In vitro rumen fermentation characteristics due to *Acanthopora muscoides* (L.) Bory supplementation

Nur Hidayah [1,2], *Firda Nasywa* Sofia[3], Kustantinah[4], *Andriyani* Astuti[4], *Chusnul* Hanim[4], and *Cuk Tri* Noviandi[4*]

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

Abstract. In this study, a red macroalgae species, *Acanthopora muscoides* (L.) Bory was processed as a supplement *Pennisetum purpureum* cv. Gama Umami was used to evaluate their *in vitro* ruminant methane characteristics. This parameter will give us insight into the fermentation product from the feed degradation process condition in the rumen. A complete randomized block design with four treatments and four replications was used, with a time of rumen fluid collection as a block. The treatments consisted of control (only *Pennisetum* purpureum cv. Gama Umami as a basal diet) and supplementation *Acanthopora muscoides* (L.) Bory at 2.5, 5.0, and 7.5% organic matter (OM). The result showed that supplementing *Acanthopora muscoides* (L.) Bory up to 7.5.0% OM on *Pennisetum purpureum* cv. Gama Umami increased ($P<0.01$) total volatile fatty acid (VFA) production, propionate proportion, and microbial protein synthesis. Those treatments decreased ($P<0.01$) the proportion of acetate, butyrate, and acetate propionate ratio without disturbing pH value and NH3 concentration. The conclusion was *Pennisetum purpureum* cv. Gama Umami supplemented with *Acanthopora muscoides* (L.) Bory 7.5% OM is the best treatment in this study to increase rumen fermentation characteristics.

1. Introduction

The Food and Agriculture Organization [1] reported that Indonesia is the second largest seaweed producing country in the world (38.7%) after China (47.9%) and has the largest species diversity in the world [2]. Indonesia's seaweed exports in 2022 reached 232,081.2 tons [3] and became one of the world's main producers of wet seaweed [4]. Soetjipto et al. [4] and Darmawati et al. [5] stated that seaweed in Indonesia is used as raw material for food, medicine, and cosmetics industries. However, up to now the number of species of seaweed that is used and commercialized is still very limited. This is because of reliance on a few species, challenges in sustainable cultivation and harvesting, and the need for technological advancements to exploit a broader range of species [6, 7]. The two most common seaweed species used are the *Euchema* spp. and *Gracilaria* spp. So, many seaweed species have not been explored [8]. Kasanah et al. [8] reported that the Gunungkidul Yogyakarta area is one of the coasts in the South Coast region of Java which has a beach length of 13 km which has the potential for diversity and availability of natural seaweed sources.

Several studies conducted in Europe, Australia, America, New Zealand, Korea, and Africa report that seaweed as feed ingredients or additives can reduce methane emissions in ruminants and increase productivity [9, 10, 11]. This effort is very important because ruminant methane has two negative effects, contributing to increased greenhouse gas emissions and decreased ruminant productivity. Ruminants contribute 17% of all anthropogenic emissions during the feed fermentation process in the rumen [12]. Mayberry et al. [13] stated that three to twelve percent of the feed's digestible energy that could be utilized for production is lost as methane.

However, studies on using various tropical seaweed species for ruminants are still very limited. The results of initial research conducted by Hidayah *et al*. [14] and Hidayah *et al*. [15] reported that one of the tropical seaweeds originating from coastal areas in Gunungkidul Regency, Yogyakarta, Indonesia was *Acanthopora muscoides* (L.) Bory could be used as an additive to ruminants to reduce methane production and increase rumen fermentation characteristics. However, that result is only based on seaweed as a single substance, not as a supplement in basal feed. *Pennisetum purpureum* cv. Gama Umami is commonly grass that farmers give to ruminants as a basal diet. But the high grass on the feed will have higher enteric methane production than mixed with concentrate. Therefore, in this study want to evaluate the effect of supplementing *Acanthopora muscoides* (L.) Bory to *Pennisetum purpureum* cv. Gama Umami (grass-based diets) on fermentation characteristics need to be investigated.

