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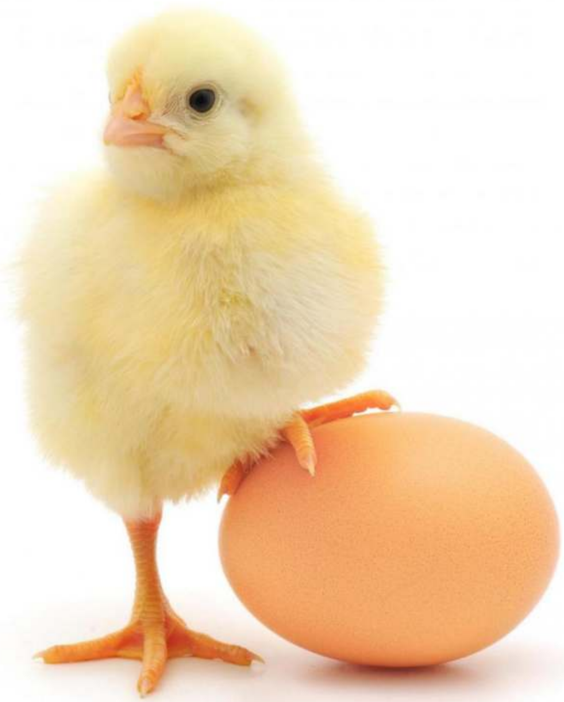

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Research Article
Immersion of *Sargassum binderi* Seaweed in River Water Flow to Lower Salt Content before Use as Feed for Laying Hens

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Abstract
Background and Objective: In certain coastal areas of Indonesia, *Sargassum binderi* drifts to the shore because of ocean waves and because people do not use it, becomes useless waste. This seaweed could potentially be used as feed for laying hens because certain bioactive compounds in seaweed, such as alginate, fucoidan, fucoxanthin and poly-unsaturated fatty acids (PUFA), are useful for poultry health. High salt content is a problem with using *Sargassum binderi* as poultry feed because it causes diarrhea and death in poultry. Therefore, the salt content of *Sargassum binderi* should be reduced before it is fed to poultry. The purpose of this study was to reduce the salt content of *Sargassum binderi* for use as feed for laying hens. **Materials and Methods:** The experiment was arranged in a completely randomized design with *Sargassum binderi* immersed in flowing river water for durations of 0, 3, 5, 7, 9, 11, 13, 15, 19, 21 and 23 h, each treatment was repeated 3 times. The measured variables were salt, crude protein, total dry matter, organic matter and ash. **Results:** The results showed that the different immersion durations of *Sargassum binderi* in flowing river water significantly affected ($p<0.05$) the reduction of salt, total dry matter and ash content and also significantly affected ($p<0.05$) the increase in organic matter and crude protein. **Conclusion:** The immersion of *Sargassum binderi* in flowing river water for 15 h was the best treatment to lower salt, total dry matter and ash and to increase the organic matter and crude protein content.

Key words: *Sargassum binderi*, bioactive compound, immersion in flowing water, feed for laying hens, organic matter

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Seaweed is one of the potential marine resources that is abundant in the ocean near Indonesia. The Ministry of Maritime Affairs and Fisheries[1] reported that the production of seaweed in Indonesia reaches 4,305,027 ton/year. The wide indicative of seaweed in Indonesia's ocean reaches 769,452 ha and the wide effective reaches 384,733 ha[2].

Various species of seaweed are available in Indonesia's ocean. Kadi[3] reported the availability of *Sargassum* species in Selat Sunda and on the South coast of Java to be 5-10 and 5-15 t km^{-2}, respectively. In addition, in the area of Kambuno Island in South Sulawesi, the harvesting of *Sargassum* reaches 33 g m^{-2}, with the amount of biomass reaching 50% and the density reaching 17 g m^{-2} [4]. Furthermore, Mubarak *et al.*[4] described the area at Siburu, Marak and Pisang in Mentawai Islands in West Sumatra Province, where *Sargassum* seaweed harvesting was 5, 5 and 50 g m^{-2}, respectively, with biomass amounts of 3, 3 and 13%, respectively and with densities of 1, 1 and 7 g m^{-2}, respectively.

