Effect of *Palisada perforate* (Bory) K.W.Nam supplementation on gas production and fermentation kinetics in total mixed ration

Nur Hidayah [1,2], Kustantinah[3], *Cuk Tri* Noviandi[3], and *Andriyani* Astuti[3*]

[1]Graduate student of Animal Science. Universitas Gadjah Mada. Jl. Agro Karangmalang. Yogjakarta 55281. Indonesia
[2]Department of Animal Science. Agriculture Faculty. Tidar University. Jl. Barito 1. Magelang. Central Java 56114. Indonesia
[3]Animal Science Faculty. Universitas Gadjah Mada. Jl. Agro Karangmalang. Yogjakarta 55281. Indonesia

Abstract. This research aimed to evaluate *Palisada perforate* (Bory) K.W.Nam as a supplement on total mixed ration to their gas production and fermentation kinetics. A factorial randomized block design with two factors and five replications. The first factor was kinds of total mixed ration (TMR 1, 80% grass (G) and 20% concentrate (C) based on dry matter (DM); TRM 2, 70% G and 30% C; TMR 3, 60% G and 40% C) and the second factor was level of *P. perforate* (Bory) K.W.Nam supplementation (0 and 5% organic matter (OM)). There was no interaction between kinds of TMR and level of *P. perforate* (Bory) K.W.Nam supplementation on gas production and fermentation kinetics. The TMR 3 increased ($P<0.01$) gas production in early incubation (2 and 12 h) but was similar to all treatments on 24 and 48 h of incubation. Meanwhile, increased concentrate proportion on TMR 2 and 3 increased ($P<0.01$) easily degraded fraction (a). Supplementation of *P. perforate* (Bory) K.W.Nam up to 5% OM on all TMR doesn't affect gas production and fermentation kinetics. TMR consisted of 60% grass and 40% concentrate is the best basal diet to increase gas production in early incubation and easily degraded fraction on fermentation kinetics.

1. Introduction

In general, seaweed or macroalgae are aquatic organisms classified with specific characteristics of not having true roots, stems, and leaves [1]. Darmawati et al. [2] explained that in the plant kingdom, seaweed is included in the *Thallophyta* division with all parts called thallus. While the part that resembles a root is called a holdfast, the stipe is the stem, and the blade is the leaf part [3]. Tanna and Mishra [4] stated that seaweeds are incredibly diverse in terms of species, habitat, maturity, harvesting period, and environmental factors, and they are also great sources of various nutrients.

Seaweeds are divided into three groups: red seaweed (Rhodophyta), brown seaweed (Ochrophyta, Phaeophyceae), and green seaweed (Chlorophyta). This classification is based

* Corresponding author: andriyaniastuti@ugm.ac.id

on several characteristics, including pigmentation, other morphological features, photosynthetic membrane structure and elements, and the chemical nature of photosynthetic storage products. Seaweeds get their color from their chlorophyll content; green seaweed color from chlorophyll a and b which amounts to higher plants. Phycoerythrin and phycocyanin are dominant in red seaweed. Whereas, xanthophyll and fucoxanthin pigments, which responsible for the color of brown seaweed [5]. Tanna and Mishra [6] also explained that seaweed is a rich source of bioactive compounds, including dietary fibers, proteins, minerals, and vitamins. Additionally, they contain bioactive substances that may have therapeutic applications in treating inflammation, cancer, oxidative stress, allergies, diabetes, thrombosis, obesity, hypertension, lipidemia, and many degenerative diseases. These substances include polysaccharides, polyphenols, and phytochemicals.

In the animal sector, using seaweed as a feed supplement and substitution increases growth rate, feed conversion efficiency, and ruminant productivity [7]. Besides that, it has been proven very effective and efficient in decreasing ruminant methane emissions and increasing productivity [8, 9, 10]. Bromoform, one of the volatile halogenated compounds found in seaweed, has contributed to this effect. Allen et al. [11] and Costa and Leigh [12] explained that bromoform inhibits or blocks the activity of key methanogenic enzymes (methyl coenzyme M reductase (MCR) and coenzyme M transferase). The halogenated molecules have different molecular weights and are kept in gland cells. These cells release the molecules into the environment to act as a chemical defense against bacteria that are antagonistic to herbivores [13]. Various biological activities, including antioxidant, anti-inflammatory, antiprotozoal, and antibacterial effects, have been demonstrated for these compounds [14]. Ahmed & Nishida [15] stated the anti-methanogenic efficaciousness may also be attributed to additional secondary metabolites. Seaweed also produces a wide range of other non-volatile metabolites, including flavonoids, halogen compounds, and other phenolic compounds [14].