*Corresponding author: c.t.noviandi@ugm.ac.id

2. Materials and Methods

2.1. Experimental design

This study used a complete randomized block design with four treatments and four replications, which a time of rumen fluid collection as a block. The treatments consisted of control (only *Pennisetum purpureum* cv. Gama Umami as a basal diet) and supplementation *Acanthopora muscoides* (L.) Bory at 2.5, 5.0, and 7.5% OM.

2.2. Preparing *Acanthopora muscoides* (L.) Bory

This natural seaweed is from Gunungkidul District, Yogyakarta Province, Indonesia. The seaweed was rinsed with water to remove sand, grit, and other seaweed species. Subsequently, the sample was arranged on a freeze dryer (Buchi, Lyovapor, L-200) to dry. Meanwhile, the *Pennisetum purpureum* cv. Gama Umami collected Animal Science Farm at Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia, and then dried it in an oven at 55ºC. The dehydrated substance was ground into a fine powder (80–100 mesh) using a Willey mill and then stored in freezer-sealed plastic bags for further analysis.

Fig. 1. *Acanthopora muscoides* (L.) Bory

2.3. *In vitro* rumen fermentation characteristics analysis

The rumen fermentation characteristic analysis consisted of pH, VFA, NH_3, and rumen microbial protein, which were collected after 72 hours of *in vitro* incubation. The pH of the supernatant was determined using a pH meter (Hanna pH-meter portable, Hanna Instruments, USA). Ammonia (NH_3) concentration was determined using the procedure outlined by Chaney & Marbach [16]. Using a UV-vis microplate spectrophotometer set to 750 nm and a standard curve (y = 0.028018x - 0.01108, r2 = 0.99, (NH4)2 SO4, Merck). The method described by Plummer [17] for measuring microbial proteins was used, with a UV-vis microplate spectrophotometer set to 630 nm and a standard curve (y = 2.5075x + 0.0824 and r2 = 0.99, BSA/Bovine Serum Albumin, Sigma). Cottyn and Boucque [18] method was applied to determine VFA using gas chromatography (GC 2010 Plus, Shimadzu Crop., Kyoto, Japan, HP-FFAP column (50 m × 0.2 mm × 0.3 µm) and FID detector).

2.4. Statistical analysis

The statistical analysis was performed using ANOVA, and the treatment mean differences were examined using Duncan's multiple range test. IBM SPSS Statistics version 26 was used to conduct the statistical analysis.

3. Result and Discussion

The highest total VFA production (202.75 mM) was noted at P4 treatment (*Acanthopora muscoides* (L.) Bory supplementation at 7.5.0% OM, P<0.01, Table 1). However, this high VFA production did not negatively affect rumen pH condition, which ranged from 6.86 to 6.92. This treatment also significantly improved propionate proportion (20.95%), which resulted in the lowest acetate, butyrate, and acetate to propionate ratio (71.00%, 8.05%, and 3.97; P<0.01). This result indicated more energy saving, which will increase ruminant productivity. Table 2 shows that supplementing *Pennisetum purpureum* cv. Gama Umami as a basal diet with *Acanthopora muscoides* (L.) Bory supplementation at 7.5.0% resulted in the highest microbial protein synthesis, averaged at 69.62 mg/100 mL (P<0.01).

Table 1. *In vitro* total and proportion VFA due to *Acanthopora muscoides* (L.) Bory supplementation with different levels on *Pennisetum purpureum* cv. Gama Umami

Treatments	Total VFA (mM)	Proportion (%)		
		Acetate (A)	Propionate (P)	Butyrate (B)
P1	$32.81^a \pm 2.70$	$78.97^d \pm 0.68$	$5.24^a \pm 1.04$	$15.79^c \pm 0.83$
P2	$62.11^a \pm 5.90$	$74.05^c \pm 0.44$	$14.41^b \pm 1.59$	$11.54^b \pm 1.21$
P3	$146.82^b \pm 7.41$	$72.44^b \pm 1.39$	$19.12^c \pm 1.40$	$8.44^a \pm 0.62$
P4	$202.75^c \pm 51.50$	$71.00^a \pm 0.60$	$20.95^c \pm 0.28$	$8.05^a \pm 0.35$

Different superscripts in the same column show very significant differences (*P*<0.01);
P1: *Pennisetum purpureum* cv. Gama Umami;
P2: P1 + *Acanthopora muscoides* (L.) Bory 2.5% OM;
P3: P1 + *Acanthopora muscoides* (L.) Bory 5% OM;
P4: P1 + *Acanthopora muscoides* (L.) Bory 7.5% OM

