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Nutrients content of four tropical seaweed species from Kelapa Beach, Tuban, Indonesia and their potential as ruminant feed

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Abstract. *Kustantinah, Hidayah N, Noviandi CT, Astuti A, Paradhipta DHV. 2022. Nutrients content of four tropical seaweed species from Kelapa Beach, Tuban, Indonesia and their potential as ruminant feed. Biodiversitas 23: 6191-6197.* Indonesia is one of the tropical countries with the largest diversity of seaweed, but studies on identifying the nutrients content of numerous seaweed species from tropical oceans and their potential as ruminant feed have not been widely reported. This study was designed to evaluate the nutrient, macro and micromineral content of brown (*Laminaria* sp. and *Padina australis*) and red seaweed (*Eucheuma cottonii* and *Gracilaria* sp.) from Kelapa Beach, Tuban, East Java. All tests were carried out in duplicate from individual seaweed and data were analyzed descriptively by calculating the average of data obtained. The result showed that all of the seaweed species in this experiment had a high content of water (69.41-86.33%) and ash/mineral (23.42-65.63%). The brown seaweed *Padina australis* had the highest dry matter (30.59%) and crude protein (12.57%). The red seaweed of *Gracilaria* sp. had the highest ash (65.63%) and the lowest organic matter (34.37%), ether extract (0.21%), crude fiber (2.49%), nitrogen-free extract (19.95%), neutral detergent fiber (21.74%), acid detergent fiber (5.46%), and gross energy (1,083 Cal/g); however, in contrast, *Eucheuma cottonii* had the highest organic matter (76.58%), ether extract (2.85%), crude fiber (8.80%), nitrogen-free extract (56.38%), neutral detergent fiber (56.93%), acid detergent fiber (11.44%), and gross energy (2,911 Cal/g) but the lowest dry matter (13.67%) and crude protein (8.55%). The brown seaweed *Padina australis* had the highest Na (0.89%), Mg (4.90%), and Cd (3.73 ppm) contents. The red seaweed of *Gracilaria* sp. had the highest K (2.18%), Ca (5.80%), Fe (7,596 ppm), Mn (818 ppm), Cu (5 ppm), Zn (52 ppm), and Pb (60.38%); however, in contrast, *Eucheuma cottonii* had the lowest P (0.01%), K (2.02%), Ca (0.64%), Mg (1.09%), Fe (400 ppm), Mn (38 ppm), Pb (17.31 ppm), and Cd (1.53 ppm) but the highest S (0.46%). Based on this study, brown seaweed (*Laminaria* sp and *Padina australis*) and red seaweed (*Eucheuma cottonii* and *Gracilaria* sp.) from Tuban Regency, East Java, Indonesia have the potential as ruminant feed, especially as soluble carbohydrates and an organic mineral source that requires attention for heavy metals to prevent toxicity to ruminants.

Keywords: Chemical composition, macro minerals, microminerals, ruminant feed, tropical macroalga

INTRODUCTION

Ruminants have an important role in the provision of food and the sustainability of agricultural production systems. Ruminant livestock products provide essential nutrients for humans, including amino acids, fatty acids, vitamins, and minerals. However, the production of ruminants must be accompanied by good feeding management to increase livestock productivity. Nowadays, the animal feeding sector is interested in using seaweed as a supplement in ruminant feed. Seaweed is a macroalga that belongs to the Tallophyta division which has a body skeleton structure in the form of a stem (thallus) and does not have leaves and roots. Brown et al. (2014) stated that seaweed has novel bioactive compounds that improve health-promoting properties that are not found in terrestrial plants. Several studies conducted in Europe, Australia, America, New Zealand, Korea, and Africa reported that seaweed is very efficient to increase the productivity of ruminants. The secondary bioactive compounds of alga have antiemthanogenic abilities (Roque et al. 2019). Alga contains protein, carbohydrates, fats, vitamins, minerals, oils, amino acids, and other secondary compounds (phlorotannin, iodine, and halogenated compounds), which are beneficial for livestock health (Gaillard et al. 2018; Pirian et al. 2017). Besides that, seaweed are very efficient in reducing methane emissions in ruminants due to the content of their secondary metabolites, especially halogen compounds and phlorotannin (Choi et al. 2021; Kinley et al. 2020). Halogen compounds such as bromoform are antimethanogenic compounds that can inhibit the methyl-coenzyme reductase (MCR) enzyme in the methanogenesis process (Allen et al. 2014). Pandey et al. (2021) reported that ruminants such as cattle, goats, and sheep contribute to methane production by 17% of the total anthropogenic emissions, where enteric methane (CH_4) is produced from the feed fermentation process in the rumen. Enteric fermentation produces carbon dioxide (CO_2) and hydrogen (H_2) gas, which play an important role in CH_4 formation in the reduction pathway by archaea microbes during the methanogenesis process (Broucek 2014). The effort to reduce ruminant methane emission is important to do because methane emission has two negative effects: it contributes to greenhouse gas production which has a global potential 28 times that of carbon dioxide (IPCC 2014) and reduces ruminant production because approximately 3-12% of the digested energy from the feed is lost as methane) as stated by Mayberry et al. (2019)

