



Fermentation of *Sargassum binderi* Seaweed for Lowering Alginate Content of Feed in Laying Hens

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ABSTRACT

The object of this study was to reduce the alginate content of *Sargassum binderi* by fermentation of *Bacillus megaterium* S245 in feed of laying hens. The experiment was arranged in a factorial with completely randomized design. The first factor was different inoculum dosage (1, 3, 5, 7, and 9%), while the second factor was different fermentation period (1, 3, 5, 7 and 9 days), and each treatment was replicated for five times. The measurements were total dry matter, organic matter, ash, alginate, and crude protein. The results showed there were no significant effect of inoculum dosage, and interaction between inoculum dosage and fermentation period on alginate, total dry matter, organic matter, ash and crude protein content of *Sargassum binderi* while fermentation period reduced the alginate and total dry matter content. Fermentation period also increased the crude protein content significantly. Besides that, fermentation period didn't effect on organic matter and ash content significantly. The fermentation of *Sargassum binderi* with *Bacillus megaterium* S245 at inoculum dosage of 1% and fermentation period of nine days was the best combination for lowering alginate content in *Sargassum binderi* and this treatment had positive effect on nutrient content of *Sargassum binderi*.

Key words: Alginate, *Bacillus megaterium* S245, Fermentation, Laying hens, *Sargassum binderi*

INTRODUCTION

Brown seaweed (*Phaeophyceae*) is one of the seaweed divisions that has variety of species, such as *Ascophyllum*, *Durvillaea*, *Ecklonia*, *Laminaria*, *Lessonia*, *Macrocystis*, and *Sargassum* (Mc Haugh, 2003). Mc Haugh (2003) explained that brown seaweed strain *Ascophyllum* is founded in seas of Scotland, Iceland, Norway, and Canada, and strain *Durvillaea* is founded in seas of Tasmania and Australia, while strain *Ecklonia* is founded in seas of South Africa South Korea. The brown seaweed strain *Laminaria* is founded in seas at France, Norway, Scotland, and Iceland, and brown seaweed *Lessonia* is founded in seas of Chile, while brown seaweed strain *Macrocystis* is founded in seas of United States of America, Mexico, Chile, Argentina, and South Africa, and brown seaweed strain *Sargassum*, *Turbinaria*, and *Padina* are widely found and quite abundant in Indonesian seas (Rachmaniar, 2005).

Seaweed is a marine resource that has the potential to be developed as a nonconventional feed for poultry. They are considered the most important food supplement of the 21 century as a source of proteins, lipids, polysaccharides, minerals, vitamins, and enzymes (Rimber, 2007). According to Rasyid (2004), seaweed can be used as a mixture of animal feed, especially in maritime countries. Now the availability of seaweed as animal feed increased with the production of seaweed feed in form of powder seaweed (Mc Haugh, 2003).

Sargassum binderi is species of brown seaweed (*Phaeophyceae*). *Sargassum binderi* contains crude protein 6.93%, alginate 20.89% (Dewi et al., 2018), crude fat 1.07%, crude fiber 7.76%, metabolism energy 2179.63 kcal/ kg, Ca (Calsium) 0.64%, and P (Phospor) 0.62% (Analysis of Nutrition of Non Ruminant Laboratory, 2019). Brown seaweed contain alginate (Dewi et al., 2018), fukoidan (Synytsya et al., 2010; Song et al., 2012), fucoxanthin (Haugan et al., 1995; Matsuno, 2001), and

unsaturated fatty acid (PUFA/Poly-Unsaturated Fatty Acid) (Al-Harathi and El-Deek, 2012a; Carrillo et al., 2012; Pal et al., 2014). This bioactive compound has hypocholesterolemic activity, antiviral, antibiotic, anti-inflammatory, antithrombin, anticoagulation, antilipemic, and stimulant (Al-Harathi and El-Deek, 2012a). Based on previous research showed that cholesterol in yolk was lowered by alginate and fucoidan compounds in brown seaweed (Carrillo et al., 2012). In addition, alginate as water soluble fiber can lower cholesterol in the blood especially LDL (Low Density Protein) and act as antihyperlipidemic compound (Mao et al., 2004). Furthermore, Al-Harathi and El-Deek (2012b) reported fucoxanthin may reduce cholesterol, but it is also known to increase pigmentation in egg yolks. Fatty acids in seaweed are reported that have a role in reducing the cholesterol level in egg yolks (Carrillo et al., 2012).

