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Effect of *Palisada perforate* (Bory) K.W.Nam Dietary Supplementation on Goat Nutrient Digestibility, Nitrogen Balance and Fermentation Characteristics

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ABSTRACT

In this study, a red macroalgae species, *Palisada perforate* (Bory) K.W.Nam, was processed as a supplement *total mixed ration* (TMR), which was used to evaluate the nutrient digestibility, nitrogen balance, and rumen fermentation characteristics of goat. For this purpose, the impact of different levels of *P. perforate* (Bory) K.W.Nam supplementation were compared: *P. perforate* (Bory) K.W.Nam supplements at 0.0, 2.5 and 5.0% organic matter (OM). Twelve Kacang goats (body weight 16.85-31.80kg) were used in this study and grouped in a complete randomized block design with three treatments and four replications. The results showed that nutrient intake and digestibility (except crude protein) increased ($P<0.05$) with *P. perforate* (Bory) K.W.Nam supplementation at 2.5-5.0% OM. Adding 5.0% OM *P. perforate* (Bory) K.W.Nam on the basal diet also increased ($P<0.05$) nitrogen balance. Supplementing that seaweed at 5.0% OM increased ($P<0.01$) total VFA production, propionate proportion, and microbial protein synthesis. That treatment also decreased ($P<0.01$) acetate proportion followed by acetate propionate ratio, and methane calculation without affecting NH_3 concentration and pH value still in normal range. It is concluded that the total mixed ration supplemented with *P. perforate* (Bory) K.W.Nam 5.0% OM resulted in the highest nutrient intake and digestibility, nitrogen balance, VFA total, propionate proportion, and microbial synthesis protein. This treatment also resulted in the lowest acetate proportion, acetate propionate ratio, and methane prediction but did not affect NH_3. This combination has the potential to increase ruminant productivity.

Key words: Red seaweed, *Palisada perforate* (Bory) K. W. Nam, Digestibility, Small ruminant

INTRODUCTION

Macroalgae, also called seaweed, are marine autotrophic organisms that are classified into three categories based on pigmentation: brown seaweed (Phaeophyta), red seaweed (Rhodophyta) and green seaweed (Chlorophyta) (Min et al. 2021). Dini (2023) stated that seaweed generates nutrients like protein, carbohydrates, etc. as well as non-nutritive molecules such as dietary fibers and secondary metabolites that can enhance their physiological processes. Seaweeds are rich in minerals because of their ability to take in inorganic materials from their environment, they are rich in polysaccharides but only contain trace amounts of lipids, primarily polyunsaturated fatty acids (PUFAs) (Morais et al. 2020). Compounds from seaweed that have antibacterial, antiviral, antioxidant and anti-inflammatory properties include polysaccharides, fatty acids, peptides, terpenoids, pigments, and polyphenols (Min et al. 2021). Seaweed's composition is highly variable and is influenced by various factors such as species, habitat, length of collection, light intensity, and nutrient concentration in the water (Misurcova 2011). Dominguez (2013) revealed that genetics (species) and environment (e.g., location, nutrient abundance, salinity, and light) affect the seaweed metabolite composition diversity. These compositions have been proven to have an anti methanogenic, antiparasitic, and antioxidant properties, which are responsible for increasing the health and productivity of livestock (Pereira 2018; Angulo et al. 2020; McGurrin et al. 2023).

Morais et al. (2020) stated that green and red seaweed generally have higher nutrient value than brown seaweed, and red seaweed has abundant protein content (El-Beltagi et al. 2022). The largest varieties of seaweed most commonly found are green and red seaweed, while brown seaweed is mostly found in cold and warm temperatures (Kasanah et al. 2022). Indonesia is a tropical nation with the highest species diversity in the world (Erniati et al. 2016)

