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Mineral composition of a sub-tropical seaweed Gracilaria sp collected Muara Gembong, Java Sea, Indonesia

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ABSTRACT

The aims of the research were to find out mineral composition of seaweed Gracilaria.sp macro mineral elements i.e of Ca, Mg, Na, and K, and micro mineral elements Cu, Zn, Fe, Co, I, Mn and Se. Gracilaria.sp was destroyed by dry digestion and wet digestion and mineral content was measured using atomic absorption spectroscopy (AAS). The results showed that dry digestion produced more minerals than wet digestion. Gracilaria.sp contains various minerals, each of which is sodium (3816 ppm), potassium (869.75 ppm), calcium (905 ppm), magnesium (0.231 ppm). For micro minerals the content of Cu(1.06 ppm), Zn(2.16 ppm), Fe(0.678), Co(0.15) and Mn(6.70 ppm). Apart from Gracilaria.sp various other plants such as turi leaves, cassava leaves, Imperata cylindrica and grass also contains macro minerals and micro minerals.

Keywords: Gracilaria.sp, Mineral Content, Atomic Absorption Spectrometry

I. INTRODUCTION

Every day goats need forage as much as 10% of their body weight. Goats have the nature of choosing the feed that is served, so forage needs to be given twice the amount provided in the morning and evening. Some types of forage that are commonly used include: Caliandra calothyrsus (ii) Leucaena leucocephala, elephant grass and seaweed.

The mineral content in forage feeds must be available in sufficient quantities because the animal's body cannot make its own minerals [3]. Minerals are one of the nutritional components that have an important role in the growth, health, production, reproduction and immunity of animals [4], for the synthesis of steroid hormones as well as thyroid hormones [5], and also acts as a regulator in all metabolic processes [6]. Therefore, minerals are needed for the survival of livestock, therefore the mineral content in forage needs to be considered. Cattle at experience mineral deficiencies usually have signs of decreased body weight and milk production in lactating animals, cramps in the tendons, licking of the stomach. lick what's around it for salt.

Young cattle that are deficient in Ca and P minerals experience Rickettsia, while adult cattle experience Osteoditropy which then results in symptoms of

Osteomalacia and deviant eating behavior (biting bones, wood, hair, and other objects). If there is a lack of magnesium, you will experience hypomagnesia and tetany. o Sodium/Na deficiency in the long term will cause lack of appetite, laziness, and allotriophagy, o Micromineral deficiency in the long term also increases the typical symptoms, such as: Hypocobaltosis, causes emaciation, and eating behavior deviates, o Deficiency of Co and Mo, causes diarrhea, skeletal osteoporosis, Zn deficiency, causes parakeratosis (skin eczema in pigs), oMn deficiency in chicks and hens, causes perosis, I deficiency, causes damage to the thyroid gland manifested as goiter and exophthalmia, and Nickel excess, causes eye disease and blindness.

Minerals can be divided into two main groups, namely macrominerals and microminerals. Ruminants need macrominerals Ca, Mg, P, K, Na, Cl and S, while the microminerals needed by ruminants are chromium (Cr), cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn). Although macromineral elements in the body are few in number compared to other nutrients such as protein and fat, macrominerals are closely related to the reproductive ability of ruminants [7, 8], regulation of acid balance, osmotic pressure of the body and being part of the building blocks of the body, such as bones and teeth [9].

One of the plants that can be used as forage for livestock is seaweed. Seaweed is a plant with high ash content and contains minerals Na, K, I and Cl [9]. Despal et al [10]. (2016) reported that the ash content of some seaweeds was more than 50%, while Murakami et al [11] reported that the ash content of one species of seaweed, Sargassum polycystum, was 42.4±0.07%. High ash content was also found by Matanjun et al. [12] on Eucheuma cottonii species. Meanwhile, in Gelidium pusillum, the ash content was found to be lower at 21.15±0.74% [13]. The high ash content can be used as a mineral source for livestock. But the balance of minerals it contains needs to be studied first. The study of the mineral composition of seaweed is not only important to determine its contribution to the fulfillment of livestock needs, but more than that to prevent the occurrence of toxicity. As is known, minerals are micronutrients, meaning that their needs for livestock are only in small amounts. Overfeeding can cause livestock poisoning.

This study was designed to assess the feasibility of using seaweeds from the Galician coast as a source of minerals in organic dairy cattle. The main objective was to evaluate content and solubility of macro minerals (Ca, P, Mg, Na) and micro minerals from several species of seaweed.