Sargassum binderi contains high salt, it reaches 17.20% (Dewi et al., 2018). The immersion of *Sargassum binderi* in water flow was the best method for lowering high salt content in *Sargassum binderi*. Dewi et al. (2018) reported, the immersion of *Sargassum binderi* for 15 hours in water flow could reduce salt content in *Sargassum binderi* from 16.86% to 0.94%. However, this method has not been able to reduce dominant carbohydrates (alginate) in seaweed. Alginate is a family of linear binary copolymer of β -1,4-D-manuronic acid and β -1,4-L-guluronic acid residue and contained widely varied composition and sequence (Draget, 2009). Alginate is a polyuronic saccharide that is isolated from the cell walls of a number of brown seaweed species around the world, and it is also produced as an extracellular matrix by certain bacteria (Stokke et al., 2000; Draget et al., 2005; Draget, 2009; Nalamothu et al., 2014). Alginate compound reach up 40% from the dry matter of seaweed (Draget et al., 2005). This high alginate content in the poultry feed will bind nutrients and inhibit absorption in the gastrointestinal tract, so that it will interfere performance of poultry (Riski, 2015; Hendro, 2015; Zulhaqqi, 2015; Zahara, 2015). On the other hand, proper concentration of alginate in poultry ration will help for lowering fat digestion by binding fat in the digestive tract, and then alginate with undigested fat will excreted as feces (Kasahara et al., 2018). Mushoilaeni et al. (2015) reported, that feeding of alginate to mice in hypercholesterolemia conditions as much as 0.75-1% lowering cholesterol in blood serum of mice by 53%.

Feed stuff fermentation process with microorganism can improve quality of feedstuff nutrient, digested, and elongate of feedstuff storage.

According to Rizal et al. (2013) palm kernel cake fermented with *Aspergillus niger* improve the nutrient content and nutritional quality. Crude fiber in pineapple peel waste was reduced by fermentation using cellulolytic local microorganism solution derived from bamboo sprouts (Adrizar et al., 2017). Heryandi et al. (2018) reported fermented of pineapple peel waste could be used in broiler diet up to 12% without negative effects on organ development and carcass performance.

Subaryono et al. (2016) reported, bacterium *Bacillus megaterium* S245 produce alginate lyase This bacterium includes of negative gram bacterium which was isolated from *Sargassum crassifolium* brown seaweed. Furthermore, it is explained this enzyme can depolymerize polymanuronate or polyguluronate from alginate structure, this bacterium also was used to produce oligosaccharides alginate from alginate (Subaryono et al., 2016). So far, there is no report regarding the utilization of *Bacillus megaterium* S245 as inoculum to ferment *Sargassum binderi* brown seaweed for lowering its alginate content thought fermentation method, in feed of laying hens. We are already performed the experiment for lowering alginate in *Sargassum binderi* by using *Bacillus megaterium* S245 as inoculum.

MATERIALS AND METHODS

Collection of *Sargassum binderi* seaweed

Sargassum binderi seaweed was collected by a simple random sampling method at Nipah Beach, Pesisir Selatan District, West Sumatra, Indonesia. Whole individuals of *Sargassum binderi* were used in this experiment.

Fermentation of *Sargassum binderi* with *Bacillus megaterium* S245

The fermentation process in this study was liquid fermentation type. The total substrate (consist of *Sargassum binderi* seaweed flour, palm sugar, and water) in this experiment was 300 ml, and the ratio of *Sargassum binderi* seaweed flour with water was 1: 5, and palm sugar was added 3% from the total volume of substrate. The levels of *Bacillus megaterium* S245 as inoculum were 3, 6, and 9% from the substrate. Fermentation period was 1, 3, 5, 7, and 9 days for each treatment. This experiment was performed in a factorial completely randomized design. The first factor was inoculum dosage of *Bacillus megaterium* S245 consist of 3, 6, and 9%, and the second factor was fermentation period was 1, 3, 5, 7, and 9 days, and each treatment was replicated for five times.

Preparation of *Sargassum binderi* samples as product of fermentation for analysis

After fermentation, the *Sargassum binderi* seaweed was dried in oven, then crushed to a dry powder with blender (Philips). Furthermore, samples were analyzed for alginate by Zaelanie et al. (2001) method, total dry matter, organic matter, ash, and crude protein content of *Sargassum binderi* seaweed by AOAC (1990) method.