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and the second-largest seaweed production rate in the world (38.7%) after China (47.9%) (FAO, 2018). Many Indonesian seaweed species are still unexplored, especially as ruminant feed (ingredient and or additive). Eight species of tropical seaweeds from coastal areas in Gunungkidul District, Yogyakarta Province, Indonesia, were evaluated for their production of methane *in vitro* study and secondary metabolites by Hidayah et al. (2023) and Hidayah et al. (2024). The outcome demonstrates that the secondary metabolites of red tropical seaweed species, *Palisada perforate* (Bory) K.W. Nam, have potential to be used as additives to lower methane production in ruminants. Additionally, Hidayah et al. (2024) mentioned that *P. perforata* (Bory) K.W. Nam is a common seaweed species in coastal regions. The *in vitro* study shows that *P. perforata* (Bory) K.W. as supplementation on a basal diet (grass based and TMR ration based) is effective in decreasing methane production. However, there have been no *in vivo* studies regarding *P. perforate* (Bory) K.W. Nam supplementation as a goat feed additive. Therefore, in this experiment, we want to determine how supplementing *P. perforata* (Bory) K.W. Nam to basal diets affects goat nutrition digestibility, nitrogen balance, and fermentation characteristics.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Ethics Committee of the Faculty of Veterinary Medicine UGM Yogyakarta, number: 143/EC-FKH/Eks./2024.

Experimental animals and diets

This study was conducted during the period from January to June 2024. The animals included in the study were 12 Kacang goats and aged 2-3 years. These goats were divided into 3 treatment groups (n=4 per group) based on their live weight (LW), as follows: 26.96±4.10kg (control group), 25.16±4.15kg (2.5% organic matter (OM) *P. perforate* (Bory) K.W.Nam supplemented group), and 23.84±5.26kg (5.00% OM *P. perforate* (Bory) K.W.Nam supplemented group). Goats were kept in individual pens equipped with feeding and water troughs.

The base daily diet for the goats consisted of 60% Napier grass (*Pennisetum purpureum* cv. Gama Umami) and 40% concentrate (TMR) on a dry matter (DM) basis. Daily feed and drinking water were provided *ad libitum*, and the diet consisted of a 60:40 ratio of Napier grass to concentrate, based on DM. The TMR ingredients and chemical composition are shown in Table 1. This diet was a control treatment and two treatments with supplementation of *P. perforate* (Bory) K.W.Nam at 2.5 and 5.0% OM. The *P. perforate* (Bory) K.W.Nam was collected from Gunungkidul, Yogyakarta, Indonesia in October 2022. Seaweed samples were spread out on the bamboo shelf and allowed to dry under the roof, which was partially exposed to sunlight for the shade drying method (25-30^{o}C for 4 days) after being rinsed with water to remove sand and other dirt. The dry samples were grounder in a Willey mill with a 2mm screen until to a fine powder form. Moreover, this *in vivo* experiment was conducted for 60 days, 14 days for adaptation, and 46 days for treatment period.

Nutrient intake and digestibility determination

During the collection period, which lasted 14 days, each animal's sample feed (given and refused) and faces were collected and weighed every 24 hours after morning feeding. Those samples were oven dried at 55^{o}C for 96 h, weighed, and grounded to pass a 2mm screen using a Willey mill for proximate analysis (AOAC 2005). The following nutrients were measured: DM (dry matter), EE (ether extract), CF (crude fiber), CP (crude protein), NFE (nitrogen free extract), and TDN (total digestible nutrient). Feed intake was calculated by subtracting amount of given and refused feed per animal. The nutrient digestibility was determined based on feed intake minus total feces, all calculated on a DM basis (Sallam et al. 2023).

Nitrogen balance determination

During the collection period (14 days), urine samples were taken and measured from each animal every 24 hours after morning feeding. Urine was taken in individual buckets. Before beginning the urine collection process, 100mL of sulfuric acid was added to each bucket to prevent ammonia loss. A pH indicator strip non-bleeding pH 0–6.0 (MColorpHast™, Merck, Germany) was used to measure the acidity of urine every day. The feed intake, faces, and urine samples were analyzed for crude protein according to the AOAC (2005) method. The nitrogen value was calculated by dividing crude protein by 6.25. Nitrogen (N) balance was calculated based on the difference between the total N in feed intake and the total N in feces and urine (Sinz et al. 2018). The feed N content multiplied by the feed intake yielded the total N intake. The retained N value was calculated by multiplying the total volume of daily feces and urine by the N content of those materials. This yielded the total N in feces and urine.