In Indonesia, studies on using various tropical seaweed species for ruminants are still very limited, although it has the highest diversity and is the second producer of seaweed in the world [16, 17]. This is due to a number of factors, including over-reliance on a small number of species, difficulties with sustainable farming and harvesting, and the requirement for technological advancements to exploit a wider variety of species [18, 19]. The two most often utilized seaweed species are *Gracilaria* spp. and *Euchema* spp. Thus, a large number of seaweed species remain unexplored [1]. Kasanah et al. [1] also explained that one of the coasts in Java's South Coast region is the Gunungkidul Yogyakarta area, Indonesia. Its 13 km of beach has the potential to be diverse and has an abundance of natural seaweed sources. One of them is red seaweed, *Palisada perforate* (Bory) K.W.Nam, which has the potential as a supplement or feed additive to reduce ruminant methane production [20]. It should be noted that the result is based solely on seaweed as a single ingredient and not on its addition to basal feed. Because of that, the purpose of this study is to assess the impact of *Palisada perforate* (Bory) K.W.Nam supplementation on total mixed rations (TMR) as basal diets on gas production and fermentation kinetics. This variables are important to evaluate as an indicator for determining rumen fermentation patterns and feed degradation process by rumen microbes.

2. Materials and Methods

2.1. Experimental design

This study used a factorial randomized block design with two factors and five replications, which a time of rumen fluid collection as a block. The first factor was the kinds of total mixed ration (TMR 1, 80% Napier grass (G) and 20% concentrate (C) based on dry matter (DM); TRM 2, 70% G and 30% C; TMR 3, 60% G and 40% C) and the second factor was level of *P. perforate* (Bory) K.W.N supplementation (0 and 5% OM).

2.2. Preparing *Palisada perforate* (Bory) K.W.Nam and total mixed ration

Natural red seaweed, *P. perforate* (Bory) K.W.Nam, was harvested in October 2022 from Gunungkidul, Yogyakarta, Indonesia. To get rid of grit, sand, and other seaweed species, the seaweed was thoroughly cleaned with water. Afterward, the sample was dried on a freeze dryer (Buchi, Lyovapor, L-200) and grounded with a Willey mill until a fine powder (80–100 mesh).

The TMR consisted of grass (*Pennisetum purpureum* cv. Gama Umami) and concentrate (rice brand, wheat pollard, soybean meal, coconut cake meal, corn gluten feed, molasses, and premix). The sample dried in an oven at 55ºC for 3 days and grounded until powder formed with a Willey mill (80–100 mesh). The concentrate formula and nutrient content of TMR are shown in Tables 1 and 2. The nutrient content of TMR as recommended by Kearl [21] as follows crude protein 10.69% and total digestible nutrient 69.93% based on DM. The TMR nutrient content has iso crude protein and iso total digestible nutrient.

Table 1. Concentrate formula

Ingredients (%)	Concentrate 1	Concentrate 2	Concentrate 3
Rice bran	13.25	17.25	20.25
Wheat pollard	45	45	45
Soybean meal	11.25	7.25	4.25
Coconut cake meal	14.50	14.50	14.50
Corn gluten feed	10	10	10
Molasses	5	5	5
Premix	1	1	1
Total	100	100	100

Table 2. TMR nutrient content (%)

TMR / Nutrient	1 (80% G + 20% C)	2 (70% G + 30% C)	3 (60% G + 40% C)
OM	83.85	83.60	86.48
Ash	15.95	16.10	13.52
CP	13.03	13.03	12.91
EE	2.64	3.13	2.62
CF	28.88	26.45	27.52
NFE	41.02	43.52	43.43
TDN	58.79	61.35	63.23

TMR: total mixed ration; G: grass; C: concentrate; OM: Organic matter; CP :Crude protein ; EE : Ether extract ; CF : Crude fiber ; NFE : Nitrogen free extract ; TDN : Total digestible nutrient ; NFE = 100 – (% CP + % CF + % EE + % ash) ; TDN = 70.6 + 0.259 CP + 1.01 EE - 0,76 CF + 0,091 NFE (Sutardi, 2001)

2.3. *In vitro* process and gas production evaluation

In vitro process, we used a piston and glass syringe of 100 mL according to the Menke & Steingass [22] method. The glass syringe was filled with rumen liquor and buffer solution (total at 30 mL, 1:2 ratio vol/vol), and also 200 mg sampled (TMR with or without *P. perforate* (Bory) K.W.Nam supplemented) as substrates. Using a vacuum tube, the rumen

liquor was collected and mixed from the Bali cow and bull fistulated. The Animal Care Committee, Veterinary Faculty, Universitas Gadjah Mada, Indonesia approved the rumen collection process. The *in vitro* process was carried out under anaerobic conditions until 48 h of incubation. We evaluated and calculated total and kinetic gas production with the Neway program [23].