Indonesia is a tropical country with abundant seaweed availability (amount and species diversity) and has the potential to be used to increase ruminant productivity. The diversity of seaweed in Indonesia is the largest compared to other countries (Erniati et al. 2016). The expedition carried out by Van Bosse in the Siboga Sea in 1899-1900 reported that Indonesian waters had a source of seaweed germplasm of about 555 species from about 8000 types of seaweed in the world and could grow well in the territory of Indonesia (Soetjipto et al. 2019). Regarding to their pigmentation, seaweed is divided into three different groups, namely green seaweed (Chlorophyta), brown seaweed (Phaeophyta), and red seaweed (Rhodophyta) (Min et al. 2020). Indonesia has become one of the world's major producers of seaweed. Indonesian waters are in accordance with the biological needs and growth of seaweed, making Indonesian seaweed of good quality (high nutrient like as polysaccharides, protein, amino acids, polyunsaturated fatty acids, macro and micro minerals). Fresh seaweed production in Indonesia in 2018 reached 10.32 million tons (KKP 2018). Meanwhile, the export value of fresh seaweed in 2020 reached 177 thousand tons (BPS 2021). Tuban Regency is one of the cities coasts in the North Coast (Pantura) area of East Java which has a beach length of 65 km that has the potential of natural seaweed sources.

There are limited studies about the nutrient, macro and micromineral content of tropical seaweed species, especially from Tuban Regency, Indonesia. Therefore, there is a need to study the potential of tropical seaweed from Tuban Regency, East Java, Indonesia with different species as an alternative for ruminant feed. The objective of this study was to identify the nutrient, macro and micromineral content of four tropical algal species (*Laminaria* sp., *Padina* australis, *Gracilaria* sp., and *Eucheuma cottonii*) from Indonesia, especially on the north coast of Java Island Tuban Regency.

MATERIALS AND METHODS

Study area

The natural seaweed species used in this research were collected from Kelapa Beach, Tuban, East Java, Indonesia, in April 2022 (Figure 1). There were two species of brown seaweed (*Laminaria* sp. and *Padina* australis) and two species of red seaweed (*Gracilaria* sp. and *Eucheuma cottonii*) for sample in this study (Figure 2).

