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Processing of *Sargassum binderi* Seaweed for Supplementation in Poultry Diet

Yelsi Listiana Dewi[1], Ahadiyah Yuniza[2], Nuraini[2], Kesuma Sayuti[3], and Maria Endo Mahata[2*]

[1]*Department of Livestock Breeding, Faculty of Military Logistics, Indonesia Defense University, Belu, Indonesia*
[2]*Department of Animal Nutrition and Technology, Faculty of Animal Science Andalas University, Padang, Indonesia*
[3]*Department of Agricultural Technology, Faculty of Agricultural Technology, Andalas University, Padang, Indonesia*

*Corresponding author's Email: maria@ansci.unand.ac.id

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ABSTRACT

Sargassum binderi has been potentially used as laying hen feed since it contains bioactive compounds useful for poultry health. In addition, the high alginate content of *S. binderi* has made it inappropriate for the poultry diet. Therefore, the alginate content should be reduced before its use in poultry feed. This study aimed to reduce the alginate of *S. binderi* for use as laying hen feed. The experiment was performed in two phases in a completely randomized design. The first phase included heated *S. binderi* in the autoclave and the second phase entailed the immersion of *S. binderi* in whiting filtrate. The treatments in the physical method contained a control group, and four treatment groups heating for 15, 30, 45, and 60 minutes. The treatments in the chemical method had a control group and four treatment groups with immersion periods of 1, 2, 3, and 4 hours. Each treatment was repeated five times, and the investigated parameters were crude protein, total dry matter, organic matter, ash, and alginate, respectively. The heating durations of *S. binderi* in an autoclave and different immersion periods of *S. binderi* in whiting filtrate did not significantly affect total dry matter, organic matter, ash, alginate, and crude protein. The results of this study showed that physical treatment (heat treatment) and chemical treatments (whiting filtrate immersion) did not have a significant effect on the alginate content, crude protein, ash, dry matter, and organic matter.

Keywords: Alginate, Heating, Laying hen, *Sargassum binderi*, Whiting filtrate

INTRODUCTION

As a tropical region, Indonesian waters have seaweed germplasm resources of 6.42% of the world's total seaweed biodiversity (Nofriya, 2015) with an area of 12.123.383 ha, which is the habitat of seaweed or the largest in the world (Kementerian Kelautan dan Perikanan, 2020). The total seaweed production in Indonesia reached 9.746.946 tons (Fu, 2021). Seaweed is a marine resource that can be developed as a non-conventional feed ingredient for poultry. According to Jacob (2022) and Morais et al. (2020), seaweed can be used as animal feed.

Advantages of seaweed are vitamin, mineral, fiber, and bioactive compound source (Mahata et al., 2015; Reski et al., 2022), sea instead of arable land-based production, and high productivity in terms of biomass produced per unit of surface area (Buschmann et al., 2017). According to Mahata et al. (2015), *S. binderi* seaweed contains 6.93% crude protein, 7.76% crude fiber, 20.89% alginate, 1.07% crude fat, 0.64% Ca, and 0.62% Phosphor. Brown seaweed is reported to contain bioactive compounds, such as alginate (Sanjeewa et al., 2017; Pereira and Cotas, 2020), fucoidan (Thanh et al., 2013; Laeliocattleya et al., 2020; Ponce and Stortz, 2020), fucoxanthin (Muradian et al., 2015; Zhang et al., 2015; Seo et al., 2016; Sulistiyani et al., 2021), and polyunsaturated fatty acids (PUFA; Carrillo et al., 2012; Siahaan et al., 2018). This bioactive substance exhibits stimulant, anti-inflammatory, antiviral, antibiotic, anti-thrombin, anticoagulant, hypocholesterolemic, and anti-thrombin actions (Pal et al., 2014; Hakim and Patel, 2020). Nonetheless, the inclusion of seaweed in animal diets is hampered by the high content of ash and poorly digestible carbohydrates (Sharma et al., 2018), high content of salt (Dewi et al., 2018; Reski et al., 2020), low digestibility (Bikker et al., 2016, 2020), limited shelf life (Paull and Chen, 2008; Stevant et al., 2017), and high cost of production (van den Burg et al., 2013). To address some of these disadvantages, seaweed can be immersed in water to reduce the ash (minerals + sand + salt) content outside the leaves (Dewi et al., 2018)

Alginate in brown seaweed is an abundant polysaccharide that reaches 25-45% dry weight (Rinaudo, 2014). The alginate content in poultry rations is limited because it will bind nutrients and inhibit the absorption of food substances in the digestive tract, interfering with the performance of poultry production. However, the right alginate concentration in the ration will trap fat and cholesterol in the digestive tract, then alginate and fat are excreted with the feces to reduce the fat content in the body and eggs of poultry (Dewi et al., 2023). Mushoilaeni et al. (2015) reported that giving alginate 0.75-1% to rats reduced serum cholesterol by 53%.

