IOP Conference Series: Earth and Environmental Science

PAPER • **OPEN ACCESS**

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To cite this article: H Hasanah *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **346** 012069

Evaluation of water spinach (*Ipomoea aquatica*) as forage substitution on *in vitro* gas production, digestibility, and kinetic fermentation

H Hasanah[1,2,4], J Achmadi[2], E Pangestu[2] and A Agus[3]

[1]Department of Biology, Faculty of Mathematic and Natural Science, Yogyakarta State University, Yogyakarta, Indonesia
[2]Faculty of Agriculture and Animal Science, Universitas Diponegoro, Semarang, Indonesia
[3]Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia

E-mail: hasanahhimmatul07@gmail.com

Abstract. The aim of this study was to evaluate water spinach as forage substitution on *in vitro* gas production, digestibility and kinetics fermentation. The experiment was arranged in a completely randomized design with four treatments and five replications. The Treatments were: T1= control of *Pennisetum purpureum* cv Mott grass + 0% of water spinach, T2= + 20% of water spinach, T3: 40% of water spinach and T4: 60% of water spinach. Results showed that substitution of 20% Water Spinach as forage increased gas production after 48 h of incubation. There is no difference in the potential fraction degraded by the addition of a water spinach at the level of 20% compared to the control, although there is a decrease in the addition of 40 and 60% of the water spinach. Substitution of water spinach was not significant on kinetics fermentation of readily soluble fraction (a) and rate constant per hour of gas production (c). The substitution of 20% water spinach increased dry matter digestibility reaching 10% compared to control. The water spinach substitution increased organic matter digestibility ($P<0.05$). It is concluded that 20% of water spinach had the highest gas production and nutrient digestibility and thus, it can be used as forage alternative for ruminant.

1. Introduction

Ruminant animals have the ability to utilize forage into meat and milk. The quality of forage will affect consumption, nutrient utilization, and quality of product. The challenge of tropical countries, such as Indonesia is the low quality of forage due to high fiber content which leads to the inefficiency of nutrient utilization causing a decrease in the productivity of ruminants [1-4]. Therefore, forage alternative with high nutrient content is needed that it can be utilized by ruminants.

Water spinach is forage as source of fiber which is widely grown in Indonesia. The area planted with water spinach reached 47,805 ha with production reaching 276,976 ton or 5.79 ton per ha [5]. According to its nutrient content, water spinach is potential to be forage. The results of Khamparn and Preston [6], Opene et al. [7], Umar et al. [8] showed that water spinach contained crude protein ranging from 5.18 to 24.6%, fiber 13-17.67%, dry matter 10.3% and organic matter 87.6%.

The high quality of nutrient content makes water spinach forage alternative that can be used to supply ruminant requirements. However, the effects of water spinach substitution in the diet is needed to be carried out to evaluate water spinach as forage substitution on *in vitro* gas production, digestibility and kinetics fermentation. The aim of this study was to evaluate water spinach as forage substitution on *in vitro* gas production, feed nutrient digestibility and kinetics fermentation

2. Materials and method

2.1 Experimental site

The study was carried out at the Laboratory of Nutritional Biochemistry, Department of Animal Nutrition and Feed Science, Faculty of Animal Scicence, Universitas Gadjah Mada, Yogyakarta, Indonesia.

2.2 Experiment design and basal diet

Analysis of nutrient content carried out on feed ingredients includes dry matter (DM), organic matter (OM), crude fiber (CF), crude protein (CP), ether extract (EE), hemicellulose, cellulose and lignin by AOAC [9]. The nutrient content of feedstuffs used in this study are shown in table 1.

Table 1. Nutrient content of feedstuffs used in the study (% DM)*.

Feedstuffs (%)	*Pennisetum purpureum* cv Mott grass	Concentrate	Water spinach
DM	92.93	88.87	89.42
OM	83.43	89.77	84.60
CF	31.49	17.59	21.62
CP	6.70	12.60	10.65
EE	1.35	2.66	1.86
Ash	16.57	10.23	15.4
Hemicellulose	21.18	28.48	27.24
Cellulose	30.70	25.35	26.88
Lignin	12.67	11.47	12.22

DM =Dry Matter, OM =Organic Matter, CF =Crude Fiber, CP =Crude Protein, EE =Extract Ether
*Analysis in Laboratory of Nutritional Biochemistry, Faculty of Animal Science

The study consists of four treatments, T1: control or 0% water spinach, T2: with 20% water spinach, T3: 40% water spinach, and T4: 60% water spinach. This experiment was designed in completely randomized design with 5 replicates per treatment. The formulation and nutrient composition of diets are shown in table 2.

Table 2. Nutrient composition of diet substituted by adding water spinach.

