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EFFECTS OF DIFFERENT DIETARY CONCENTRATION OF FERMENTED BROWN ALGAE *SARGASSUM BINDERI* ON PLASMA LIPID PROFILES, YOLK LIPID, AND CHOLESTEROL TOTAL OF LAYING HENS

Y. L. Dewi[1], A. Yuniza[2], K. Sayuti[3], Nuraini[2], and M. E. Mahata*[2]

[1]Department of Livestock Breeding, Faculty of Military Logistics, Indonesia Defense University, Belu, 85752, Indonesia
[2]Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Padang, 25163, Indonesia
[3]Department of Agricultural Product Technology, Faculty of Agricultural Technology, Andalas University, Padang, 25163, Indonesia
*Corresponding author's email: maria@ansci.unand.ac.id

ABSTRACT

Sargassum binderi is a brown algae abundantly available, good nutrient, cheap, does not compete with human, and has not been used optimally as feed for laying hens. Therefore, the purpose of this study was to study the effect of using fermented *S. binderi* on plasma lipid profiles, fat content, and cholesterol in yolk in laying hens. This study used 200 laying hens (1571-1586 g), aged from 60 to 68 weeks randomized into five different concentrations (0%, 4%, 8%, 12%, and 16%) with a length of 6 weeks. At the end of the study, blood samples were randomly taken from 40 laying hens (2 laying hens per treatment) and 80 eggs were collected randomly (4 eggs per treatment). Variables measured were plasma lipid profile (triglycerides, total cholesterol, and LDL), lipid, and yolk cholesterol. The results showed that the treatment of *S. binderi* had a significant effect on the lipid profile of laying hens blood serum for total cholesterol and LDL but had no significant impact on blood serum triglycerides. In addition, there was no significant effect on yolk fat and significantly on yolk cholesterol. Thus, the provision of *S. binderi* to 16% in the laying hen's diet can reduce total blood serum cholesterol from 211.60 to 152.49 mg/dl and LDL from 95.55 to 49.05 mg/dl, with a decrease of 27.93%, and 48.66%, respectively, and decreased yolk cholesterol from 1,279.54 to 1,074.30 mg /100 g with a reduction of 16.04%.

Keywords: Fermentation, hens laying, plasma lipids, *S. binderi,* yolk cholesterol
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INTRODUCTION

Algae is used as a food ingredient, extraction of phycocolloids, extraction of compounds that have antiviral, antibacterial, anti-tumor activity, and as a biological fertilizer in various countries (Pereira, 2016). Algae production in the world is 30.4 million tonnes (FAO, 2018). Furthermore, it was stated that the most extensive algae producing countries in the world (in the lowest ranking order), namely Chile, China, and Norway for wild brown and red algae, and Chilean kelp; China, Indonesia, South Korea, and the Philippines for cultivated algae, especially Euchema, Japanese kelp, Gracilaria, and *Undaria pinnatifida*.

Algae is a marine resource that has the potential to be developed as a non-conventional feed for poultry. The algae has the potential as a source of nutrients that contain high protein, amino acids, carbohydrates, lipids, vitamins A, B (especially B12), and C, pigments, antioxidants, and antimicrobials (Al-Harthi and El-Deek, 2011, 2012). Algae is safe to use as animal feed because the heavy metal content is shallow according to feed standards (Michalak *et al*., 2010). According to Rasyid (2004), Valderrama *et al*. (2013), and Jacob (2022), algae can be used as animal feed.Algae in Indonesia consist of about 452 species of red seaweed (Rhodophyceae), 196 species of green seaweed (Chlorophyceae), and 134 species of brown seaweed (Phaeophyceae) (Suparmi and Sahri, 2009). Algae found in Indonesian sea include red algae (Rhodophyta), brown algae (Phaeophyta), and green algae (Chlorophyta) (Rachmaniar, 2005). Brown algae (*Phaeophyceae*) is one of the algae divisions that has variety of species, such as *Ascophyllum, Durvillaea, Ecklonia, Laminaria, Lessonia, Macrocystis*, and *Sargassum* (Mc Haugh, 2003). *S. binderi* is species of brown algae (*Phaeophyceae*).