The *Acanthopora muscoides* (L.) Bory supplementation did not interfere with the fermentation activity, as indicated by the pH values for all treatments falling within the normal range. Normal pH values can range from 5.5 to 7.5 depending on the type of feed and feeding frequency, according to Zheng et al. [19]. A pH value below 6.2 has a negative effect on fiber fraction digestibility, while a pH value below 5.5 will impact the incidence of rumen acidosis, reduce consumption, and even in extreme conditions the livestock will not want to eat (off feed) and can cause serious problems [20]. Similar research reported by Canul-Ku et al. [21], which was inclusion different levels of *Sargassum* on a basal diet (Stargrass hay; *Cynodon nlemfuensis*) up to 30% dry matter (DM) did not affect rumen pH value.

The NH_3 concentration in this study ranged from 13.12 to 14.23 mg/100 mL, which were enough to support microbial protein synthesis. NH_3 concentrations were obtained when supplementation *Acanthopora muscoides* (L.) Bory was above the level limiting *in vitro* ruminal microbial growth (5 mg/100 mL) as stated by Satter and Slyter [22]. The supplementation of *Acanthopora muscoides* (L.) 7.5.0% OM also produced the highest microbial protein synthesis which could be attributed to the mineral content of *Acanthopora muscoides* (L.) Bory contained sulfur and phosphorus minerals at 0.17% DM and 0.11% DM [14]. The high microbial protein synthesis is important to increase feed degradation in the rumen. According to Pathak [23], vitamins and minerals particularly the minerals phosphorus and sulfur, which are crucial for microbial growth—can boost the synthesis of proteins in microorganisms. Methionine and cysteine synthesis require sulfur [24], while rumen microbes use phosphorus for ATP synthesis and protein synthesis [20] Pathak [23] added that microbiological growth may be restricted by a deficiency in phosphorus stock. Rajauria et al. [25] stated that one such natural product is macroalga, also known as seaweed, which is rich in antioxidants, polyphenols, protein, minerals, and vitamins.

Table 2. *In vitro* pH, NH_3, and rumen microbial protein concentration due to *Acanthopora muscoides* (L.) Bory supplementation with different levels on *Pennisetum purpureum* cv. Gama Umami

Treatments	pH	NH_3 (mg/100 mL)^{ns}	Rumen microbial protein (mg/100 mL)
P1	$6.86^{a} \pm 0.05$	13.12 ± 0.20	$63.11^{a} \pm 0.25$
P2	$6.91^{b} \pm 0.05$	14.23 ± 0.91	$65.88^{b} \pm 1.23$
P3	$6.91^{b} \pm 0.04$	14.09 ± 1.29	$67.86^{bc} \pm 1.25$
P4	$6.92^{b} \pm 0.05$	13.86 ± 0.68	$69.62^{c} \pm 2.99$

Different superscripts in the same column show very significant differences ($P<0.01$); ns: non significant;
P1: *Pennisetum purpureum* cv. Gama Umami;
P2: P1+ *Acanthopora muscoides* (L.) Bory 2.5% OM;
P3: P1+ *Acanthopora muscoides* (L.) Bory 5% OM;
P4: P1+ *Acanthopora muscoides* (L.) Bory 7.5% OM

The different responses reported by Park et al. [26], which using green (*Ulva* sp.) and brown (*Sargassum horneri*) seaweed that has been bloomed in Korea. The result showed that inclusion of 4% *Ulva* sp. decreased rumen NH_3 concentration and total volatile fatty acid production. Meanwhile, the total volatile fatty acid production did not affect with inclusion of *S. horneri*, but decreased rumen NH_3. The different result migh be due to different species that have different bioactive compound.

4. Conclusion

Supplementing *Acanthopora muscoides* (L.) Bory 7.5% OM on *Pennisetum purpureum* cv. Gama Umami as basal diet, which is the best treatment in this study to increased rumen fermentation characteristics.