Analysis of content

Alginate was analyzed by Zaelani et al. (2001) method. One g of *Sargassum binderi* seaweed flour was soaked in 10 ml of HCl 0.5% for 30 minutes, and then, it soaked in 10 ml of 0.5% NaOH for 30 minutes. Furthermore, sample was extracted with 10 ml Na₂CO₃ 7.5% at a temperature of 50 °C for two hours in a water bath. The sample filtered, and then the filtrate was precipitated by adding 10 ml of 5% HCl, 10 ml of NaOCl to oxidize the pigments of seaweed. The gel formed was separated by centrifuge for 15 minutes with speed 3500 rpm. Furthermore, the gel precipitate was dissolved with 10ml of 5% NaOH to convert alginate acid to alginate salt, after that, it was precipitated again with 95% isopropanol solution to form alginate salts. The obtained precipitate is dried at a temperature of 60 °C and weighed with a digital scale to a constant weight. Crude protein, dry matter, organic matter, and ash were analyzed by proximate analysis (AOAC, 1990).

Statistical analysis

Data were in a factorial completely randomized design and statistically analyzed via ANOVA test. The difference among treatment means was determined using the Duncan Multiple Range Test (DMRT) (P<0.05) (Steel and Torrie, 1980).

RESULT AND DISCUSSION

The result of fermentation of *Sargassum binderi* with *Bacillus megaterium* S245 on total dry matter, organic matter, ash, alginate, and crude protein is presented in table 1. The different of inoculum dosage of *Bacillus megaterium* S245 (3, 6, and 9%), and interaction between inoculum dosage with fermentation period (1, 3, 5, 7, and 9 days) did not affect (P>0.05) on alginate, total dry matter, organic matter, ash, and crude protein, while fermentation period affected (P<0.05) the alginate and crude protein of *Sargassum binderi*. Ash and organic matter did not affect (P>0.05) by inoculum dosage,

fermentation period, and interaction between dosage inoculum, and fermentation period significantly.

The effect of increasing of inoculum dosage in fermentation process in this experiment did not increase of microbes ability to degrade dry matter of seaweed. It was expected that a high inoculum dosage should accelerate fermentation process, because large number of microbes will highly produce enzymes, and some dry matter will be degraded. In fact, present study found the increasing of inoculum dosage from 3 to 6 and 9% in fermentation process did not affect degradation of dry matter, because the increasing of inoculum dosage, increasing the bacteria mass which caused by high competition to obtain nutrients for growth, so that the availability of substrate nutrient decreased faster in the fermentation process in comparing with fermentation process with lower inoculum dosage (3%). According to Maier (2009), microbial growth depends on the availability of substrate nutrition, if nutrient availability of the substrate decreases, the microbial growth rate decreases. The microbial growth consist of four phases are lag phase, exponential, stationary, and dead phase, and at stationary phase showed microbial growth and dead microbial is balance. Maier (2009), explained that there are several reasons why microbes reach the stationary phase, that are decrease of substrate nutrition because it has been used by microbes to growth, and accumulation of products produced by microbes was maximum where these products inhibit microbial growth. Liu et al. (2012), also explained that the seaweed waste could not be fully decomposed by microorganisms with little microbial agents, otherwise too many microbial agents competed and did not conducive to fermentation of seaweed waste. Therefore, inoculum dosage at 6 and 9% were similar with inoculum dosage 3% to degrade dry matter of *Sargassum binderi*. According to Hardini (2010), microbes need substrate as source of carbon, nitrogen, and minerals.

Total dry matter of *Sargassum binderi* after fermented with fermentation period at 5, 7 and 9 days lower than fermentation period at 1 and 3 days. It means some compound in *Sargassum binderi* fermented such as alginate was degraded in fermentation process. Prolong of fermentation period to 5 days showed increasing dry matter degradation, but prolong of fermentation period to 7 and 9 days, the activity of alginate lyase enzyme that produced by *Bacillus megaterium* S245 in fermentation process did not increase to degrade dry matter. In accordance with Correa and Villena (2010), prolong of incubation period increase growth of microbes until the stationary phase is reached.