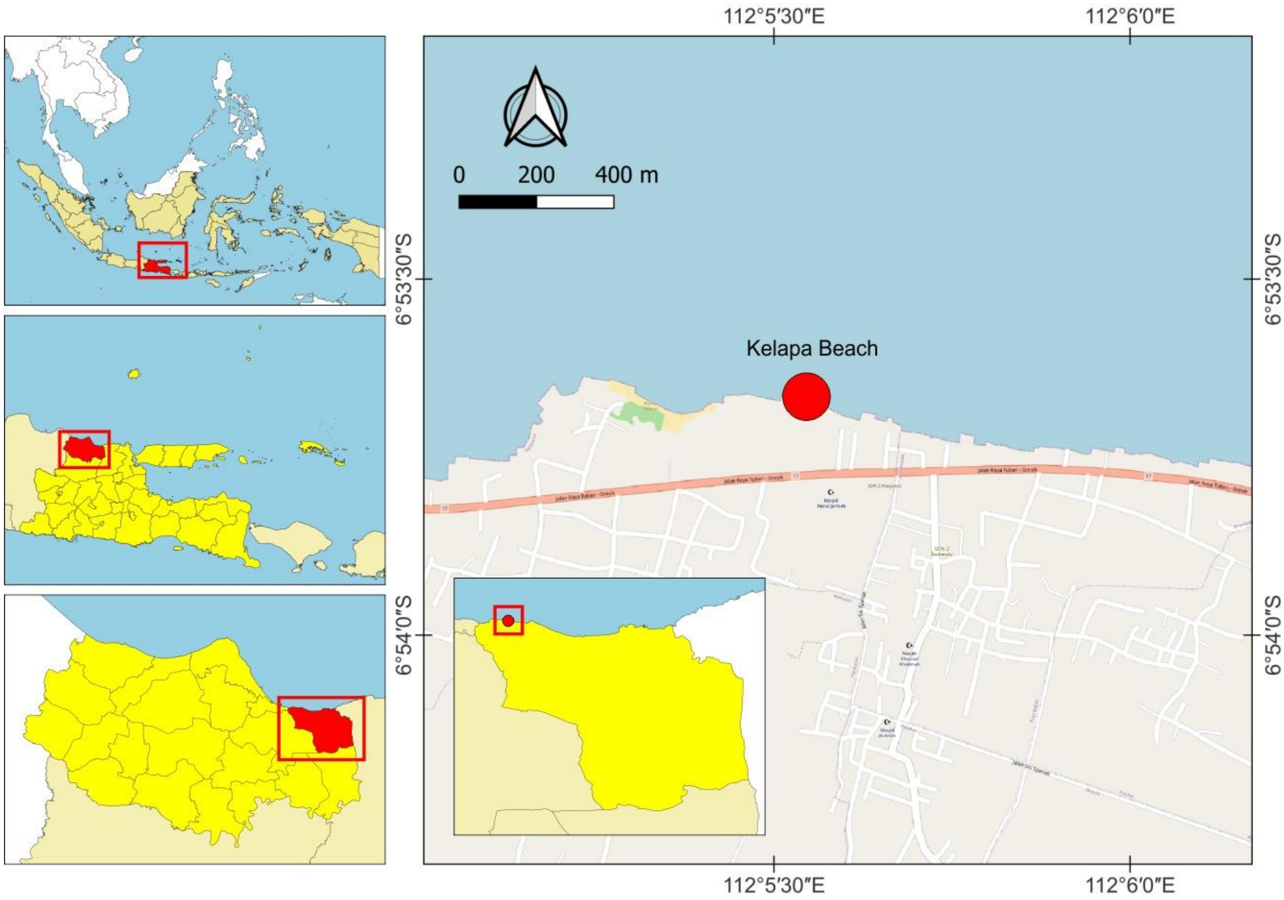

Figure 1. Location of the survey (Tuban District, East Java Province, Indonesia)

Figure 2. Tropical seaweed species (A. *Padina* australis; B. *Gracilaria* sp.; C. *Laminaria* sp.; D. *Eucheuma cottonii*)

Preparation of sample

The seaweed consisted of 4 species (*Laminaria* sp., *Padina* australis, *Gracilaria* sp., and *Eucheuma cottonii*) were cleaned with fresh water and dried under the sun (average 25-30ºC) for 5 days until they reached a constant weight. After that, all the algae were ground using hammer mill to a fine powder with 80-100 mesh size.

Nutritional component analysis

The chemical analysis using dry sample 4 species seaweed included proximate analysis: dry matter (DM), ash, organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), and nitrogen free extract (NFE)) measured according to AOAC (2005); Van Soest analysis: neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al. 1991); gross energy was determined with a bomb calorimeter (Parr, 6400 Calorimeter), macro- and micromineral contents were determined using an atomic absorption spectrophotometer (AA-610S Shimadzu). All tests were carried out in duplicate from individual algae.