Processing *S. binderi* seaweed to reduce its alginate content can be done by physical methods, such as heat treatment. Several researchers have carried out this processing method. Processing of seaweed *Sargassum* spp. and *S. dentifebium* with physical methods using heat treatment have been reported by Al-Harthi et al. (2011), El-Deek et al. (2011), and Al-Harthi and El-Deek (2012). Physical methods affect the decrease in viscosity and depolymerization of alginate. According to Mishra (2019), the decrease in alginate viscosity is influenced by temperature, alginate concentration, and heating time. In addition to using physical methods, alginate can also be derived using chemical methods, such as soaking with the whiting filtrate. Mishra (2019) reported that an alkaline solution could degrade alginate. Furthermore, alginate in an alkaline media would be broken down by an elimination reaction catalyzed by OH- to produce 4,5-unsaturated uronic acid. Heating and soaking treatment with whiting water filtrate is easy to do and inexpensive.

The following is information about how processing *S. binderi* seaweed physically and chemically affects the amount of alginate it contains and how this affects the nutritional value of the seaweed. The main objective of this study was the reduction of the alginate content of *S. binderi* seaweed before being used in the poultry diet.

MATERIALS AND METHODS

Preparation of *Sargassum binderi* seaweed samples

The simple random sampling technique was utilized to obtain *S. binderi* seaweed samples from Sungai Nipah Coast, Pesisir Selatan Regency, West Sumatra, Indonesia, which were then composited into one. All parts of this seaweed were taken (talus, bladder, and holdfast). Furthermore, this seaweed was bathed in flowing water for 15 hours to minimize its salt content, and the seaweed was ready for treatment.

Heating *Sargassum binderi*

The *S. binderi* seaweed was soaked in running water for 15 hours, weighed as much as 200 g for each treatment, and then put into a plastic bag. Afterward, it was steamed in an autoclave with a hot steam pressure of 2 atm and a temperature of 121^{o}C. The time of applying hot steam pressure was adjusted according to the length of treatment time. After the procedure was finished, the seaweed was taken from the autoclave and allowed to cool in the open air for 20 minutes before being dried in a 60°C oven until the moisture content was roughly 14%. The current study used a completely randomized design with five treatments containing a control group and four treatment groups heating for 15, 30, 45, and 60 minutes. Each treatment group has five replicates.

The immersion of *Sargassum binderi*

The *S. binderi* seaweed was soaked in flowing water for 15 hours and weighed as much as 200 g for each treatment. This seaweed was immersed in a 0.07% whiting filtrate with a pH of 12. The seaweed was soaked according to the duration of the immersion treatment. After the soaking process was completed, the seaweed was removed from the area and then dried in an oven at 60°C until the water content was approximately 14%. This experiment was conducted using a completely randomized design with five treatments containing a control group and four treatment groups with immersion periods of 1, 2, 3, and 4 hours. Each treatment group has five replicates.

Sample preparation and analysis

After *S. binderi* seaweed was processed by physical (heat treatment) and chemical methods (soaking in whiting filtrate), the dried seaweed was finely ground into powder. After physical and chemical treatment, the seaweed powder was ready to be analyzed for the content of total dry matter, organic matter, ash, alginate, and crude protein.

Parameters

Alginate was analyzed using the method of Dewi et al. (2019). Dry matter, organic matter, ash, and crude protein were assessed using the AOAC method (1990).

Analysis of alginate content

One gram of dried seaweed was soaked in 10 ml of 0.5% HCl for 30 minutes, followed by immersion in 10 ml of 0.5% NaOH for 30 minutes. Then, the sample was extracted with 10 ml of 7.5% Na2CO3 at 50°C for 2 hours in a water bath. The next step was filtering the sample. Furthermore, the filtrate obtained was precipitated by adding 10 ml of 5% HCl, then 10 ml of NaCl was added to oxidize the seaweed pigments or color carrier groups. After that, the gel formed was vortexed and separated using a centrifuge for 15 minutes at a speed of 3500 rpm. The gel precipitate obtained was dissolved with 10 ml of 5% NaOH to convert alginic acid into alginate salt, after which it was precipitated with 95% isopropanol solution to form alginate salt. The precipitate was dried at 60°C and weighed until a constant weight was obtained. Content analysis of the dry matter, organic matter, ash, and crude protein used proximate analysis (AOAC, 1990).