Feedstuffs	T1	T2	T3	T4
Formulation (%)				
Pennisetum purpureum cv Mott grass	60	40	20	0
Water Spinach	0	20	40	60
Concentrate	40	40	40	40
Total	**100**	**100**	**100**	**100**
Nutrient composition (%)				
DM	90.74	90.39	90.13	88.08
OM	86.27	87.91	89.94	89.23
CP	13.72	14.36	15.06	15.04
EE	2.14	2.02	1.87	2.18
CF	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; T2:+water spinach 20%; T3: + water spinach 40%; T4: + water spinach 60%
DM =Dry Matter, OM =Organic Matter, CP =Crude Fiber, EE = Extract Ether, CF = Crude Fiber

2.3 In vitro fermentation
In vitro method used the [10] method for gas production. Rumen fluid was collected from fistullated cows fed with forage and concentrate (60:40) for one week before collecting it.

2.4 Sampling and analysis
After 48 h of fermentation process, the gas production was calculated using Neway Program (Excel), a program by Chen [11]. The calculation of gas production gained the equation $Y= a+b(1-e^{-ct})$, where Y = gas volume produced at time 't', *a* = soluble fraction, *b* = fraction of potential degraded or potential gas production, *c* = degradation rate of fraction *b* [11,12]. The residue of *in vitro* fermentation was used to determine nutrient degradability of dry matter (DMD) and organic matter (OMD) based on AOAC [9].

2.5 Statistical analysis
The data of gas production, fermentation parameter, and feed nutrient digestibility were statistically measured using a completely randomized design (*One Way ANOVA*). Then, to determine the difference of means, *Duncan's Multiple Range Test* (DMRT) was applied [13].

3. Results and discussions
The results of the effect of water spinach substitution as fiber source on feed nutrient digestibility and kinetics fermentation of feed are shown in table 3. This study indicates that 20% of water spinach substitution increased DMD up to 10% compared to the control. The other levels of substitution at 40%, 60% of water spinach did not significantly have any effect. Increasing of OMD occurred in feed substituted by water spinach (P<0.01) which ranged from 3 to 4% (T3 and T4) compared to the control. The substitution of 20% water spinach did not show difference in OMD compared control, T3 and T4.

Table 3. Digestibility and feed degradation rate with substitution of water spinach as fiber sources.

Parameter	T1	T2	T3	T4	SEM	P value
DMD (%)	57.97b	64.03a	60.15b	57.80b	0.74	<0.01
DOM (%)	81.42a	83.79ab	86.39bc	87.53c	0.37	<0.01
a (%)	-0.77	-0.88	-1.11	-0.38	0.15	0.45
b (%)	81.10a	84.06a	76.67b	76.78b	0.87	<0.01
c (%)	0.046	0.047	0.049	0.052	0.00	0.06

^{a,b,c} Different superscripts in the same column show significant difference among treatments
T1: control; T2:+water spinach 20%; T3: + water spinach 40%; T4: + water spinach 60%
a: soluble fraction; b: degraded potential fraction c: rate constant per hour of gas production.
DMD = dry matter digesbility, OMD = organic matter digesbility

The study conducted by Aderao et al. [14] stated that the substitution of forages by the leaves produced higher DMD than hay (79,5-82,0%). The enhancement of DM and OM digestibility were possible because the content of water spinach lignin was lower than that of forage in control treatment. The result study of Jančík et al. [15] and Jagadeesh et al. [16] represent that the distinction of forages cell wall content would affect the DMD and OMD in rumen. Higher cell wall content lowers the feed digestibility. Chumpawadee and Pimpa [17] mentioned that the high lignin content in fiber sources led to the difficulty of microorganism to attach with low fermentation rates, low digestibility and limited feed consumption.

The substitution of water spinach as fiber sources influenced fraction b (degraded potential fraction), although it did not affect fraction a (soluble fraction in feed) and fraction c (gas production rate). The substitution level of 20% water spinach did not show the difference in fraction b, while the level of substitution 40% and 60% was lower in fraction b. Gado et al. [18] reported that forage with difference fiber content has variation in fraction b. Tan et al. [19] claimed that kinetics parameters of feedstuff degradation were affected by the type of carbohydrate in forages and ratio of structural carbohydrate and non-structural carbohydrate.

The data of gas production can be seen in table 4. The highest gas production ($P<0.01$) was produced by diet with 20% water spinach substitution at 48 h. On the other side, the substitution level of 40% and 60% water spinach resulted in lower gas production than control. The highest increase of gas production from feed degradation occurred at 12 – 24 h (figure 1). The difference of gas production in each treatment started at 2 h.

Table 4. *In vitro* gas production of feed with the water spinach substitution as fiber sources.