Fermentation characteristics analysis

Samples for fermentation characteristics (pH, VFA, NH_3, and rumen microbial protein) were taken from rumen

Table 1: Chemical compositions of substrate experiment on dry matter basis with TMR supplemented *Palisada perforate*

Ingredients	%	Chemical Composition *Total Mixed Ration*	DM %
Pennisetum purpureum cv. Gama Umami grass	60		
Concentrate	40		
Cassava by product	20.25		
Wheat pollard	45	OM	85.35
Soybean meal	4.25	EE	2.06
Coconut cake meal	14.5	CF	27.52
Corn gluten feed	10.00	CP	10.58
Molasses	5	NFE[1]	45.19
Premix	1	TDN[2]	58.62

Organic Matter (OM), Crude Fiber (CF), Crude Protein (CP), Extract Ether (EE), Nitrogen-free Extract (NFE), Total Digestible Nutrient (TDN); [1] NFE = 100 – (%CP + %CF + %EE + %ash); [2] TDN = 70.6 + 0.259 CP + 1.01 EE – 0.76 CF + 0.091 NFE (Sutardi 1980).

fluid from three goats for each treatment, on final days in total collection periods. A vacuum pump was attached to one of two flexible polyvinyl chloride tubes, each with a diameter of approximately 3 and 1cm, to collect rumen fluid samples from each goat's esophagus before morning feeding. The tubes were used to extract roughly 50mL of rumen fluid. Collected samples of rumen fluid were filtered through three layers of gauze and pH was measured after filtering using a pH meter (Hanna pH-meter portable, Hanna Instruments, USA). VFA profile (acetate, propionate and butyrate) was determined using gas chromatography (GC 2010 Plus, Shimadzu Crop., Kyoto, Japan, HP-FFAP column (50.0m × 0.20mm × 0.30μm) and FID detector) according to Cottyn and Boucque (1968) method. Ammonia determination was done according to the method described by Chaney and Marbach (1962) with a UV-vis microplate spectrophotometer at a wavelength of 750nm and a standard curve ($(NH_4)_2$ SO4, Merck) (y = 0.028018x - 0.01108, r^2 = 0.99). Microbial protein measurements were carried out according to the method described by Plummer (1967) using a UV-vis microplate spectrophotometer at a wavelength 630nm and standard curve (BSA/Bovine Serum Albumin, Sigma) (y = 2.5075x + 0.0824, r^2 = 0.99). Methane gas calculation using two prediction equations was tested by Williams et al. (2019). The following two equations were used to predict the methane yield (MY): (1) MY=4.08×(acetate/propionate) + 7.05 and (2) MY = 316/propionate + 4.4.

Experiment design and data analysis

Twelve Kacang goats were used in this study and arranged in a complete randomized block design with three treatments and four replications. The body weight is blocked due to a CV of >10%, which might affect nutrition consumption. All other data were statistically analyzed using ANOVA and the differences between treatments were assessed using Duncan's multiple range test. All analyses were performed using the SPSS software and data are presented as mean±SEM.

RESULTS

Nutrient intake and digestibility

Supplementation of *P. perforate* (Bory) K.W.Nam at 2.5-5.0% OM on TMR as a basal diet increased (P<0.05) goat nutrient intake and digestibility (except crude protein). The nutrients consisted of total dry matter, organic matter, ash, ether extract, crude fiber, nitrogen free extract and total digestible nutrients, which are shown in Table 2-4.

Nitrogen balance

Adding 5.0% OM *P. perforate* (Bory) K.W.Nam on the basal diet also increased (P<0.05) nitrogen balance, but was not able to increase nitrogen intake, absorbed, and retained in the animal body (Table 5).

Fermentation characteristics

Total VFA production improved (P<0.01) with *P. perforate* (Bory) K.W.Nam supplementation at 5.00% OM (P<0.01). However, this high VFA production did not negatively affect the rumen pH condition, which ranged from 6.74 to 6.96. This treatment also significantly improved (P<0.01) propionate proportion and decreased acetate proportion, which resulted in the lowest acetate to propionate ratio and methane prediction (P<0.01). Table 6 showed that supplementing *P. perforate* (Bory) K.W.Nam at 5% OM on TMR resulted in the highest (P<0.01) microbial protein synthesis and did not affect the NH_3 concentration (P>0.05).