Data analysis

Data were analyzed by analysis of variance using the WPS Excel-Statistics 2022 software (version 11.2.0.11254) for a completely randomized design. Before analysis, all percentages were subjected to logarithmic transformation log10 x + 1 to normalize data distribution. Mean values for each treatment were further tested by Duncan Multiple Range Test (DMRT), and the significance was declared when $p < 0.05$.

RESULTS AND DISCUSSION

Processing of *Sargassum binderi* seaweed by using heat treatment

The average content of dry matter, organic matter, ash, alginate, and crude protein of *S. binderi* seaweed treated by heat treatment is shown in Table 1.

The results of the analysis of variance showed that the application of heat treatment had a significant effect ($p < 0.05$) on the total dry matter content and a very significant effect ($p < 0.01$) on the content of organic matter, ash, and crude protein. However, it had no significant effect on the alginate content of *S. binderi* seaweed ($p > 0.05$). Based on the results of the DMRT further test, the total content of dry matter, organic matter, ash, and crude protein of *S. binderi* seaweed that was not treated with heat treatment (control) was significantly different ($p < 0.05$) from *S. binderi* seaweed which was not treated. Alginate content of the control and treatment was not significantly different ($p > 0.05$). There was no significant difference among the treatments (15, 30, 45, and 60 minutes of heating) in terms of dry matter, organic matter, ash, alginate, and crude protein ($p > 0.05$).

Table 1. Effects of *Sargassum binderi* seaweed processing by a physical method on total dry matter, organic matter, ash, alginate, and crude protein

Heating treatment (minute)	Total dry matter in fresh weight (%)	Organic matter (%)	Ash (%)	Alginate (%)	Crude protein (%)
Control	11.74^a	81.52^b	18.48^a	41.98	14.49^b
15	10.49^b	83.21^a	16.79^b	39.80	16.00^a
30	10.51^b	83.24^a	16.76^b	40.16	15.58^a
45	10.74^b	83.66^a	16.34^b	41.52	15.70^a
60	10.73^b	83.62^a	16.38^b	40.86	15.89^a

[a,b] Different superscript letters in the same column show significantly different ($p < 0.05$)

As can be seen in Table 1, dry matter was lower in heat treatments, compared to the control. The heat treatment led to the loss of some nutrients, such as vitamins, crude fats, dyes (fucoxanthin), and some minerals in seaweed. Similarly, previous studies indicated negative effects of heat treatment, including loss of vitamins (Jakobsen and Knuthsen, 2014; Lee et al., 2018), crude fat (Barszcz et al., 2014; Mosisa, 2017), and ash (Mosisa, 2017). In addition, fucoxanthin in seaweed is also damaged by heating, as described by Oryza (2011) and Susanto et al. (2017). According to Zhang et al. (2015), fucoxanthin is the dominant pigment in brown seaweed, and high temperatures easily damage this pigment. The results reported by Yip et al. (2014) indicated that fucoxanthin was stably stored at 4-25°C, and Oryza (2011) stated that fucoxanthin was relatively stable at 80°C for an hour and was damaged above 100°C.

Seaweed heat treatment for 15, 30, 45, and 60 minutes contained lower ash and higher organic matter than seaweed without treatment (control). It could be influenced by the heat application process, which causes the loss of some of the minerals contained in the seaweed.

The results of this study agree with those of Mosisa (2017), as the ash content for all treatments (dehulled traditional cooking, traditional unhulled cooking, dehulled pressure cooking, and unhulled pressure cooking) on black climbing (*Phaseolus coccineus* L.) generally decreased. This study indicates that minerals such as calcium, phosphorus, iron, and zinc are lost during the heating treatment process. Udensi et al. (2010) reported that heat treatment (autoclaving) on *Mucuna flagellipes* caused the ash content to decrease. The heat application causes a weakening of the bond structure, and the texture hardens (Sharma et al., 2012). This is thought to cause the loss of some minerals during the heating process. The decreased ash content caused the percentage of seaweed organic matter to increase in this treatment.