Table 1. Effect fermentation of *Sargassum binderi* with *Bacillus megaterium* S245 on total dry matter, organic matter, ash, alginate and crude protein

| Treatment | Total Dry Matter (%) | Organic Matter (%) | Ash (%) | Alginate (%) | Crude Protein (%) | |
|--|----------------------|--------------------|---------|--------------------|---------------------|-------|
| Inoculum Dosage (ID) (%) | | | | | | |
| 3 | 81.76 | 82.38 | 17.62 | 34.47 | 12.04 | |
| 6 | 81.88 | 82.12 | 17.88 | 34.66 | 12.09 | |
| 9 | 81.87 | 82.06 | 17.94 | 34.65 | 12.13 | |
| Fermentation Period (FP) (days) | | | | | | |
| 1 | 83.98 ^a | 82.42 | 17.58 | 36.39 ^a | 11.62 ^c | |
| 3 | 82.13 ^b | 82.03 | 17.97 | 34.85 ^a | 12.10 ^b | |
| 5 | 81.38 ^{bc} | 81.75 | 18.25 | 34.82 ^a | 12.16 ^{ab} | |
| 7 | 81.31 ^{bc} | 82.16 | 17.84 | 34.76 ^a | 12.10 ^b | |
| 9 | 80.41 ^c | 82.55 | 17.45 | 32.13 ^b | 12.44 ^a | |
| Interaction between Inoculum Concentrations (IC) and Fermentation Period (FP) | | | | | | |
| IC (%) | FP (days) | | | | | |
| 3 | 1 | 84.83 | 83.00 | 17.00 | 35.91 | 11.44 |
| | 3 | 82.10 | 82.42 | 17.58 | 34.78 | 11.88 |
| | 5 | 81.15 | 81.58 | 18.42 | 34.62 | 12.25 |
| | 7 | 80.54 | 82.42 | 17.58 | 35.24 | 12.00 |
| | 9 | 80.20 | 82.46 | 17.54 | 31.77 | 12.62 |
| 6 | 1 | 83.08 | 82.46 | 17.54 | 36.02 | 11.58 |
| | 3 | 81.48 | 82.10 | 17.90 | 34.19 | 12.37 |
| | 5 | 81.61 | 81.78 | 18.22 | 35.01 | 12.16 |
| | 7 | 82.77 | 81.48 | 18.52 | 35.81 | 12.04 |
| | 9 | 80.45 | 82.76 | 17.24 | 32.61 | 12.30 |
| 9 | 1 | 84.02 | 81.82 | 18.18 | 37.23 | 11.83 |
| | 3 | 82.79 | 81.57 | 18.43 | 35.58 | 12.06 |
| | 5 | 81.38 | 81.89 | 18.11 | 34.84 | 12.07 |
| | 7 | 80.62 | 82.59 | 17.41 | 33.22 | 12.26 |
| | 9 | 80.56 | 82.44 | 17.56 | 32.36 | 12.41 |
| Analysis of variance | | | | | | |
| ID | ns | ns | ns | ns | ns | |
| FP | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | |
| ID × FP | ns | ns | ns | ns | ns | |

^{a,b,c}Means in a column under similar treatment not sharing the same superscript are significantly different at (P<0.05); IC: Inoculum Concentrations; FP: Fermentation Period; ns: not significant; ID: Inoculum Dosage

Nutrient composition in *Sargassum binderi* changed along fermentation period such as reducing of alginate, and increasing of crude protein. In this experiment fermentation process did not affect on ash and organic matter content in *Sargassum binderi*. Ash content in fermented *Sargassum binderi* increased after fermented for all fermentation period (1, 3, 5, 7, and 9 days), compared with ash content in unfermented *Sargassum binderi*, while organic matter content in *Sargassum binderi* for all fermentation period (1, 3, 5, 7, and 9 days) was decrease compared with organic matter in unfermented *Sargassum binderi*. According to Ardiansyah et al. (2018) fermentation of *Sargassum flour* with

Aspergillus niger, *Saccharomyces cerevisiae*, and *Lactobacillus* spp increased ash content. Aslamyah et al. (2017) reported that fermentation of *Kappaphycus alvarezii* green strain, *K. alvarezii* brown strain, *Gracilaria gigas*, *Sargassum sp.*, and *Caulerpa sp* could decreased ash content of seaweed. It is reported the ash content increased in fermented *Sargassum* due to contribution of fermentation microorganisms in the degradation of organic components during fermentation (Oseni and Ekperigin, 2007). Djunaidi and Nasir (2001) obtained organic matter decreased because it is used as an energy source for microbes. This result differs from research of Felix and Brindo (2014) that expressed the ash

content of fermented *Kappaphycus alvarezii* decreased as compared with unfermented *Kappaphycus alvarezii*.