II. METHODS AND MATERIAL

A. Materials

Salt mineral eq: $CaSO_4$, $CuSO_4$, $CoCl_3$, $FeCl_3$, I_2, KNO_3, $MgCl_2$, $MnCl_2$, NaCl and

$ZnSO_4$, was purchased from Merck. Stock solution prepared by dissolving salt mineral in aquabidest. All standard solutions were freshly prepared by diluting the stock solution with aquabidest to appropriate concentrations.

Reagent for qualitative test salt mineral are:

Ammonium oxalate, Nitroso R, Excess ammonia, O phenantrolin, Sodium thiosulfate, Tartaric acid, Sodium Hydroxide, Titan yellow, Ammonium phosphate and Dithizon were supplied by Merck.

Instrumentations

Atomic absorption spectrophotometric, hot plate, oven, glassware.

B. Methods

Sample collection

All the samples have been collected from unpolluted locations at Muara Gembong, Bekasi, Indonesia. The average rainfall in this area is 1,697 mm and the highest is in January and February. The west monsoon blows from the north (coast) in early December to February. The topography of the land is flat with a height of 0-5 m above sea level. The sampling area is at coordinates 5º59" South Latitude and 107º68" East Longitude

The collection was made during low tide from upper littoral rocks of above mentioned locations in the third week of September 2019.

Preparation sample

Samples were brought to the laboratory and washed thoroughly with tap water to remove attached epiphytes and adhered dirt particles if any and dried in the shade. The material was kept in the oven at 110 ^{0}C for 12 h, pulverized in the grinder and sieved through a screen with an aperture of 0.5 mm. This powdered material was kept in airtight plastic bottles at room temperature until analysis.

Creating standard curves

Prepare 50 mL of 50 ppm ionic solution. Then a certain amount of the solution was taken and diluted with distilled water to obtain a solution of each maing with concentrations of 0.1 ppm, 0.3 ppm, 0.5 ppm, 0.8 ppm and 1 ppm. Then measure the absorbance value using AAS. Create a standard curve that describes the relationship between absorbance and concentration. The calibration curve is used to determine the ion content in the sample.

Destruction sample

Sample di digester with two methods, namely the wet method and the dry method. Samples were subjected to acid digestion and analyzed according to the procedure described wet digestion method using a wet digester. Into the ashing cup, 1 gram was added, then added nitric acid and 4 mL of aquaregia solvent. Then it was destroyed using a wet digester at a temperature of 350 °C for 1 hour. After that, remove the sample from the wet digester and then cool it. Then added nitric acid and aquaregia then. Analysis of Ca, Mg, Na and K can be tested directly by titration, and minerals using Atomic Absorption Spectrophotometric (AAS).

The second method is the dry destruction method. Seaweed that has been washed is dried in an oven and then in a furnace at a temperature of 420°C for 4-6 hours until it is formed until the ash is white. After cooling, dissolve the ash with 25 mL of aqua regia and heat to dry. Then dissolve it with distilled water and then enter into a 25 mL volumetric flask and diluted with distilled water to the mark. The solution is ready to be analyzed by AAS. All determinations were performed in triplicate and data represented on dry weight basis as mean values ± standard deviation.

Qualitative test

The qualitative test aims to determine the mineral content in the sample solution. Qualitative tests were carried out using specific reagents for certain metal ions. Table 1 below shows the reagents used for the qualitative test

Table 1. Qualitative Analysis mineral on Gracilaria sp

No	Mineral	Reagent
1	Ca^{+2}	Ammonium oxalat
2	Co^{+2}	Nitroso R
3	Cu^{+2}	Excess ammonia
4	Fe^{+2}	O phenantrolin
5	I	Sodium thiosulfate
6	K	Tartaric acid
7	Mg	Sodium Hydroxide + Titan yellow 0,1%
8	Mn	Ammonium phosphat
9	Na	Burning test
10	Zn	Dithizon in acidic condition

Quantitative test

The amount of minerals in microalgae was measured using AAS. For this reason, samples that had been destroyed, diluted with distilled water, and measured with AAS.Before setting and analyzing, the atomic absorption spectrophotometer must first be calibrated using a blank containing the solvent used to dissolve the sample. Calibration with blanko aims so that at zero standard concentration there is no absorption of light so that the standard reading or sample is more precise and accurate. Keep in mind that for AAS, the solvent used must use demine water (demineral water) which is water that does not contain minerals or metals that can interfere with the solution to be made so that it will affect the result itself (inaccurately).