Table 2: Nutrient intake (g/kg $BW^{0.75}$) in Kacang goats with TMR supplemented *Palisada perforate*

Variable	Control	Control+2.5 % OM	Control+5.0% OM	P value
DM	88.49±22.08[a]	117.22±5.18[b]	119.98±11.19[b]	0.03
EE	1.98±0.46[a]	2.56±0.13[b]	2.65±0.21[b]	0.02
CF	24.26±6.04[a]	32.28±1.53[b]	32.45±2.83[b]	0.03
CP	9.11±2.77	12.21±0.66	12.87±1.31	0.07
NFE	40.04±9.57[a]	52.91±2.24[b]	54.47±5.21[b]	0.03
TDN	52.04±13.06	68.80±3.03	71.01±6.78	0.03

Different superscripts in the same rows show significant differences (P<0.05); Dry Matter (DM), Extract Ether (EE), Crude Fiber (CF), Crude Protein (CP), Nitrogen-free Extract (NFE), Total Digestible Nutrient (TDN).

Table 3: Nutrient digestibility (g/kg $BW^{0.75}$) in Kacang goats with TMR supplemented *Palisada perforate*

Variable	Control	Control+2.5% OM	Control+5.0% OM	P value
DM	73.25±16.29[a]	99.49±5.31[b]	102.11±12.56[b]	0.02
EE	1.75±0.36[a]	2.24±0.11[b]	2.32±0.22[b]	0.02
CF	20.07±4.25[a]	27.36±1.79[b]	27.45±3.23[b]	0.01
CP	7.88±2.44	10.97±0.58	11.80±1.76	0.07
NFE	33.70±7.19[a]	45.30±2.33[b]	46.75±5.42[b]	0.02
TDN	43.34±9.93[a]	58.67±2.84[b]	60.88±7.62[b]	0.02

Different superscripts in the same rows show significant differences (P<0.05); Dry Matter (DM), Extract Ether (EE), Crude Fiber (CF), Crude Protein (CP), Nitrogen-free Extract (NFE), Total Digestible Nutrient (TDN).

Table 4: Percentage of Nutrient digestibility in Kacang goats with TMR supplemented *Palisada perforate*

Variable	Control	Control+2.5% OM	Control+5.0% OM	P value
DM	83.18±3.06	84.87±2.48	84.94±2.81	0.68
EE	88.58±3.41	87.56±1.86	87.48±2.05	0.81
CF	83.26±3.44	84.75±2.91	84.43±2.84	0.82
CP	86.36±2.47	89.80±0.88	91.36±4.82	0.18
NFE	84.54±3.11	85.61±2.45	85.72±2.37	0.83
TDN	83.60±2.91	85.29±2.22	85.56±2.83	0.61

Different superscripts in the same rows show significant differences (P<0.05); Dry Matter (DM), Extract Ether (EE), Crude Fiber (CF), Crude Protein (CP), Nitrogen-free Extract (NFE), Total Digestible Nutrient (TDN).

Table 5: Nitrogen balance in Kacang goats with TMR supplemented *Palisada perforate*

Variable	Control	Control+2.5 % BO	Control+5.0 % BO	P Value
		------g of N/$BW^{0.75}$------		
N intake	1.46±0.44	1.96±0.10	2.06±0.21	0.07
N Excretion				
N feces	0.20±0.06	0.20±0.02	0.17±0.08	0.83
N urine	0.008±0.003	0.008±0.003	0.005±0.001	0.22
N balance	1.25±0.39[a]	1.75±0.10[a]	1.88±0.28[b]	0.05
N output		------% of N intake------		
Absorbed	86.36±2.47	89.80±0.88	91.36±4.82	0.18
Retained	85.83±2.67	89.38±0.98	91.15±4.85	0.18

Different superscripts in the same rows show significant differences (P<0.05); Nitrogen (N).