Data analysis

Data were analyzed descriptively by calculating the average of data obtained

RESULTS AND DISCUSSION

Chemical composition

All of seaweed species in this experiment had a high content of water (69.41-86.33%) and ash/mineral (23.42-65.63%). The dry matter (DM) and crude protein (CP) contents of brown seaweed (DM: 24.54 and 30.59%; CP: 12.28 and 12.57%) were higher than those of red seaweed (DM: 13.67 and 22.58%; CP: 8.55-11.72%). Seaweed have less than 3% ether extract, and the result is linear with the gross energy. The brown seaweed *Padina australis* had the highest DM (30.59%) and CP (12.57%). The red seaweed of *Gracilaria* sp. had the highest ash (65.63%) and the lowest OM (34.37%), EE (0.21%), CF (2.49%), NFE (19.95%), NDF (21.74%), ADF (5.46%), and gross energy (1,083 Cal/g); however, in contrast, *Eucheuma cottonii* had the highest OM (76.58%), EE (2.85%), CF (8.80%), NFE (56.38%), NDF (56.93%), ADF (11.44%), and gross energy (2,911 Cal/g) but the lowest DM (13.67%) and CP (8.55%) (Table 1).

Ahmad et al. (2016) stated that seaweed consist of the most abundant moisture. The moisture content of 15 seaweed from Semporna, Sabah, Malaysia was 75.95-96.03%. As reported by Sofiana et al. (2020), fresh *Euchema spinosum* from the coastal area of Lemukutan Island, Indonesia, consists of water at 85.43%. The water content is similar for almost all kinds of seaweed. Even though they come from different places, the water content is similar as it is common for products that come from a water environment to have a high water content. All seaweed species in this research had lower DM content than King grass used as the basis of a substrate *in vitro* evaluation (26.51%), except for *Padina australis* (30.59%). The percentage of crude protein in this research was lower than that reported by Min et al. (2021), and the crude protein of red seaweed was 18-38% and higher than that of brown seaweed. This condition is due to the different species, harvesting times, and habitats of the seaweed. Galland-Irmouli et al. (1999) reported that the protein content in seaweed is influenced by seaweed type and season period; the highest protein content is obtained during winter and spring, while the highest is recorded in summer. The crude protein content was reported by some researchers based on the dry matter, and the crude protein content of *Laminaria* sp. from sub-tropical coastal waters at 9.8-16.6% (Min et al. 2021), *Padina australis* from Kelapa Lima coastal waters, Kupang Bay, East Nusa Tenggara, Indonesia at 13.39% (Salosso et al. 2020), *Gracilaria* Sp. from Kampung Berangbang, Banten Regency, West Java, Indonesia at 9.36% (Purwaningsih and Deskawati 2020), *Eucheuma cottonii* from Kalianda beach, South Lampung, Indonesia at 5.33% (Widiawati and Hikmawan 2021) and from the coastal areas of North Borneo, Malaysia at 9.76% (Matanjun et al. 2008).

Winarni et al. (2021) stated that generally, the fat/ether extract content of seaweed is 1-3%. The ether extract was reported by some researchers. Based on the dry matter, the ether extract of *Laminaria* sp. at 0.80% (Min et al. 2021), *Padina australis* at 2.66% (Salosso et al. 2020), *Gracilaria* sp. at 0.60% (Purwaningsih and Deskawati 2020), *Eucheuma cottonii* at 2.77% (Widiawati and Hikmawan 2021) and at 1.10% (Matanjun et al. 2008). The result of the ether extract is linear with the gross energy result, in which the seaweed with the highest ether extract will have the highest energy content. Abbott et al. (1988) stated that for calculating dietary intakes, fat/ether extract has the highest caloric (9 kcal/g) estimates than carbohydrate (4 kcal/g) and protein (4 kcal/g).