The heating treatment of seaweed for 15, 30, 45, and 60 minutes caused the crude protein content to be higher than the crude protein of seaweed without heating treatment. The decrease could influence the percentage of seaweed ash content treated with heating, increasing the percentage of organic matter and crude protein. In addition, the increased crude protein content is also influenced by the characteristics of the seaweed crude protein, which is not easily damaged by heating treatment. Deniaud-Bouët et al. (2017) reported that proteins and glycoproteins are a number of additional components of brown algal cell walls. Furthermore, they explained that brown seaweed protein combines with other components, namely alginate, fucoidan, and cellulose. Therefore, the percentage of crude protein in this study increased after being given heating treatment. According to Cascais et al. (2021), heat could alter protein yield in specific species and affect other species differently.

The alginate content of unprocessed seaweed (control) and heat treatment for 15, 30, 45, and 60 minutes was not significantly different ($p > 0.05$). Likewise, the treatment duration of heating for 15, 30, 45, and 60 minutes was not significantly different ($p > 0.05$) concerning the alginate content. Factors influencing alginate degradation are temperature and heating methods (steaming, hot steam pressure, and roasting). For heat stress treatment in the current study, an autoclave with a temperature of 121^{o}C and a pressure of 1 atm were used. It is estimated that this temperature could not degrade alginate, even though the length of heat treatment was extended to 60 minutes. The results found in the current study were in accordance with the results found by Widyastuti (2009) that alginate melted at 121.77-123.11^{o}C. The results of the present study were also in accordance with the study results found by Aida et al. (2010) that alginate can be dissociated into oligosaccharides by heating treatment at a temperature of 180 to 260°C with an alginate and water ratio of 1:25 (w/v). Therefore, the alginate contained in seaweed in this study cannot be dissociated. It is estimated that the heating temperature which was given for each treatment was still below the minimum temperature level to dissociate alginate; thus, this temperature level cannot dissociate alginate. According to Yuliani and Hartati (2011), the effect of temperatures of 150°C for 60 minutes indicates Ca-alginate depolymerization. Sartal et al. (2012) also reported that alginate could not melt during the heating process.

The heating treatments of seaweed for 15, 30, 45, and 60 minutes were not significantly different on dry matter, organic matter, ash, alginate, and crude protein of seaweed ($p > 0.05$). Processing material with hot steam pressure only causes changes in the chemical structure and does not change the chemical composition (Murni et al., 2008). Furthermore, Maehre et al. (2016) stated that heating treatment increased the protein availability of dulse red grass (*Palmaria palmate*) but not brown kelp seaweed (*Alaria esculenta*). Furthermore, it can be stated that heating seaweed causes partial or complete protein denaturation; thereby, this process facilitates the enzyme reaction in the digestive tract and increases protein utilization.

Although this heat treatment did not affect the nutritional content, it improved its quality as a poultry feed ingredient to positively affect the livestock, which was in line with findings reported by Hwang et al. (2012). The positive effects of heating include increasing the availability or quality of nutrients and inhibiting anti-nutrients. According to Maehre et al. (2016), heat treatment increases protein utilization in the red seaweed dulse (*Palmaria palmata*). Similarly, El-Deek et al. (2011) is of the opinion that processing methods, such as autoclaving and boiling, can improve the quality of foodstuffs, such as seaweed, by affecting fiber and nutrient availability. They reported that seaweed processing by this method improved the nutritional value and production performance of chickens compared to raw products. Processing with the autoclave method produces better nutrition, positively affecting livestock production performance (Al-Harthi et al., 2011).

Processing of *Sargassum binderi* seaweed by using the whiting filtrate

Table 2 shows the average content of total dry matter, organic matter, ash, alginate, and crude protein of

S. binderi seaweed treated by soaking seaweed in the whiting filtrate.

The results of the analysis of variance showed that the treatment of soaking seaweed in whiting filtrate had a significant effect on the total dry matter content and crude protein ($p < 0.05$) and an insignificant effect on the organic matter content, ash, and seaweed alginate ($p > 0.05$). Based on the results of the DMRT further test, the total dry matter and crude protein content of seaweed that was not treated with long soaking in the whiting filtrate (control) were significantly different ($p < 0.05$) with the treatment duration of immersion for 1, 2, 3, and 4 hours. However, there were insignificant differences among the groups in terms of organic matter, ash, and seaweed alginate ($p > 0.05$). The treatment duration of immersion in the whiting filtrate solution for 1, 2, 3, and 4 hours was not significantly different in the total dry matter, organic matter, ash, and alginate ($p > 0.05$), but the crude protein was significantly different among the treatments ($p < 0.05$).