2.4. Statistical analysis

ANOVA was used for statistical analysis, and Duncan's multiple range test (DMRT) was used to look at treatment mean differences. The statistical analysis was performed using IBM SPSS Statistics version 26.

3. Result and Discussion

The result showed that there was no interaction between kinds of TMR and level of *P. perforate* (Bory) K.W.Nam supplementation on gas production and fermentation kinetics. The TMR 3 increased ($P<0.01$) gas production in early incubation (2 and 12 h) but was similar to all treatments on 24 and 48 h of incubation (Table 3). Meanwhile, increased concentrate percentage on TMR 2 and 3 increased ($P<0.01$) easily degraded fraction (a) (Table 4). Supplementation of *P. perforate* (Bory) K.W.Nam up to 5% OM on all kinds of TMR doesn't affect gas production and fermentation kinetics.

Increasing the proportion of concentrate and decreasing the grass proportion in the TMR as a basal diet significantly increased the gas production in early incubation (2 and 12 h). This result indicated that increasing concentrate enhances rumen activities, gas production, and improves feed degradability in early incubation. This result is linear with the easily degraded fraction on fermentation kinetics variables, which increased with increasing concentrate proportion. This described that increasing concentrate followed the easily degradable component that gives more substance for rumen microbial to degrade.

The concentrate has a more easily degradable component in the rumen than grass. The crude protein (CP) and nitrogen-free extract (NFE) of concentrate were higher than grass, 8.98% vs 18.90% and 39.36% vs 53.94% DM, respectively. Opposite to the rumen's difficult-degradable component, grass is higher than concentrate. Grass has crude fiber (CF) content higher than concentrate, 33.42% vs 18.67% DM. Kulivand & Kafilzadeh [24] stated that CP (easily degradable component) has a positive correlation ($r = 0.62$, $p < 0.05$) with gas production at 96 h incubation. Whereas, a negative correlation is observed with gas production when the difficult degradable component, like acid detergent fibre (ADF) that crude fiber part, ($r = -0.60$, $p < 0.05$). Similar results reported by Viennasay et al. [25], increased concentrate levels on a basal diet that consisted of roughage and concentrate significantly increased rumen gas production.

Supplementation of *P. perforate* (Bory) K.W.Nam up to 5% OM on TMR based as basal diet did not increase gas production and fermentation kinetics. This indicated that the percentage of seaweed did not alter microbial activity and feed degraded in the rumen. The same result was reported by Chot et al. [26], after 12, 24, and 48 h of incubation, seaweed extract supplementation reduced methane yield but did not change significantly overall gas production. The result of this research also described that the seaweed species and amount of supplementation did not disturb the microbial activity to feed degraded. A study by Künzel et al. [27] reported that the inclusion of 2.5 and 5.0% on a DM basis brown seaweed from the Icelandic ocean (*Ascophyllum nodosum* and *Fucus vesiculosus*) on total mixed ration (TMR) reduced total gas production with 5% level ($P < 0.001$). The different response might be due to the kind and percentage of secondary metabolite from the seaweed. In this research, we used red seaweed.

Table 3. Gas production with different levels of *Palisada perforate* (Bory) K.W.Nam supplementation on different kinds of total mixed ration