The sample solution from the destruction was pipetted as much as 1 ml into a 200 ml volumetric flask and filled with demineralized water to the mark line (dilution factor = 200 ml/1 ml = 200 times). Then measured the absorbance with AAS at the wavelength according to the ion to be. The absorbance value obtained must be within the range of the calibration curve. The concentration of ions in the sample is determined based on the regression line equation of the calibration curve. The measured wavelengths for measuring the absorbance of each ion are shown in Table 2 below.

Table 2. List of various ionic wavelengths for AAS

No	Mineral	Wave length(nm)
1	Ca^{+2}	422,7
2	Co^{+2}	240,7
3	Cu^{+2}	249,2
4	Fe^{+2}	248,3
5	I	766,5
6	K	766,5
7	Mg	285,2
8	Mn	279,5
9	Na	589,0
10	Zn	13

Data analysis

From the calibration curve we get the equation of the linear line Y = aX+ b

Where Y = sample concentration

X = sample absorbance

B = constant

The ion concentration was determined using the regression equation and calculated using the equation

$$\text{Ions(mg/L)} = \frac{A}{W} \times fp$$

Where

A = concentration of measurement results

W = sample weight

fp = dilution factor

III. RESULTS AND DISCUSSION

Curve standard

From the standard curve of each ion, an inear regression equation is obtained as shown in Table 3 below.

Table 3 Results of Qualitative Analysis on Gracilaria sp

No	Mineral	Persamaan regresi linear	R
1	Ca^{+2}	Y= 0.4578X + 0.00387	0.9967
2	Co^{+2}	Y= 0.2516X + 0.00334	0.9924
3	Cu^{+2}	Y= 0.1998X + 0.00156	0.9927
4	Fe^{+2}	Y= 0.2817X + 0.00223	0.9941
5	I^{-}	Y= 0.3555X + 0.00112	0.9878
6	K^{+}	Y= 0.2658X + 0.00118	0.9927
7	Mg^{+2}	Y= 0.2512X + 0.00285	0.9921
8	Mn^{+2}	Y= 0.1592X + 0.00264	0.9868
9	Na^{+}	Y= 0.3568X + 0.00218	0.9967
10	Zn^{+2}	Y= 0.4578X + 0.00387	0.9807

Destruction

In this study, two methods of destruction were compared to determine the mineral content of Glacilaria sp, namely the wet method and the dry method. Destruction by the wet method is carried out by dissolving the sample in an acid solvent, and drying is done by heating the sample at high temperature until a white ash is formed. The function of destruction is to break the bonds between organic compounds and the metal to be analyzed. Destruction is carried out to decompose the form of metal compounds into inorganic metal forms or the breakdown of compounds into their elements so that they can be analyzed (Kristianingrum, 2012). The results of the average ion concentration in wet digestion and dry digestion are shown in Table 4 below

Table 4 Ion concentrations from wet digestion and dry digestion

No	Mineral	Wet destruction	Dry destruction
1	Ca^{+2}	0.155	0.188
2	Mn^{+2}	0.146	0.164
3	Cu^{+2}	0.196	0.123
4	Na	0.214	0.107
5	K	0.186	0.112

From Table 4 above, it can be seen that with dry destruction, the metal ion content in the destruction was higher than with wet digestion. This is because the dry digestion takes longer, and uses high temperatures, resulting in a purer sample composition and the organic compounds contained in the grass samples are evaporated to become ash. While in wet digestion it is possible for organic compounds to still be present, because the heating temperature is not as high as the furnace temperature

Qualitative Analysis

Qualitative analysis was carried out as a preliminary analysis to determine the presence or absence of minerals in seaweed. Qualitative test data are shown in Table 5

Table 5. Results of Qualitative Analysis on Gracilaria sp

No	Mineral	Reagent	Result
1	Ca^{+2}	Ammonium oksalat	A white precipitate is formed
2	Co^{+2}	Nitroso R	A red solution is formed
3	Cu^{+2}	Ammonia	A dark blue solution is formed
4	Fe^{+2}	O phenantrolin	A red solution is formed
5	I^{-}	Natrium tio sulfate	A blue solution is formed
6	K^{+}	Asam tartarat	A white precipitate is formed
7	Mg^{+2}	Sodium Hydroxide+ Yellow Titan 0,1%	A red precipitate is formed
8	Mn^{+2}	Ammonium phosphat	A pink solution is formed
9	Na^{+}	Burning test	A bright yellow flame is formed
10	Zn^{+2}	Dithizon in acidic condition	A red solution is formed

From Table 5 above, it can be seen that Gracilaria sp contains minerals needed for livestock growth.