Table 2. Effects of processing *Sargassum binderi* seaweed by a chemical method on total dry matter, organic matter, ash, alginate, and crude protein

Soaking time treatment	Total dry matter in fresh weight (%)	Organic matter (%)	Ash (%)	Alginate (%)	Crude protein (%)
Control	11.74^{a}	81.48	18.52	41.98	14.49^{c}
Group 1	10.19^{b}	80.88	19.12	44.63	15.16^{b}
Group 2	10.23^{b}	81.08	18.92	43.33	15.53^{ab}
Group 3	10.34^{b}	80.95	19.05	44.62	15.51^{ab}
Group 4	10.48^{b}	81.82	18.18	44.66	15.93^{a}

^{a,b} Different superscript letters in the same column show significantly different ($p < 0.05$)

The total dry matter content of *S. binderi* seaweed after being treated for 1, 2, 3, and 4 hours of immersion in whiting filtrate showed a decrease. This decrease in total dry matter is thought to be caused by the dissolution of some inorganic seaweed materials, such as salt, sand, or other impurities that are still attached to the seaweed. In addition, the immersion also causes the dissolution of water-soluble vitamins, namely vitamins B and C. Therefore, the total dry matter content of *S. binderi* seaweed, which is treated by immersion in whiting filtrate, decreases at different soaking times, showing differences in the total dry matter content. Similarly, Martinson et al. (2012), Mack et al. (2014), and Giannakourou and Taoukis (2021) found that immersion treatment affects the loss of water-soluble macro minerals. Furthermore, Longland et al. (2014) stated that hay soaked in water caused the loss of some minerals and vitamins during the soaking process. Salt is an easily soluble compound in water (Nelson and Cox, 2013; Dewi et al., 2018). According to Hastuti et al. (2013), Vitamin C is unstable in alkaline solutions. In the current study, the whiting filtrate is an alkaline solution with a pH of 12, causing damage and dissolution of Vitamin C in the whiting water filtrate so that it impacts reducing the total dry matter of seaweed. The results of the current study were in accordance with the results found by Aregawi et al. (2014), indicating that sesame straw treated with 3% $Ca_2(OH)_2$, which is alkaline, caused the decrease of dry matter content from 89.7% to 76.6%.

The organic matter, ash, and alginate content of *S. binderi* seaweed had no effect after being treated with different immersion times in whiting filtrate. The reason can be that the concentration of whiting (0.07%) used to reach pH 12 is too low, even though the pH of the whiting filtrate has met the requirements as an alkaline solution predicted to decompose alginate. However, the filtrate concentration of 0.07% whiting water is thought to be classified as the weak base and has not been able to loosen alginate bonds or degrade alginate in seaweed. According to Owen et al. (1984), calcium hydroxide or Ca $(OH)_2$ is a weak base compared to NaOH, and the treatment conditions determine its effectiveness. The weak base treatment of Ca $(OH)_2$ to be more effective for degrading crude fiber from rice straw takes a longer and higher reaction time and dose (Trach et al., 2001). According to Anjalani et al. (2013), the effectiveness of the Ca $(OH)_2$ treatment on the nutritional content of palm leaves was due to the short duration and the low dose level.

The crude protein content of *S. binderi* seaweed increased along with the duration of soaking the seaweed in the whiting filtrate. Increasing the crude protein of seaweed after immersion with whiting filtrate is the same as the mechanism of increasing the crude protein of seaweed after immersion in running water to reduce the salt content of the seaweed.

CONCLUSION

The results of the current study showed that physical treatment (heat treatment) and chemical treatments (whiting filtrate immersion) did not significantly affect the alginate content, crude protein, ash, dry matter, and organic matter. Based on the findings, it is recommended that the temperature level should be increased and the alkaline source should be changed by other strong alkaline sources in future studies.

DECLARATIONS

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Authors' contributions

Dewi conducted paper writing, data collection, and statistical analysis. Yuniza, Nuraini, Sayuti, and Mahata contributed to the study design and development of the research idea. All authors drafted the manuscript and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

This article has been checked by all authors, and ethical issues such as plagiarism, publication consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy were not found.

Availability of data and materials

The prepared data of the present study will be sent by the corresponding author according to the reasonable requests.

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