Variable / Time incubations		Gas production (mL/200 mg DM)			
		2 h	12 h	24 h	48 h
Interaction kinds of total mixed ration (TMR) with different levels of *P. perforate* (Bory) K.W.Nam supplementation					
TMR 1	0% OM	7.75 ± 1.89	26.35 ± 4.98	42.23 ± 5.26	53.50 ± 7.16
	5% OM	6.82 ± 1.95	25.77 ± 3.38	41.03 ± 4.60	50.54 ± 7.26
TMR 2	0% OM	6.23 ± 2.64	26.71 ± 5.89	38.29 ± 7.56	47.48 ± 7.35
	5% OM	9.03 ± 1.69	28.26 ± 2.81	39.98 ± 5.74	51.63 ± 5.53
TMR 3	0% OM	10.27 ± 2.23	31.17 ± 3.14	45.82 ± 3.04	57.84 ± 2.37
	5% OM	9.22 ± 2.28	29.22 ± 3.63	42.82 ± 4.12	54.13 ± 4.93
Effect of different kinds of TMR					
TMR 1		7.34^{a} ± 1.86	26.09^{a} ± 4.09	41.70 ± 4.71	51.86 ± 6.93
TMR 2		7.63^{a} ± 2.54	27.40^{a} ± 4.58	39.14 ± 6.39	49.79 ± 6.35
TMR 3		9.68^{b} ± 2.18	30.09^{b} ± 3.36	44.15 ± 3.80	55.76 ± 4.25
Effect of different levels of *P. perforate* (Bory) K.W.Nam supplementation					
0% OM		8.06 ± 2.64	27.86 ± 5.03	41.85 ± 6.16	52.94 ± 7.07
5% OM		8.42 ± 2.15	27.86 ± 3.39	41.30 ± 4.67	52.10 ± 5.76

Different superscripts in the same rows show significant differences (P<0.05); TMR 1: 80% G + 20% C; TMR 2: 70% G + 30% C; TMR 3: 60% G + 40% C; G: grass; C: concentrate; OM: organic matter

Table 4. Fermentation kinetics with different levels of *Palisada perforate* (Bory) K.W.Nam supplementation on different kinds of total mixed ration

Variable		Kinetic gas production			
		a (mL/200 mg DM)	b (mL/200 mg DM)	c (mL/jam)	a+b (mL/200 mg DM)
Interaction kinds of total mixed ration (TMR) with different levels of *P. perforate* (Bory) K.W.Nam supplementation					
TMR 1	0% OM	1.87 ± 2.90	57.30 ± 7.31	0.055 ± 0.01	59.17 ± 9.93
	5% OM	0.34 ± 2.78	58.50 ± 5.85	0.043 ± 0.01	58.84 ± 7.85
TMR 2	0% OM	4.66 ± 4.19	57.45 ± 10.11	0.042 ± 0.00	62.10 ± 7.45
	5% OM	4.27 ± 1.10	56.37 ± 3.80	0.045 ± 0.00	60.64 ± 4.83
TMR 3	0% OM	5.05 ± 1.84	60.15 ± 1.00	0.047 ± 0.00	65.19 ± 1.46
	5% OM	4.17 ± 2.23	59.71 ± 1.40	0.046 ± 0.00	63.88 ± 2.23
Effect of different kinds of TMR					
TMR 1		1.02^{a} ± 2.77	57.97 ± 6.13	0.048 ± 0.01	58.99 ± 8.23
TMR 2		4.44^{b} ± 2.69	56.85 ± 6.77	0.043 ± 0.00	61.29 ± 5.75
TMR 3		4.61^{b} ± 1.98	59.93 ± 1.17	0.047 ± 0.00	64.54 ± 1.90
Effect of different levels of T*P. perforate* (Bory) K.W.Nam supplementation					
0% OM		3.95 ± 3.12	58.44 ± 6.42	0.048 ± 0.01	62.39 ± 6.78
5% OM		2.93 ± 2.75	58.19 ± 4.06	0.045 ± 0.00	61.12 ± 5.51

Different superscripts in the same rows show significant differences (P<0.05); TMR 1: 80% G + 20% C; TMR 2: 70% G + 30% C; TMR 3: 60% G + 40% CC; G: grass; C: concentrate; OM: organic matter

4. Conclusion

It is concluded that TMR consisted 60% grass and 40% concentrate is the best basal diet to increase total gas in early incubation and easily degraded fraction on fermentation kinetics. Using this basal diet has the potential to reduce methane production because it is more degradable in the rumen. This is good for increasing ruminant productivity and reducing environmental pollution. Future research needs to examine the higher level of this seaweed supplementation or another seaweed species on this basal diet.

5. Acknowledgments

We would like to express our gratitude and appreciation to Indonesia Endowment Funds for Education (LPDP) Center for Higher Education Funding (BPPT) for supporting this research.

