	7.52 <sup>a</sup>	5.90 <sup>b</sup>	4.75 <sup>b</sup>	0.35	<0.01
Butyrate, Mm	2.01 <sup>a</sup>	1.88 <sup>a</sup>	1.82 <sup>a</sup>	1.41 <sup>b</sup>	0.07	0.01
Acetate to propionate ratio	2.74 <sup>c</sup>	2.69 <sup>c</sup>	3.06 <sup>a</sup>	2.97 <sup>b</sup>	0.37	<0.01
CH <sub>4</sub> , Mm	10.01 <sup>a</sup>	9.18 <sup>ab</sup>	8.46 <sup>b</sup>	6.56 <sup>c</sup>	0.36	0.01
CO <sub>2</sub> , Mm	16.06 <sup>a</sup>	14.82 <sup>ab</sup>	13.23 <sup>b</sup>	10.35 <sup>c</sup>	0.60	<0.01

<sup>a,b,c</sup>Different superscripts in the same row show significant different among treatments

T1: Control or pelleted feed containing no water spinach, T2: Pelleted feed containing 20% of water spinach, T3: Pelleted feed containing 40% of water spinach, T4: Pelleted feed containing 60% of water spinach

**Table 4:** *In vitro* digestibility of pelleted feed containing water spinach

Parameter	T1	T2	T3	T4	SEM	P value
48 h,%						
Dry matter	69.14	66.27	69.14	69.44	0.76	0.45
Organic matter	67.81 <sup>b</sup>	74.91 <sup>ab</sup>	81.67 <sup>a</sup>	82.01 <sup>a</sup>	1.38	0.01
Crude fiber	51.48	52.25	57.89	53.63	1.03	0.11
72 h,%						
Dry matter	72.20 <sup>c</sup>	78.47 <sup>bc</sup>	85.46 <sup>ab</sup>	89.99 <sup>a</sup>	2.36	0.01
Organic matter	74.59 <sup>b</sup>	77.93 <sup>ab</sup>	83.47 <sup>a</sup>	84.77 <sup>a</sup>	1.38	0.05
Crude fiber	66.09	68.48	63.48	65.50	1.70	0.16

<sup>a,b,c</sup>Different superscripts in the same row show significant different among treatments

T1: Control or pelleted feed containing no water spinach, T2: Pelleted feed containing 20% of water spinach, T3: Pelleted feed containing 40% of water spinach, T4: Pelleted feed containing 60% of water spinach.

Acetate production decreased with increasing proportion of water spinach in pelleted feed. This result was not consistent with the finding of Harahap *et al.* (2019) and Maktabi *et al.* (2016), who reported that feed with high neutral detergent fiber content will impact to enhance acetate concentration in rumen. This may indicate that high cellulose content take longer time for microbe to convert substrate into VFA in the end of fermentation process. This phenomenon was supported by the lower VFA concentration in pelleted feed containing water spinach than that in control pelleted feed. In addition, Hasanah *et al.* (2019) reported that potential of degraded fraction in feed supplemented with 0 or 20% water spinach was higher compared to 40% and 60% water spinach inclusion in feed. Indeed, the lowest acetate concentration on pelleted feed containing 60% water spinach was followed by the increasing acetate to propionate ratio and decrease methane and CO<sub>2</sub> production. Methane is the result of acetate fermentation in rumen. This is because hydrogen will also be produced in the process of forming acetate. Hydrogen is the main component of producing methane other than carbon (Lan and Yang, 2018; Hook *et al.*, 2010). These conditions have an impact on reduction of methane in pelleted feed containing 60% water spinach. With regard to CO<sub>2</sub> the production of this gas decreased with the increased levels of water spinach in pelleted feed.

In this study, OM digestibility of the pelleted feed containing different levels of water spinach was not different although control feed had lower OM digestibility compared to pelleted feed containing 40% and 60% water spinach. Increasing OM digestibility in water spinach supplemented pelleted feed incubated at 48 h indicated that more feed was fermented by the microbes. This may eventually increase the microbial population in the rumen. In general, the process of rumen microbial synthesis requires carbohydrate as energy and nitrogen in the form of NH<sub>3</sub>. McDonald *et al.* (2011) and Klein (2019) stated that the number of microbes that can be synthesized in rumen is limited by the amount of energy and efficiency in the use of energy by the microbes as well as the adequacy of NH<sub>3</sub> as a source of protein. Meanwhile NH<sub>3</sub> concentration among the treatment groups was not different (Table 3). This may indicate, therefore, that the carbohydrate, as energy source for microbial synthesise, was utilized better when including water spinach. Harahap *et al.* (2019) suggested that better synchronization between ruminal NH<sub>3</sub> release and carbohydrate availability are necessary to support rumen microbial synthesise. Note that decreasing of microbial synthesis will affect the degradation of feed in rumen. On the other word, increasing DM and OM digestibility at 72 h was caused by the chloride acid (HCl) and pepsin enzyme supplementation following the ruminal fermentation

process. This was supported by McDonald *et al.* (2011) that post-rumen digestibility was accelerated by the presences of HCl and pepsin enzymes in abomasum.

## Conclusion

Water spinach may replace the portion of *Pennisetum purpureum* at a level of 20% in pellet feed because resulted in highest OMD and VFA production and also lowest ratio of acetate to propionate.

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## Author's Contributions

All authors took parts in the preparation, development and publication of this manuscript.

## Ethics

The author declares that there are no ethical issues related to this study.

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