Treatment	h 0	h 1	h 2	h 4	h 6
T1	29.60±0.89	33.30±0.76	35.80±0/91^{ab}	40.30±0.67^{b}	44.40±0.82^{b}
T2	29.50±0.50	33.62±0.65	36.50±0.86^{b}	41.38±0.82^{c}	45.63±0.96^{c}
T3	29.50±0.35	33.13±0.54	35.38±0.41^{a}	38.87±0.41^{a}	42.37±0.41^{a}
T4	29.80±0.45	33.80±0.57	36.30±0.48^{ab}	40.00±0.71^{b}	43.70±0.90^{b}
Treatment	h 8	h 12	h 24	h 36	h 48
T1	49.10±0.89^{c}	58.70±1.20^{b}	81.50±1.11^{b}	89.80±0.84^{b}	92.90±2.60^{b}
T2	50.75±0.75^{d}	61.13±1.67^{c}	84.67±1.08^{c}	91.83±1.14^{c}	96.33±0.82^{c}
T3	46.25±0.25^{a}	56.37±0.65^{b}	78.50±1.11^{a}	84.63±0.65^{a}	87.00±1.87^{a}
T4	48.00±0.70^{b}	60.00±0.70^{bc}	79.50±1.50^{a}	87.71±3.48^{a}	88.40±1.67^{a}

^{a,b,c} Different superscripts in the same column show significant difference among treatments
T1: control; T2:+water spinach 20%; T3: + water spinach 40%; T4: + water spinach 60%

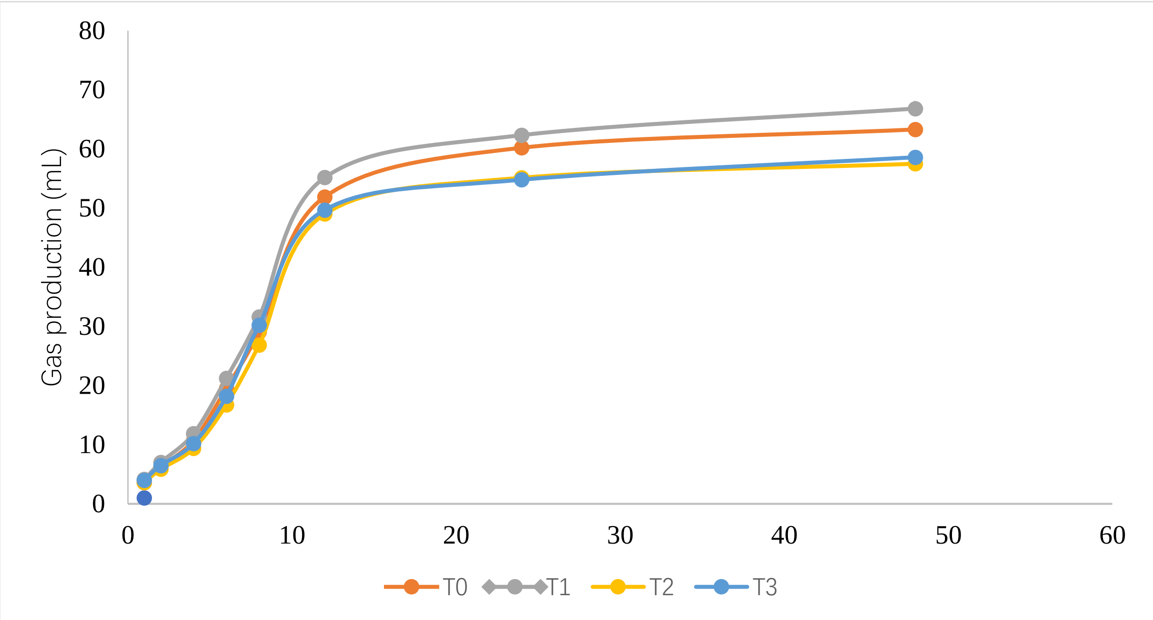

Figure 1. Gas production with water spinach substitution as fiber sources.

In this study, there was positive correlation between DMD and gas production produced in 20% water spinach substitution. The substitution of 20% water spinach resulted in highest DMD and gas production. The research had similarity the research by Sommart et al. [20], reporting that the increase of feed digestibility would affect the total increase of gas production. Soto et al. [21] stated that gas production in diet with vegetable waste substitutions showed an improvement (172-188 ml/g) for 24 h of incubation. Gas production was parameter used to estimate digestibility value and fermentation products [14,22].

4. Conclusion

The utilization of 20% water spinach as fiber sources can increase dry matter and organic matter digestibility and total gas production with no difference for rumen fermentation kinetics. Thus, it is concluded that the level of 20% water spinach substitution is potential to be fiber sources to substitute forage.

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