Table 6: Fermentation characteristics and methane prediction in Kacang goats with TMR supplemented *Palisada perforate*

Variable	Control	2.5% BO	5.0% BO	P value
Fermentation Characteristics				
pH	6.74±0.11[a]	6.96±0.12[b]	6.92±0.09[b]	0.02
VFA total (mM)	96.61±3.01[a]	99.46±5.49[a]	116.74±3.74[b]	0.00
Acetate (%)	75.08±2.09[b]	70.40±2.93[a]	70.87±0.70[a]	0.02
Propionate (%)	17.41±1.73[a]	19.54±1.07[ab]	21.08±1.33[b]	0.03
Butyrate (%)	7.52±0.70	10.06±1.89	8.05±1.64	0.11
Acetate / Propionate	4.35±0.55[b]	3.62±0.35[a]	3.37±0.20[a]	0.02
NH_3 (mg/100 mL)	13.57±1.85	13.55±1.45	12.10±1.99	0.43
Microbial protein synthesis (mg/100 mL)	277.89±31.42[a]	279.89±34.77[a]	362.30±36.62[b]	0.02
Methane prediction (g CH_4/kg DMI)				
MY 1	24.81±2.23[b]	21.81±1.44[a]	20.81±0.83[a]	0.02
MY 2	23.37±2.32[b]	20.75±1.48[b]	17.28±0.79[a]	0.00

Different superscripts in the same rows show significant differences (P<0.05); Volatile Fatty Acid (VFA); Dry Matter Intake (DMI) Williams et al 2019 (g CH4/kg DMI); MY = 4.08 × (acetate/propionate) + 7.05; MY = 316/propionate + 4.4.

DISCUSSION

This study aimed to test 2 levels of inclusion on a basal diet (TMR, 60:40 ratio of Napier grass to concentrate) of one red seaweed species, *P. perforate* (Bory) K.W.Nam at 2.5-5.0% OM compared with a control diet. The study wants to assess the effect on nutrient intake and digestibility, nitrogen balance and rumen fermentation characteristic parameters (pH value, total and partial VFA, NH_3 concentration, microbial protein synthesis, and methane prediction) of Kacang goats. To the best of our knowledge, this is the first study in which the supplementation tropical red seaweed species, *P. perforate* (Bory) K.W.Nam with different levels, were tested on Kacang goat.

The inclusion of *P. perforate* (Bory) K.W.Nam up to 5% OM increased (P<0.05) nutrient intake (total dry matter, ether extract, crude fiber, and nitrogen free extract), except for crude protein and total digestible nutrients intake. This indicates that *P. perforate* (Bory) K.W.Nam supplementation up to 5% OM on TMR as a basal diet with no refusals did not reduce its palatability, so increased the nutrient intake. A study by Rjiba-Ktita et al. (2019) showed that the inclusion of green seaweed up to 400 g/kg in concentrate has different sheep feed intake responses on different species, which are not affected by *Ulva* and decreased when *Chaetomorpha* was added. Nyloy et al. (2023) reported that supplementation of red seaweed, *Asparagopsis taxiformis* at 0.25% OM on TMR (composed of 35% concentrate feed and 65% grass silage on a DM basis) was significantly lower on dry matter intake compared to the control treatment in lactating Norwegian Red dairy cows. The different responses showed that seaweed: species, flavor (taste, smell and texture) and amount added could be attributed in part to ruminant palatability. Ginane et al. (2011) explained that all five basic tastes (sweet, bitter, salty, sour, and umami) are sensed by lingual receptors in ruminants, including sheep, cattle, and goats. The flavor that has a high positive hedonic value is umami, which elicits the most agreement in preferences. In contrast, sweet tastes appear to have a positive value in cattle and goats but not in sheep. Salty tastes can be either positive or negative depending on the needs of the body. The bitter taste appears to have a rather negative hedonic value. Last but not least, it's unclear what the sour taste is worth.