Table 1. Chemical composition and gross energy of four tropical seaweed species (%DM)

Chemical composition	Brown seaweed		Red seaweed	
	Laminaria sp.	*Padina australis*	*Gracilaria* sp.	*Eucheuma cottonii*
Water content (%)	75.46	69.41	77.42	86.33
DM (%)*	24.54	30.59	22.58	13.67
OM (%)*	43.27	58.56	34.37	76.58
Ash (%)*	56.73	41.44	65.63	23.42
CP (%)*	12.28	12.57	11.72	8.55
EE (%)*	0.93	0.37	0.21	2.85
CF (%)*	4.98	6.31	2.49	8.80
NFE (%)*	25.08	39.31	19.95	56.38
NDF (%)	29.48	22.75	21,74	56.93
ADF (%)	8.13	6.90	5,46	11.44
Hemicellulose (%)	21.35	15.85	16.28	45.49
Gross energy (Cal/g)*	1,575	1,896	1,083	2,911

Note: 1) proximate analysis (DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract, CF: crude fiber, NFE: nitrogen-free extract, *Hidayah et al. (2022)). 2) van soest analysis (NDF: neutral detergent fiber, ADF: acid detergent fiber, hemicellulose, IMT Laboratory, Animal Science Faculty, UGM, Yogyakarta (2022)

The gross energy content is higher than that of tropical marine macroalga (red and brown) from intertidal reefs around Townsville, QLD, Australia, at approximately 696-1,185 kcal/g (Dubois et al. 2013) because of different seaweed species. This is indicated that tropical seaweed from Tuban Regency more potential as an alternative energy source for ruminant.

Seaweed have less than 10% crude fiber (2.49-8.80%) and more nitrogen-free extract (19.95-56.38%). This is because algae store their food reserves in the form of carbohydrates, especially polysaccharides (Winarni et al. 2021). Makkar et al. (2016) stated that carbohydrates in brown seaweed are not starch but are stored as a laminarin (laminaran) with a polysaccharide of glucose. However, the polysaccharides of red seaweed similar to starches that do not contain amylose. The crude fiber in this research is higher than the crude fiber reported by Matanjun et al. (2008) 5.91% dry weight. Siddique (2013) reported that environmental conditions (salinity, water transparency for the synthesis of NFE, and nutrient uptake) are one factor that affects crude fiber levels. The crude fiber content of seaweed is similar to that of cereal grains (corn, barley, triticale, and oat at 2.90-11.94% DM) reported by Marin et al. (2016). The NDF, ADF, and hemicellulose of seaweed were lower than those of 226 forage plants in South Sulawesi, Indonesia, both grasses and legumes (NDF: 66.62-76.39%, ADF: 29.94-43.41%, and hemicellulose: 28.98-33.98%, except *Eucheuma cottonii* at 45.49% (Nasrullah et al. 2004). The NDF content is similar to that reported by Dubois et al. (2013) on marine macroalga (red and brown) intertidal reefs around Townsville, QLD, Australia (tropical seaweed) at approximately 12.66-54.94%.

All seaweed have more than 20% of mineral content, so potential as a mineral source for ruminants can be as mineral supplementation or replacement of tropical grass for ruminant feed. Circuncisao et al. (2018) stated that the mineral content of seaweed is more 10-100 times that of land vegetables. Tropical grass deficiencies in macrominerals are compared with the requirements of grazing and lactating dairy cattle (Costa and Leigh 2014). Nasrullah et al. (2004) reported that the ash content of forage plants in South Sulawesi, Indonesia, and 266 samples of grasses and legumes that were collected from the highland and lowland from 1998 to 2000 in the dry and rainy seasons were less than 10%. The ash content in seaweed (macrominerals and trace elements) is higher (20-50% DM) than that in land vegetables (Holdt and Kraan 2011; Pereira 2016). Similar result with high mineral content reported by some research, the mineral content (based on the dry matter) of *Padina australis* at 34.58% (Salosso et al. 2020), *Gracilaria* sp. at 24.83% (Purwaningsih and Deskawati 2020), and *Eucheuma cottonii* at 46.19% (Matanjun et al. 2008). Factors affecting the mineral content of seaweed are their age and capability to absorb inorganic substances from the environment (Rubio et al. 2017; Siahaan et al. 2018). Siahaan et al. (2018) stated that green and red seaweed have a lower absorption rate to uptake minerals than brown seaweed due to the polysaccharides in the brown algae cell walls containing alginic acid, alginate, and salt alginic acid.