References

1. Food and Agriculture Organization, The global status of seaweed production, trade and utilization (Food and Agriculture Organization of the United Nations, Rome, 2018)
2. E. Erniati, F.R. Zakaria, E. Prangdimurti and D.R. Adawiyah. Acta Aquatica. **3**, 12-17 (2016)
3. Badan Pusat Statistik, Ekspor rumput laut dan ganggang lainnya menurut negara tujuan utama, 2012-2022 (2024).
4. W. Soetjipto, R. Adriansyah, R.A.Q. A'yun, T. Setiadi, H. Susanto, A. Solah, U. Hasan, U. Khaerawati, C. Aryshandy, L. Moriansyah, N.D. Purnama, S. Wahyuni, E. Horida and I. Kurnia, Peluang usaha dan investasi rumput laut (Direktorat Usaha dan Investasi Ditjen Penguatan Daya Saing Produk Kelautan dan Perikanan Kementerian Kelautan dan Perikanan, Jakarta, 2019)
5. 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)
6. M. MacMonagail, L. Cornish, L. Morrison, R. Araújo, and A.T. Critchley. Sustainable harvesting of wild seaweed resources. Eur. J. Phycol. **52**, 371-390 (2017)
7. S. Kraan. Seaweed resources, collection, and cultivation with respect to sustainability. In *Sustainable Seaweed Technologies* (Elsevier Inc., 2020).
8. N. Kasanah, D.S. Seto, H. Khotimah, and Triyanto, Rumput laut pangan (Universitas Gadjah Mada Press, Yogyakarta, 2022)
9. 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)
10. R.D. Kinley, G. Martinez-Fernandez, M.K. Matthews, R. de Nys, M. Magnusson, and N.W. Tomkins. J. Clean Prod. **259**, 120836 (2020)
11. A.A. Mihaila, C.R.K. Glasson, R. Lawton, S. Muetzel, G. Molano, and M. Magnusson. Appl. Phycol. **3**, 274-284 (2022)
12. D. Pandey, M. Mansouryar, M. Novoa-Garrido, G. Næss, V. Kiron, H.H. Hansen, M.O. Nielsen, and P. Khanal. *Nutritional and anti-methanogenic potentials of macroalgae for ruminants. In: Lei, X. (Ed.), Seaweed and Microalgae as Alternative Sources of Protein*. (London: Burleigh Dodds Science Publishing, 2021)
13. D. Mayberry, H. Bartlett, J. Moss, T. Davison, and M. Herrero. *Agric Syst.* **175**. 13-21 (2019)

14. N. Hidayah, C.T. Noviandi, A. Astuti, and Kustantinah. JAVAR. **10**, 751-762 (2023)
15. N. Hidayah, C.T. Noviandi, A. Astuti, and Kustantinah. Nusantara Biosci. **16**, 37-42 (2024)
16. A.L. Chaney and E.P. Marbach EP. Clin. Chem. **8**, 130-132 (1962).
17. D. Plummer, An introduction to practical biochemistry, 3rd Edition (Mc. Graw-Hill Book Company Publishing, New Delhi, 1967)
18. B.G. Cottyn and C.H.V. Boucque. J. Agric. Food Chem. **16**, 105-107 (1968)
19. Y. Zheng, S. Xue, Y. Zhao, and S. Li. Animals **10**, 893 (2020)
20. R. Franzolin and B.A. Dehority. RBZ. 39, 2262-2267 (2010)
21. L.A. Canul-Ku, J.R. Sanginés-García, E.A. Urquizo, J.R. Canul-Solís, I.A. Valdivieso-Pérez, E. Vargas-Bello-Pérez, I. Molina-Botero, J. Arango, and Á.T. Piñeiro-Vázquez. Animals **13**, 1858 (2023)
22. L.D. Satter and L.L. Slyter. Br. J. Nutr. **32**, 199-208 (1974)
23. A.J.V.W. Pathak. Vet. World. **1**, 186-189 (2008)
24. National Research Council, Nutrient requirements of dairy cattle. 7th ed. (National of Sciences Press, Washington, 2001)
25. G. Rajauria, B. Foley, and N. Abu-Ghannam. Food Res. Int. (2017) **99**, 995–1001
26. K.Y. Park, Y.H. Jo, J.G. Nejad, J.C. Lee, H.G. Lee. Algal Res. **65,** 102706 (2022)