To date, the utilization of seaweed in Indonesia as an ingredient in food or feed is not optimal. In certain coastal areas in Indonesia, *Sargassum* has been underutilized and is often wasted because the coastal communities are not able to convert the seaweed into an economically valuable product. In the area of Sungai Nipah Beach at Pesisir Selatan District, West Sumatra Province, Indonesia, many *Sargassum* seaweed species were observed. *Sargassum binderi* is a dominant species of seaweed at Sungai Nipah Beach, but there is no report regarding its availability in this area. This seaweed grows and develops naturally without being cultivated by the community. The community at Sungai Nipah Beach could collect as much as 1 t of *Sargassum* seaweed every week to sell to the cosmetics industry[5]. However, since the community in the Sungai Nipah area could not communicate with a seaweed buyer, the *Sargassum* in the Sungai Nipah area was not harvested; it drifted by ocean waves to the shore and became waste.

Sargassum binderi is a species of brown seaweed (Phaeophyceae). According to Mahata *et al.*[5], *Sargassum binderi* from Sungai Nipah Beach contains 6.93% crude protein, 1.07% fat, 7.76% crude fiber, 2179.63 kcal kg^{-1} energy, 0.64% Ca, 0.62% P and 20.89% alginate. Brown seaweeds contain bioactive compounds, such as alginate[5], fukoidan[6,7], fucoxanthin[8,9] and unsaturated fatty acids (PUFA/Poly-Unsaturated Fatty acids)[10]. The bioactive compounds in seaweed have been reported to have functions including lowering cholesterol, acting as antiviral, antibiotic, anti-inflammatory, anti-thrombin, anticoagulation and antilipemic compounds and serving as enzyme inhibitors and stimulants[11]. Previous research reported that alginate and fucoidan in brown seaweed can lower cholesterol in egg yolk[10]. Furthermore, according to Al-Harthi and El-Deek[12], fucoxanthin can lower cholesterol and increase the pigmentation of egg yolk. The fatty acids in seaweed have also been reported to lower cholesterol in egg yolk[10].

The high salt content in *Sargassum binderi* is a problem facing its utilization as poultry feed[5,13,14] because the tolerance of salt content in poultry feed is 0.25-0.5%[15]. Mahata *et al.*[5] reported that *Sargassum binderi* contains as much as 17% salt. According to Mahata *et al.*[5], high salt content in poultry feed causes diarrhea and death. Therefore, a process for lowering the salt content of *Sargassum binderi* is needed before it can be fed to poultry.

The salt content of seaweed can be reduced by washing with water[16-24]. In a previous study, salt content in *Sargassum binderi* decreased from 17.23-14.89%[25] after washing with water but was still not optimal for poultry feed. Immersing the seaweed in flowing water is more effective at lowing the salt content compared to the washing method. Martin *et al.*[26] described that one of the factors that affects a solid substance's solubility is the intensity of the stirring; low stirring causes passive flow, high stirring causes turbulence and the centrifugal force of the rotation pushes the particles up and down.

There is little information regarding reducing the salt content of *Sargassum binderi* by immersion in flowing river water or concerning the effects of this treatment on nutrient content. The primary purpose of this study is to reduce the salt content of *Sargassum binderi* for use as feed for laying hens.

MATERIALS AND METHODS

Collection of *Sargassum binderi*: The seaweed *Sargassum binderi* was collected by a simple random sampling method at Sungai Nipah Beach, Pesisir Selatan District, West Sumatra, Indonesia. Whole individuals of *Sargassum binderi* (thallus, bladder and holdfast) were used in this experiment.

Immersion of *Sargassum binderi* in flowing river water: As much as 200 g of fresh *Sargassum binderi* was immersed in flowing river water at Sungai Gunung Nago, Kecamatan Pauh, Padang, West Sumatra, Indonesia with a river depth of 1.3 m and a current of 0.6745 m^3 sec^{-1}. The seaweed was immersed in accordance with a duration immersion treatment. At the end of the immersion duration, the seaweed was removed from the river and subsequently dried in an oven at 60°C until its water content was approximately 14%.

Preparation of *Sargassum binderi* samples for salt, crude protein, dry matter, organic matter and ash analysis: After immersion in flowing river water, the samples of *Sargassum binderi* were dried then crushed to a dry powder with blender. Samples were analyzed for salt content, crude protein, dry matter, organic matter and ash.

Experimental design: The experiment was performed in a completely randomized design with 13 different immersion durations (0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23 h) as treatments. Each treatment was repeated 3 times.

Measured variables: Salt content was measured by Kohman's method[27], crude protein, dry matter, organic matter and ash was measured by the method described by AOAC[28].

Data analysis: Data were analyzed statistically by one way ANOVA with a completely randomized design. The difference among treatment means was determined using the Duncan multiple range test (DMRT) ($p<0.05$) according to Steel and Torrie[29].