The umami and salty taste of *P. perforate* (Bory) K.W.Nam contributed to increasing the goat's nutrient intake. Fan et al. (2023) explained that taste modifiers can indirectly affect grazing behavior by controlling the amount of livestock feed intake. Glutamate is known to have a significant impact on the acceptability, palatability, and flavor of foods with umami taste in humans (Ginane et al. (2011). Matsumoto (2015) stated that monosodium glutamate (MSG) in seaweeds, inosine 5′-monophosphate (IMP) in meat and fish, and guanosine 5′-monophosphate (GMP) in mushrooms all contribute to the distinct flavor known as umami, which is the Japanese word is deliciousness. The umami taste indicates the presence of proteins in the substance (Ginane et al. 2011) and ingredients include peptides and amino acids (Jensen et al. 2022). Milinovic et al. (2021) explained that seaweed can be a rich source of glutamate, an umami compound, but the amounts can vary greatly depending on the type of seaweed, where it comes from, how it is stored, and how it is extracted. Whereas, the salty taste indicates the presence of minerals (Ginane et al. 2011). Hidayah et al. (2024) reported that *P. perforate* (Bory) K.W.Nam contains minerals higher than 30%, as large as 47.15% DM and crude protein at 16.05% DM. Seaweeds' high mineral content gives them a naturally salty taste (Jensen et al. 2022). Munoz and Diaz (2020) explained that seaweed may contain up to ten times more minerals than terrestrial plants. High amounts of different minerals in salt water, where seaweed is found, are the cause of this condition. A study by Guda (2018) explained that salty and sweet treatments can improve forage palatability. The salty and sweet treatments used in grasslands increase the bites number per minute, time spent at each feeding station, and the opposite effect when bitter taste agents are applied (Fan et al. 2023).

The increased nutrient digestibility (except the crude protein digestibility) with *P. perforate* (Bory) K.W.Nam supplementation might be due to the secondary metabolites (tannin) and mineral content (sulfur and phosphor) enhancing the microbial protein synthesis (Table 6) and not decreasing palatability. Tannins contribute to the decline in protozoa following the rising rumen bacteria population, so feed degradation and digestion are increasing. Protozoa population in the rumen that is either free or attached to methanogenic archaea, can be decreased by tannin and phlorotannin (Piñeiro-Vázquez et al. 2015). Newbold et al. (2015) explained that to obtain protein, protozoa engage in

predatory behaviour towards rumen bacteria. Besides that, the bitter taste of tannin from this treatment can still be tolerated by goats so it does not interfere with their palatability. Tanin compounds are commonly associated with the organoleptic properties of astringency and bitterness (Soares et al. 2020). This condition is because goats have a higher tolerance for bitterness than sheep or cattle. Hofmann (1989) reported that goats have a habit of eating dicotyledonous plants, which are rich in cell content, and also repeatedly produce bitter-tasting secondary compounds, so the goats came across bitterness flavor more frequently than sheep or cattle. Ginane et al. (2011) stated that compared to other mammals, fewer genes coding for the bitter receptors are found in cows, which means they are less tolerant of this taste.

In this research, tannin also protected protein from rumen microbial degradation, so the protein digestibility did not show an increase. Tannins are natural protectors of protein in ruminants, which form stable complexes with proteins in the rumen and are unstable in the abomasum (Getachew et al. 2000; Rodríguez et al. 2014). Polyphenols (like tannin) slow down the rumen's ability to break down protein and fiber by keeping microbes from attaching to them (Makkar 2003). Tannins can form complexes with protein and fiber fractions in the rumen, which decreases the degradability of both of them (Muir 2011). This result is linear with an NH_3 concentration that does not increase (Table 6) with supplementation of *P. perforate* (Bory) K.W.Nam up to 5.0% OM, which indicates that the CP in this ration was not more degraded by rumen microbial population. Hidayah et al. (2024) reported the tannin content of *P. perforate* (Bory) K.W.Nam at 0.76 mg/g DM.

Meanwhile, minerals, particularly sulfur and phosphorus, which are crucial for microbial growth can boost the protein microbial synthesis (Pathak 2008). This mineral contributed to increasing feed degradation and digestion. *P. perforate* (Bory) K.W.Nam in this research contains sulfur and phosphorus of 0.81 and 0.09% DM. This mineral percentage can increase the protein microbial synthesis and is safe for palatability. In the study reported by Terry et al. (2023), the inclusion of 1 or 2% red seaweed (*Mazzaella japonica*) on TMR decreased the OM digestibility of mature beef heifers. *M. japonica* is relatively high in sulfur at 6.46% and phosphorus at 0.16% (Terry et al. 2022). Nasem (2016) declared that the majority of ruminants need between 0.18 and 0.24% DM of dietary sulfur. Meanwhile, the inclusion rates of *M. japonica*>2%, may raise concerns due to its high sulfur concentration, particularly when combined with other feed ingredients like distillers' grains or high sulfur concentration water (Terry et al. 2023).