S.binderi contains 6.93% crude protein, 7.76% crude fiber, and 20.89% alginate (Dewi *et al*., 2018). In addition, this algae contains 1.07% crude fat, 0.64% Ca, and 0.62% P (Non-Ruminant Nutrition Laboratory Results, 2015). Brown algae is reported to have bioactive compounds, such as alginates (Dewi *et al*., 2018), fucoidan (Song *et al*., 2012; Udani and Hesslink. 2012; Thanh *et al*., 2013), fucoxanthin (Udani and Hesslink, 2012; Beppu *et al*., 2012; Mikami and Hosokawa, 2013; Muradian *et al*., 2015; Zhang *et al*., 2015), and

unsaturated fatty acids (PUFA/ Poly-Unsaturated Fatty Acid) (Carrillo *et al*., 2012). These bioactive compounds has hypocholesterolemic (Pal *et al*., 2014). Based on the results of previous research, several researchers have reported several bioactive compounds found in brown algae and their use limitations in poultry diets. Carillo *et al*. (2012) said alginates and fucoidan found in *Sargassum* spp. can decrease yolk cholesterol. In addition, alginate as a water-soluble fiber can reduce cholesterol content in rat blood, especially LDL (Low-Density Lipoprotein), and act as an antihyperlipidemic compound (Wikanta *et al*., 2002; Wikanta, 2003; Mao *et al*., 2004; and Astawan *et al*. al., 2005). Furthermore, Al-Harthi and El-Deek (2012) reported that fucoxanthin could lower cholesterol and increase pigmentation in the yolk. PUFA fatty acids in algae are informed to lower cholesterol in the yolk (Carillo *et al*., 2012). *Porphyridium* sp., *Sargassum* spp., *S. dentifebium* is reported to be able to be given 2–10% in the diet of laying hens and reduce yolk cholesterol by 6–26% (Ginzberg *et al*., 2000; Carrillo *et al*., 2012; and Al-Harthi and El-Deek, 2012). The use of *Gracilaria edulis*, *Enteromorpha prolifera*, *Cladophora* sp., *Sargassum* sp., and *S. dentifebium* in laying hens diet can increase yolk pigmentation at the level of 2.5% - 15% (Horhoruw *et al*., 2009; Michalak *et al*., 2010; Carrillo *et al*., 2012; and Al-Harthi and El-Deek, 2012). Provision of *Porphyridium sp*. with a level of 5–10%, *S. dentifebium* with a group of 6%, and Schizochytrium by 0.1–0.5% in the diet can lower blood serum cholesterol in laying hens (Ginzberg *et al*., 2000; Al-Harthi and El- Deek, 2012; and Park *et al*., 2015). 2% *G. vermiculophylla* in the diet of laying hens can increase the eggshell thickness (Ozaki *et al*., 2013). The production performance of laying hens can be maintained by administering *S. dentifebium*, *C. vulgaris*, *Chondrus crispus*, and *Sarcodiotheca gaudichaudii* with levels of 0.5–3% in the diet (Halle *et al*., 2009; Al-Harthi and El-Deek, 2011; and Kulshreshtha *et al*., 2014). In addition, giving *S. dentifebium* to laying hens affected the increase in oleic fatty acid in eggs at a rate of 3% (Al-Harthi and El-Deek, 2012).

The use of algae as an ingredient in the poultry diet has limitations (Ghosh *et al*., 1981; Moen *et al*., 1999). According to Dewi *et al*. (2018; 2019), high salt and alginate are some of the limiting factors of algae as poultry feed. *S. binderi* contains high levels of salt and alginate, namely 17.20% and 20.68%, respectively (Dewi *et al*., 2018). According to Lichtenwalner (2018), the salt (NaCl) content in the poultry diet is limited to 0.1–0.4%. Jacob (2022) added that algae contains carbohydrates that are difficult to digest. The salt content of *S. binderi* can be maximally reduced by immersion in water flow for 15 hours (Dewi *et al*., 2018), and alginate can be reduced by the fermentation method using *Bacillus megaterium* S245 (Dewi *et al*., 2019). The effect of using fermented *S. binderi* in the laying hen's diet on plasma lipid profiles and crude fat and yolk cholesterol in layer hens is presented in the following information. The purpose of this study was to study the effect of using fermented *S. binderi* on plasma lipid profiles (triglycerides, total cholesterol, and LDL) and crude fat and yolk cholesterol content in laying hens.

MATERIALS AND METHODS

Ethical Approval: The animal experiments were carried out in accordance with the guidelines laid by institutional Ethics committee for the care of animals and were approved by Animal Ethics Committee of the Universitas Andalas, Padang, Indonesia with No:577/KEP/FK/2019.

Procurement and Processing *Sargassum binderi* seaweed: S. *binderi* was collected by a simple random sampling method at Nipah Beach, Pesisir Selatan Regency, West Sumatra, Indonesia in October–November 2018 and the seaweed fermentation process was carried out at Industri Teknologi Industri Pakan Laboratory, Faculty of Animal Science, Universitas Andalas in February–March 2019.