Analysis of salt content in *Sargassum binderi*: Five grams of dried *Sargassum binderi* powder was placed in a 250 mL glass beaker and 50 mL boiling water was later poured into the beaker and stirred with a spatula for 3 min. The mixture was diluted with 500 mL water in a volumetric flask and filtered using Whatman paper no. 41. Next, 10 mL of this filtrate was placed in a 250 mL Erlenmeyer flask along with 3 mL of K_2CrO_4. The filtrate was titrated with $AgNO_3$; the titration process was stopped when the color of the filtrate turned red. The salt content was calculated by Kohman's method[27].

Analysis of crude protein, dry matter, organic matter and ash: Crude protein, dry matter, organic matter and ash were analyzed by proximate analysis[28].

RESULTS AND DISCUSSION

Effect of immersion time on salt, crude protein, total dry matter, organic matter and ash: The effects of the duration of *Sargassum binderi* immersion in flowing river water on salt, crude protein, total dry matter, organic matter and ash are shown in Table 1 and 2. Processing *Sargassum binderi* with immersion in flowing river water significantly lowered salt, total dry matter and ash content ($p<0.05$) and significantly increased organic matter and crude protein ($p<0.05$).

Salt parameter: The mean salt content of *Sargassum binderi* was significantly affected ($p<0.05$) by different durations of immersion in flowing river water. *Sargassum binderi* shows a linear decrease in salt (NaCl) content along with immersion durations of 0-15 h, but salt content does not show a significant decline after additional immersion from 15-23 h as shown in Table 1. The decrease in salt content seen in this experiment was affected by several factors: immersion duration, water as the medium of immersion and the speed of water flow. Therefore, a long duration of immersion in flowing

Table 1: Effects of treatments on salt content

Immersion duration (h)	Salt (NaCl) (%)
Control (0)	16.86^{a}
1	6.50^{b}
3	4.60^{c}
5	4.37^{cd}
7	3.54^{de}
9	3.11^{e}
11	2.92^{ef}
13	2.28^{f}
15	0.94^{g}
17	1.14^{g}
19	1.04^{g}
21	1.56^{g}
23	1.14^{g}
SEM	0.25

SEM: Standard-error of the mean, means with different superscript at the same column are significantly different ($p<0.05$)

Table 2: Effects of treatments on crude protein, total dry matter, organic matter and ash content

Immersion duration (h)	Crude protein (%)	Total dry matter (DM %)	Organic matter (%)	Ash (%)
Control (0)	5.64^{f}	19.20^{a}	55.41^{f}	41.59^{a}
1	6.84^{e}	12.92^{b}	70.39^{e}	29.61^{b}
3	7.03^{e}	12.35^{bc}	77.06^{d}	22.94^{c}
5	7.12^{e}	12.25^{bc}	77.10^{d}	22.90^{c}
7	6.91^{e}	12.01^{c}	77.82^{d}	22.18^{cd}
9	9.19^{cd}	9.28^{e}	78.53^{c}	21.41^{de}
11	8.85^{d}	9.28^{e}	79.70^{c}	20.30^{ef}
13	8.84^{d}	9.28^{e}	79.92^{c}	20.08^{f}
15	9.16^{cd}	9.28^{e}	81.58^{b}	18.42^{g}
17	9.51^{abc}	10.42^{d}	85.98^{a}	14.02^{h}
19	9.42^{bcd}	9.29^{e}	85.91^{a}	14.09^{h}
21	9.80^{ab}	9.27^{e}	85.12^{a}	14.88^{h}
23	10.04^{a}	9.44^{e}	85.70^{a}	14.23^{h}
SEM	0.19	0.27	0.43	0.43

SEM: Standard-error of the mean, means with different superscript at the same column are significantly different ($p<0.05$)

river water caused a large portion of the salt contained in *Sargassum binderi* to dissolve in water. In accordance with Ari[30], in an ionic solution, such as salt solution, water molecules weaken the ionic bonds of salt, thereby dissolving it as Na and Cl ions. In addition, the speed of water flow in this experiment was 0.6745 m^3 sec^{-1}, which accelerated the dissolution of salt from *Sargassum binderi*. Martin *et al.*[26] reported that one of the factors affecting a solid substance's solubility is the intensity of the stirring; low stirring causes passive flow, while high stirring causes turbulence. The centrifugal force of the rotation pushes the particles up and down.