Supplementing *P. perforate* (Bory) K.W.Nam at 5% OM tended to increase ($P<0.1$) nitrogen (N) intake, did not differ ($P>0.05$) N excretion (feces and urine), and increased ($P<0.05$) N balance. This is a good result because N excretion did not increase with seaweed supplementation, which will minimize environmental pollution and enhance ruminant productivity. This condition might be due to the *P. perforate* (Bory) K.W.Nam supplementation that contains tannin, which made the CP in the ration not more degraded by rumen microbial. Besides that, the degradation product from dietary CP by rumen microbes (NH_3) was used for ruminal microbial protein synthesis, so the N urine was not increased. This result is linear with the microbial protein synthesis data, which increased with 5% OM supplementation of of *P. perforate* (Bory) K.W.Nam (Table 6). Terry et al. (2023) explained that ruminal microbes break down some dietary CP into NH_3-N, amino acids, and peptides in the rumen. NH3-N serves as the main source of nitrogen for ruminal microbial protein synthesis. Similar results were reported by Belanche et al. (2016) and Terry et al. (2023). Adding 5% of brown seaweed (*Laminaria digitata*) to the basal diet (50:50 forage-to-concentrate ratio) improved 9.9% of the microbial protein synthesis efficiency per unit of degradable OM (Belanche et al. 2016). In the study by Terry et al. (2023), supplementation up to 2% *M. japonica* on TMR (barley silage at 52%, barley straw at 44%, and vitamin and mineral supplement at 4% on a DM basis) as a basal diet linearly ($P<0.001$) increased the heifer N intake. The seaweed supplementation also linearly increased ($P=0.020$) fecal N excretion and did not affect total urinary N excretion, N fractions (allantoin, uric acid), total purine derivatives, microbial purine derivatives absorbed, microbial N flow, or retained N.

Adding *P. perforate* (Bory) K.W.Nam 2.5-5% OM on TMR increased pH value compared to the control treatment (6.75 vs 6.91-6.97). But, the seaweed supplementation did not disturb the rumen environment, because the pH values of all treatments were still in the normal range. Normal pH values can range from 5.50 to 7.50 depending on the type of feed and how often it is fed (Zheng et al. 2020). The highest in total VFA and propionate production was when TMR supplemented with *P. perforate* (Bory) K.W.Nam at 5% OM. This treatment also had the lowest acetate and acetate propionate ratio. This result indicated higher propionate production compared to acetate, which means higher energy savings for production. Linear results showed higher microbial protein synthesis and lower methane prediction (Table 6) which could contribute to the sulfur and phosphorus content of *P. perforate* (Bory) K.W.Nam.

The *in vitro* study showed that supplementation of *P. perforate* (Bory) K.W.Nam decreased ($P<0.01$) protozoa population followed by low methanogenic archaea and increased the number of rumen bacteria and VFA production. Tannin *P. perforate* (Bory) K.W.Nam contributed to a decline in protozoa, which are symbiosis with methanogenic archaea, so decreased methanogenic archaea followed by reduced methane production. Scalbert (1991) also reported that methanogenic archaea are toxic to tannin monomers like pyrogallol, gallic acids, and tannic acids. Meanwhile, the lower methane prediction indicated more hydrogen is used in forming propionate than methane. Wang et al. (2023) explained that rumen metabolic hydrogen primarily through methanogenesis to forming methane and propionate synthesis. Theoretically, a promising strategy for lowering greenhouse gas emissions from ruminants could involve diverting hydrogen from methanogenesis to propionate formation, which would also likely increase animal productivity. Whereas, the NH_3 concentration did not decrease when the basal diet added *P. perforate* (Bory) K.W.Nam up to 5% OM. The ranged NH_3 concentration from 12.16 to 13.45 mg/100 mL was sufficient to support the microbial protein synthesis

process. According to Schwab et al. (2005), depending on the circumstances surrounding fermentation, the ideal range of ammonia-N for bacterial growth is 5–11 mmol/L.

Conclusion

The total mixed ration supplemented with *Palisada perforate* (Bory) K.W.Nam 5.0% OM resulted in the highest nutrient intake and digestibility, nitrogen balance, VFA total, propionate proportion, and microbial synthesis protein. This treatment also resulted in the lowest acetate proportion, acetate propionate ratio, and methane prediction but did not affect NH_3 concentration. This combination has the potential to increase ruminant productivity.

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