Quantitative Analysis of Mineral Content in Gracilaria sp

The concentration of minerals (mg/L) contained in Gracilaria sp is determined based on the regression line equation of the calibration curve of the standard solution for each mineral, then the unit is changed to mg/Kg using the equation. The results of the mineral content analysis in Gracilaria sp are shown in Table 6

Table 6 Mineral concentration in Gracilaria sp

No	Mineral	Concentration(mg/Kg)
1	Ca^{+2}	2.07
2	Co^{+2}	3.82
3	Cu^{+2}	4.07
4	Fe^{+2}	1.45
5	I^{-}	0.88
6	K^{+}	19.38

7	Mg^{+2}	22.07
8	Mn^{+2}	23.82
9	Na^{+}	14.07
10	Zn^{+2}	0.46
11	Ca^{+2}	9.55

Mineral content of various forages

Apart from Gracilaria sp, mineral content tests were also carried out on various plants. Various mineral rich plants, which can be used as forage for goats are shown in Table 7 below

Table 7 Content of macro elements(ppm) in forage

No	Forage	P	S	K	Ca
1	Corn straw	0.26	0.12	3.01	0.32
2	Turi leaves	0.22	0.33	2.49	2.13
3	Cassava leaves	0.18	0.24	1.28	0.74
4	King grass	0.36	0.14	2.79	0.45
5	Puzzle grass	0.80	0.19	6.07	0.25
6	Reed	-	0.25	3.49	0.4
7	Field grass	-	0.12	3.33	0.44

In Table 7, it is shown that in reeds and field grass the concentration of P is not readable. This does not mean that the two plants do not contain P at all, but that the concentration may be below the minimum detection limit of the instrument. The highest concentration of P was found in teki grass, which was 0.80%, while in other plants it was between 0.18 and 0.36%.

Elemental P in plants is usually bound in the form of phytate salts, especially in grains. Phytate salt (phytin-phosphorus) is very difficult for monogasth cattle to digest, because the phytase enzyme in their digestive tract is limited. This does not apply to ruminants (9, 10). In ruminants in the rumen there are microbes that are able to break down phosphorus-phytin, so that the phosphorus contained in these plants can be used by livestock.

Table 8 Content of micro elements (ppm) in forage

No	Forage	Si	Cr	Mn	Fe	Co	Cu	Zn
1	Corn straw	1.14	10	9	1034	27.6	4.92	42.80
2	Turi leaves	-	10	14	423	nd	4.10	46.20
3	Cassava leaves	0.34	nd	62.8	280	nd	316	63.50
4	King grass	2.00	nd	168	1230	nd	568	64.00
5	Puzzle grass	3.09	nd	69	315	nd	560	51.00
6	Reed	2.4	nd	8	682	nd	645	58.20
7	Field grass	3.8	nd	167	1880	nd	415	67.10

The results of the micro-mineral analysis are shown in Table 8. All the elements contained in these plants are needed by livestock for normal growth, as well as rumen microbes. Rumen microbes are very important for ruminants, because they are able to digest crude fiber into useful products for their host. The results of the analysis show that some plants have very low elemental content, namely Cr, Mn, and Co. According to McDonald [14] grasslands with Co concentrations of less than 0.10 ppm can lead to deficiency in doniba and calves. Co deficiency in the long term can reduce the concentration of Co in the liver and kidneys as well as its concentration in the renal fluid. If the concentration of Co in the rumen fluid is less than 0.5 ng/ml, the process for the formation of Vit. B12 by rumen microbes is inhibited (7, 8). So that livestock are not disturbed in the formation of Vit. B12, then in the feed it is necessary to add Co from the outside. Elements of Cu and Zn were found evenly in all plants, ranging from 3.90 to 6.84 ppm and 43.30 to 73.50 ppm. Both of these elements are needed by livestock for normal growth.

IV. CONCLUSION

Gracilaria sp type seaweed, which is often cultivated in Indonesia has the potential to be used as a source of macro minerals, especially Mg and K minerals. The use of seaweed as a different mineral source is highly dependent on the type and location of planting the seaweed. Utilization of seaweed as a mineral source has a limiting factor in the form of a high possibility of toxicity due to high metal content in Indonesian waters. Suggestion Mapping of environmental conditions and types of seaweed on the dominant mineral content in grass needs to be done. This is intended to avoid the possibility of underestimating or overestimating the use of ingredients in dairy cattle rations. This condition is further aimed at achieving optimization of the use of materials to support the achievement of the desired performance and production. Evaluation of the content of other minerals, especially metals, still needs to be done to estimate the safe use of seaweed as a mineral source.

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