Crude protein content: Protein contents of *Sargassum binderi* are depicted in Table 2. The protein content of this seaweed increased significantly ($p<0.05$) with increasing duration of immersion in flowing river water. The result of this experiment agrees with the result reported by Martinson *et al.*[31], who indicated that prolonging the immersion of orchardgrass in its vegetative phase in flowing river water increased crude protein content. These researchers also stated that the increase of crude protein in orchardgrass in its vegetative phase was due to indirect mechanisms, where the leaching of other water-soluble nutrients concentrates crude protein levels and affects several minerals, such as potassium, magnesium and phosphorus, which affected the change in crude protein concentration. It was dependent on forage type, maturity and soaking duration. Collins[32] also stated that crude protein of grass and alfalfa, which were treated by immersion for 60 min in cold water, was higher than crude protein in un-treated grass and alfalfa. Other research by Kajihausa *et al.*[33] reported that the protein content of sprouted sesame seed flour increased when soaking in water was prolonged from 8-12 h with crude protein content of 26.09% to 45.64-48.70%, respectively. The increase of crude protein in *Sargassum binderi* in this research was estimated to be influenced by the dissolution of soluble chemicals, such as salt, soluble vitamin and soluble carbohydrates. According to Linsley *et al.*[34], salt is a soluble chemical in water. Lehninger[35] stated that vitamin B and C are soluble in water. Protein is soluble in water, but the protein in seaweed does not dissolve in water because the crude protein in seaweed cell walls is combined with other compounds, such as cellulose, alginate and fucoidan and is difficult to dissolve in water. Kloareg *et al.*[36] reported that cell wall of brown seaweed contains cellulose, alginate, fucoidan and protein. Furthermore, Horn[37] stated that protein of seaweed combines with structural components (alginate, fucoidan and cellulose).

Total dry matter: The mean dry matter of *Sargassum binderi* after treatment by immersion in flowing river water is depicted in Table 2. The decreasing dry matter was influenced by the dissolution of organic and inorganic compounds such alginate and soluble vitamins (vitamins B and C) during immersion processing. The result of this research is in accordance with the results of Martinson *et al.*[38], who reported that immersion duration affected the loss of dry matter in orchard grass hay in its vegetative phase and alfalfa hay due to dissolved carbohydrate content. The results of this research are also supported by Warr and Petch[39], who stated that soaking hay for 12 h caused the loss of dry matter in soluble carbohydrates of as much as 1.5-2%. In addition, the soaking treatment also affected the loss of soluble macrominerals[31,40]. Other research mentioned by Longland *et al.*[41] notes that certain minerals and vitamins are lost during the process of soaking hay. In this experiment, the process of immersing *Sargassum binderi* in flowing river water also dissolved a portion of its salt content. According to Linsley *et al.*[34], salt is a soluble chemical in water.

Effect on organic matter and ash: The mean total organic matter and ash of treated *Sargassum binderi* is depicted in Table 2. The result of this experiment is in accordance with Kajihausa *et al.*[33], who stated that the ash content of sprouted and un-sprouted sesame seed flour was reduced by soaking in water for a duration of 8-16 h. The increasing organic matter and decreasing inorganic matter (ash) content in this research is suspected to be due to the dissolution of salt and other minerals when *Sargassum binderi* is immersed in the water flow. Salt is the most abundant inorganic matter in seaweed; therefore, treated *Sargassum binderi* lost salt, which increased the organic matter and decreased the inorganic matter (ash). According to Linsley *et al.*[34], salt is a soluble chemical in water. In addition, immersion duration also influenced the loss of inorganic matter, such as phosphorus (P), potassium (K) and magnesium (Mg)[38]. This finding agrees with Nsa *et al.*[42], who stated that the reduction of ash content in castor oil seed (*Ricinus communis*) after immersion in water could be attributed to the leaching of mineral elements into the water. Therefore, the processing of *Sargassum binderi* seaweed by immersion in flowing river water for 15 h can be beneficial for breeders. Treated *Sargassum binderi* can be used as poultry feed because this processing can optimally reduce the salt content, does not disturb the nutritional content of the seaweed and is applicative. Such results of this study can be used for processing seaweed for poultry feed and the methods in this study can be further developed to identify technology to reduce the salt content of seaweed with a shorter immersion duration.

CONCLUSION

The immersion of *Sargassum binderi* in flowing river water for 15 h was the best treatment to reduce salt, total dry matter and ash and to increase organic matter and crude protein content.

SIGNIFICANCE STATEMENT

This study discusses the problem with using the seaweed *Sargassum binderi* as poultry feed, its high salt content and shows that it can be optimally reduced with immersion for 15 h in flowing river water. This can be beneficial for breeders who can now use *Sargassum binderi* as poultry feed. We provide new information for the world of farms. This study will help researchers uncover critical areas regarding the problem of *Sargassum binderi* seaweed as poultry feed and its processing, which many previous researchers were not able to explore. Thus, we arrive at a new theory on the immersion of *Sargassum binderi* in flowing river water for 15 h to optimally reduce salt content.

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