Brown seaweed (*Laminaria* sp. and *Padina australis*) and red seaweed (*Eucheuma cottonii* and *Gracilaria* sp.) from Tuban Regency, East Java, Indonesia have potentials as ruminant feed. They are available in high quantity (amount and variety) and quality. In terms of nutritional value, brown and red seaweed have high mineral content potentially as an organic mineral source that can be used as an alternative mineral supplement on ruminant feed. Also high in soluble carbohydrates that can support the growth of ruminant rumen microbial to increase the feed degradability that is linear with high productivity. Based on the chemical composition, *Eucheuma cottonii* has the highest organic matter, which is 1.5-2x higher than other seaweeds in this study. The OM of *Eucheuma cottonii* was dominated by EE, NDF, NFE, and hemicellulose which reflected high energy. Quickly available energy was described by NFE (56.38%) and not quickly available energy was described by hemicellulose (45.49%) that in a balanced condition, so there is no need to worry become toxic to ruminants when the ruminant only feed with *Eucheuma cottonii*.

Macro and micromineral composition

The brown seaweed *Padina australis* had the highest Na (0.89%), Mg (4.90%), and Cd (3.73 ppm) contents. The red seaweed of *Gracilaria* sp. had the highest K (2.18%), Ca (5.80%), Fe (7,596 ppm), Mn (818 ppm), Cu (5 ppm), Zn (52 ppm), and Pb (60.38%); however, in contrast, *Eucheuma cottonii* had the lowest P (0.01%), K (2.02%), Ca (0.64%), Mg (1.09%), Fe (400 ppm), Mn (38 ppm), Pb (17.31 ppm), and Cd (1.53 ppm) but the highest S (0.46%) (Table 2). All of the seaweed in this research were sufficient macro (P, K, Na, Ca, Mg, and S) and micro (Fe and Mn) minerals for ruminant (beef, dairy, and sheep) requirements (Table 2).

The use of seaweed for ruminants is not enough for Cu and Zn requirements, so it is necessary to add other mineral sources. Careful attention should be given paid to the number of seaweed used in ruminant feed to prevent toxicity to livestock. The National Research Council (NRC 2005) suggested that the maximum tolerable dietary levels of K for sheep and cattle be 2%. Clinical signs of K toxicosis include cardiac insufficiency, edema, muscle weakness and death (NRC 1980). The maximum tolerance levels for Ca and P are for cattle (1.5%; 0.7%) and sheep (1.5%; 0.6%). Excess of either of these minerals may cause bone disorders and reduce feed consumption and gain. Cattle and sheep should be able to tolerate 0.6% Mg (NRC 2005). Excess Mg causes diarrhea, depressed nutrient utilization and progressive degeneration of the stratified squamous epithelium of ruminal papillae (Chester-Jones et al. 1990). The National Research Council (NRC 2005) suggested maximum tolerable dietary levels of S for cattle and sheep at 0.3% with a high concentrate diet or 50% high forage diet. Most livestock species have a high tolerance for Fe. Cattle can tolerate Fe and Mn at 500 and 2000 ppm, respectively (NRC 2005).

This seaweed also contains heavy metals (Pb and Cd), which need attention. This condition indicated high concentrations of heavy metals in Tuban East Java ocean water. The range of heavy metal levels in livestock feed from FDA CVM CY15-17 was 0-1.4 ppm for Cd and 0-12.2 ppm for Pb (Deemy 2019). Masters et al. (1999) stated that Cd and Pb in grazing mammals have a tendency to accumulate in the liver and kidneys and in bone for Pb. The National Research Council (NRC 2005) suggested a maximum tolerance of Cd at 25 ppm DM in the diet for a few days. A study by Lane et al. (2015) reported that impaired reproduction in dairy cows occurred with long-term exposure to low levels of Cd. However, ruminants have a tolerable level of Pb at 250 ppm (Chester-Jones et al. 1990). Blood and Henderson (1968) stated that the most commonly affected by Pb poisoning are ruminants. Pb particles have a tendency to settle in the reticulum and be converted to soluble Pb acetate by the acidic medium from the forestomach. The toxicity of Pb can cause diarrhea, anorexia, decreased animal performance, and weight loss (NRC 2005).