6. References

1. N. Kasanah, D.S. Seto, H. Khotimah, and Triyanto, Rumput laut pangan (Universitas Gadjah Mada Press, Yogyakarta, 2022)
2. Darmawati, I. Sutinah, Ode, B.D.H. Setyono, S. Laheng, T. Mujtahidah, Y.P. Sari, I. Wahyuni, Z. Abidin, W. Sukendar, S.T.M. Sulthoniyah, A.B. Marda, M.A. Yusuf, A.N.N. Suci, A. Abdullah, and V. Setyaka, Kiat agribisnis rumput laut (CV. Tohar Media, Makasar, 2023)
3. C.M. Buchholtz, G. Krause, and B.H. Buch, Seaweeds and man in seaweed biology ecological studies (Springer-Verlag, Berlin, 2012)
4. B. Tanna, A. Mishra, Compr. Rev. Food Sci. Food Saf. **17**, 1613–1624 (2018)
5. B. Kılınç, S. Cirik, G. Turan, H. Tekogul, E. Koru, Seaweeds for food and industrial applications, *In Food industry*, ed. I. Muzzalupo (Intech Open, London, 2013)
6. B. Tanna, A. Mishra, Compr. Rev. Food Sci. Food Saf. **18**, 817–831 (2019)
7. B.R. Min, D. Parker, D. Brauer, H. Waldrip, G. Lockard, K. Hales, A. Akbay, S. Augyte, Anim. Nutr. **7**, 1371-1387 (2021)
8. B.M. Roque, C.G.J. Brooke, J. Ladau, T. Polley, L.J. Marsh, N. Najafi, P. Pandey, L. Singh, R. Kinley, J.K. Salwen, E. Eloe-Fadrosh, E. Kebreab, and M. Hess. Anim. Microbiome **1**, 1-3 (2019)
9. R.D. Kinley, G. Martinez-Fernandez, M.K. Matthews, R. de Nys, M. Magnusson, and N.W. Tomkins. J. Clean Prod. **259**, 120836 (2020)
10. A.A. Mihaila, C.R.K. Glasson, R. Lawton, S. Muetzel, G. Molano, and M. Magnusson. Appl. Phycol. **3**, 274-284 (2022)
11. K.D. Allen, G. Wegener, R.H. White, Appl. Environ. Microbiol. **80**, 6403-6412 (2014)
12. K.C. Costa, J.A. Leigh, Curr. Opin. Biotechnol. **29**, 70-75 (2014)
13. C. Parchemin, D. Raviglione, H. Ghosson, M.V. Salvia, C. Goossens, P. Sasal, E. Faliex, N. Tapissier-Bontemps, Algal Res. 72, 103138 (2023)
14. F.S.-L. Keng, S.-M. Phang, N. Abd Rahman, E.C. Leedham Elvidge, G. Malin, W.T. Sturges, J. Appl. Phycol. **32**, 1377-1394 (2020)
15. E. Ahmed, T. Nishida, Anim. Feed Sci. Technol. **318**, 116118 (2024)
16. Food and Agriculture Organization, The global status of seaweed production, trade and utilization (Food and Agriculture Organization of the United Nations, Rome, 2018)
17. E. Erniati, F.R. Zakaria, E. Prangdimurti and D.R. Adawiyah. Acta Aquatica. **3**, 12-17 (2016)
18. M. MacMonagail. L. Cornish. L. Morrison. R. Araújo. and A.T. Critchley, Eur. J. Phycol. **52**. 371-390 (2017)
19. S. Kraan. Seaweed resources. collection. and cultivation with respect to sustainability. In *Sustainable Seaweed Technologies* (Elsevier Inc., United Kingdom, 2020).

20. N. Hidayah, C.T. Noviandi, A. Astuti, and Kustantinah. Nusantara Biosci. **16**, 37-42 (2024)
21. L.C. Kearl, Nutrient requirements of ruminants in developing countries (International Feed Stuffs Institute, Utah Agriculture Experimental Station, Utah State University, Utah, 1982)
22. H.H. Menke, H. Steingass, Anim. Res. and Dev. **28**, 7-55 (1988)
23. X.B. Chen, Neway program international feed resources unit (Rowett Research Institute, Backburn, 1994)
24. M. Kulivand, F. Kafilzadeh, Acta Sci. **31**, 9-14 (2015)
25. B. Viennasay, P. Totakul, M. Matra, B. Phestcha, M. Wanapat, Agric. Food Sci. **102**, 4927-4932 (2022)
26. Y. Choi, S.J. Lee, H.S. Kim, J.S. Eom, S.U. Jo, L.L. Guan, . . .S.S. Lee, Sci. Reports. **11**, 24092 (2021)
27. S. Künzel, T. Yergaliyev, K.J. Wild, H. Philippi, A.H. Petursdottir, H. Gunnlaugsdottir, . .M. Rodehutscord, Front. Microbiol. **13**, 889618 (2022)