Alginate is a polysaccharide group widely found in brown seaweed (*phaeophyceae*), and this carbohydrate as calculated as dry matter in analysis of food substances by the proximate analysis method. In this experiment, there is no effect of all of inoculum dosage and interaction of inoculum dosage with fermentation period on the alginate content in *Sargassum binderi*. According to Draget et al. (2005), and Draget (2009), alginate is a polysaccharide compound found in brown seaweed (*phaeophyceae*), its compound is composing of salts of alginic acid with β -1,4-D-mannuronic acid and α -1, 4-L-guluronic acid bonds.

Table 1 showed, the fermentation period of *Sargassum binderi* by alginate lyase which produced by *Bacillus megaterium* S245 in 1, 3, 5, and 7 days fermentation period were not enough to reduce alginate content in *Sargassum binderi*. Therefore, fermentation period on 3, 5, and 7 days did not affect the alginate ($P > 0.05$). However the extension of fermentation period to 9 days caused a significant decrease in alginate ($P < 0.05$). According to Ostgaard et al. (1993), alginate from *Laminaria saccharina* brown seaweed was degraded by combination of *Ascophillum nodosum* and cow manure as much as 10% still be found after 30 days fermentation process. It is mean degradation of alginate need a long period. The best fermentation period to reduce alginate content in *Sargassum binderi* in this experiment was found at nine days with alginate content was 32.13%. According to Draget (2005), alginate lyase catalyzes the depolymerization of alginate by splitting the 1-4 glycosidic linkage in β -elimination reaction, leaving an unsaturated uronic acid on the non-reducing end of the molecules. The research by Zhang et al. (2012) and Liu et al. (2012) show that fermented from seaweed waste need long fermentation period for 15 days to improve its quality of nutrition. The result of this research was different with findings of Moen (1997) that indicated the alginate degradation of *L. hyperborea* started after 50 hours, the viscosity of the polymer rapidly decreased within the period 50-100 hours, and after 100 hours no alginates could be isolated since the extractable part was completely depolymerized.

In this experiment, the increasing of fermentation period would increase crude protein content in fermented *Sargassum binderi*. The increasing of crude protein in fermented *Sargassum binderi* was from bacterial mass and enzymes that produced by *Bacillus megaterium* S245. Bacteria mass and enzymes are proteins. According to Azhar (2016), bacterial cell wall is composed of proteins,

and enzymes produced by bacteria are proteins. This result agrees with Zhang et al. (2012) that reported fermented seaweed waste could be increase of crude protein as much as 34.56%, and also Ardiansyah et al. (2018) reported that fermentation of *Sargassum flour* with *Saccharomyces cerevisiae*, and *Lactobacillus* spp increased crude protein content. Felix and Brindo (2014) reported that crude protein content of raw and fermented *Kappaphycus alvarezii* are 14% and 23.86% respectively. This result showed that increased of crude protein content of fermented *Kappaphycus alvarezii*. Furthermore, Tangendjaya (1993) explain that increase amount of microbial mass will cause increase content of fermented products, where protein content is reflection of cell mass microbe, which in fermentation process, microbe produces enzymes that will degrade complex compounds into simple material, and microbes will also synthesize proteins which are protein enrichment process, that are enrichment of crude protein.

CONCLUSION

The fermentation of *Sargassum binderi* with *Bacillus megaterium* S245 with inoculum dosage 1% and fermentation period nine days were the best combination for lowering alginate content in *Sargassum binderi* and this treatment has positive effect on nutrient content of *Sargassum binderi*.

DECLARATIONS

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Competing interests

The authors declared that they have no competing interests.

Author's contribution

Dewi wrote the paper, collected data, and performed statistical analysis. Yuniza, Nuraini, Sayuti, and Mahata created the idea and designed the study. Dewi and Mahata drafted the manuscript and approved the final manuscript.

Consent to publish

All authors informed their consent prior to inclusion in the study.

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