Supplementation of 0.1 kg DM seaweed (composed of Sea Lettuce (*Ulva rigida*) (as flakes, 80%), Japanese Wireweed (*Sargassum muticum*) (flakes, 17.5%), and Furbelows (*Saccorhiza polyschides*) (powder, 2.5%)) from the Galician coast, Spain, on concentrate 4.4 kg DM on Holstein Friesian lactating cows, improved the mineral Fe status on feed and the heavy metal (Cd and Pb) concentrations in milk did not statistically differ from the control (no poisoned in cattle) (Rey-Crespo et al. 2014). The postpartum phosphor concentration on blood serum dairy cow increased with the addition of calcareous marine seaweed on diet as a replacement of limestone at 0.42% and 0.47% DM (Neville et al. 2022). Similar result reported by Boccardo et al. (2022), calcareous marine seaweed (CMA) obtained from *Lithothamnium calcareum* had the faster peak of blood total Ca concentration compared to calcium carbonate (CC) and calcium propionate (CP) on dairy heifers. This result indicated that CMA can be replaced CC and CP for oral Ca supplementation for dairy.

Table 2. The mineral content of four tropical seaweed species (%DM) and the mineral requirement for ruminant

Mineral	*Laminaria* sp.	*Padina australis*	*Gracilaria* sp.	*Eucheuma cottonii*	Beef* (all classes)	Dairy* (lactating)	Sheep* (all classes)
Macrominerals							
P (%)	0.12	0.13	0.13	0.01	0.17-0.59	0.31-0.40	0.16-0.38
K (%)	2.09	2.04	2.18	2.02	0.60-0.70	1.00-1.04	0.50-0.80
Na (%)	0.76	0.89	0.76	0.81	0.06-0.08	0.19-0.23	0.09-0.18
Ca (%)	3.66	3.07	5.80	0.64	0.16-1.53	0.43-0.60	0.20-0.82
Mg (%)	2.38	4.90	1.48	1.09	0.10-0.20	0.18-0.21	0.12-0.18
S (%)	0.20	0.36	0.30	0.46	0.15	0.20	0.14-0.26
Microminerals							
Fe (ppm)	5710	4553	7596	400	50	15-43 (growing)	30-50
Mn (ppm)	498	590	818	38	20-40	14-22 (all classes)	20-40
Cu (ppm)	4	4	5	4	10	9-11 (all classes)	7-11
Zn (ppm)	44	28	52	21	30	47-55 (all classes)	20-33
Pb (ppm)	52.15	44.58	60.38	17.31	-	-	-
Cd (ppm)	3.52	3.73	3.36	1.53	-	-	-

Note: 1) mineral analysis (P: phosphor, K: potassium, Na: sodium, Ca: calcium, Mg: magnesium, S: sulfur, Fe: ferrum, Mn: manganese, Cu: cuprum, Zn: zinc, Pb: lead, Cd: cadmium, BPTP Yogyakarta (2022); (2) * NRC (1980).

Brown seaweed (*Laminaria* sp. and *Padina australis*) and red seaweed (*Eucheuma cottonii* and *Gracilaria* sp.) were sufficient macro (P, K, Na, Ca, Mg, and S) and micro (Fe and Mn) minerals for ruminant requirements with need requires attention for heavy metals (Pb and Cd) to prevent toxicity to ruminants. For that reason, seaweeds from Tuban Regency, East Java, Indonesia for feed must concerning their content in trace elements. The range of heavy metal levels in livestock feed from FDA CVM CY15-17 was 0-1.4 ppm for Cd and 0-12.2 ppm for Pb.

Based on this study, it is concluded that brown seaweed (*Laminaria* sp and *Padina australis*) and red seaweed (*Eucheuma cottonii* and *Gracilaria* sp.) from Tuban District, East Java, Indonesia have the potential as ruminant feed, especially as soluble carbohydrates and an organic mineral source that requires attention for heavy metals to prevent toxicity to ruminants.

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