Whole individuals of *S. binderi* (talus, bladder, and holdfast) were used in this experiment. Then, this algae was immersed in water flow at Sungai Gunung Nago, Kecamatan Pauh, Padang, West Sumatra, Indonesia with a depth of 1.3 m and a current of 0.6745 m^3 sec for 15 hour. At the end of the immersion, the seaweed was removed from the water and dried in an oven at 60^{o}C until its water content was approximately 14%, and the dried algae was crushed to a dry powder. The continued process is algae flour was fermented with *Bacillus megaterium* S245 with inoculum dosage 1% and fermentation period nine days. After fermentation, the w*et al*gae fermentation was dried, then crushed to a dry powder. The *S. binderi* fermentation meal was for conservation and placed in paper bags (reinforced with plastic). After this, proximate analysis, alginate, metabolizable energy, and fucoxanthin were carried out (Table 1), and then the amino acid content determination was carried out (Table 2), according to the analytical procedure described by AOAC (1990), Zaelanie *et al*. (2001), Sibbald (1986), Limantara dan Heriyanto (2010), and Marino *et al*. (2010), respectively.

Birds and Diets: The study conducted at The Zulfani Laying Farm in Korong Taluk Nibung, Nagari Sunur, Padang Pariaman Regency, West Sumatra on 16th May–6th July 2019. Two hundred Isa Brown layers (1571-1586 g) were divided into five treatment groups with four (4) replicates of 10 birds each at age 60 to 68 weeks. The treatments were assigned randomly and consisted of 0%, 4%, 8%, 12%, and 16% fermented *S. binderi*. The diets were calculated to meet the recommendations of the National Research Council (NRC, 1994). Feed and water were provided *ad-libitum*.

Plasma Lipid Profile: Plasma lipid profile measured at the Laboratory of Klinik Fitria, Padang, West Sumatra in July–August 2019. Blood samples were taken from 40 laying hens randomly (2 layers from each replication of each treatment) were taken on the last day of week 6 of treatment. The blood of laying hens was taken by the intravenous blood sampling method in the Pectoral vein using a 3 ml syringe. Blood from the needle was collected in 4 ml volume Clot Activator Vacuum Tube Pro-coagulation tube (JUN NUO® RD-Vtube, China). The laying hen blood that has been collected from each treatment in a centrifuge (Heraeus ™ Multifuge ™ X3 Centrifuge Series, Thermo Fisher Scientific, Robert-Bosch-Straße 1 D-63505, Langenselbold, Germany) at a speed of 3000 rpm for 15 minutes to obtain serum. The serum obtained was put into an Eppendorf tube (BCN-1700-BP Goldengate Bioscience, USA) volume of 1.7 ml which would be used to analyze the lipoprotein content. Analysis of the total cholesterol, triglycerides, and LDL content of laying hens blood serum using the enzymatic calorimetry method according to the DiaSys Diagnostic System (2014) with a semi-auto chemistry analyzer (RT-9200, Rayto). The working principle of this instrument, mixing each reagent for total cholesterol, triglycerides, and LDL with a blood serum sample, then the absorbance is measured.

Table 1. The nutritional profile of fermented *Sargassum binderi.*

Nutrient	In dry wet
Dry matter	92.57 %
Ash	16.18%
Organic matter	76.34%
Crude protein	11.68%
Crude lipid	0.80%
Crude fiber	15.17%
Calcium	1.19%
Phosphor	0.26%
Natrium	0.37%
Metabolism Energy	477.87 kkl/kg
Alginate	29.13%
Fucoxanthin	2.3458 µg/g

Yolk Crude Lipid and Cholesterol: Yolk crude lipid measured at the Nutrisi Non Ruminasia Laboratory, Faculty of Animal Science, Andalas University in July 2019 and yolk cholesterol measured at Biotechnology Laboratory, Faculty of Animal Science, Andalas University in September–October 2019 Eighty eggs were randomly collected (4 eggs from each replication of each treatment) taken on the last two days at week 6 of the handling used to analyze crude lipid and yolk cholesterol samples.

Yolk Crude Lipid: Analysis of yolk crude lipid using the proximate analysis method (AOAC, 1990) with a set of Soxhlet instruments based on lost fat content.

Table 2. Amino acid profile of fermented *Sargassum binderi*

Amino Acid	(%)
Aspartic acid	0,56
Glutamic acid	0,32
Serine	0,28
Histidine	0,74
Glysine	0,31
Threonine	0,37
Arginine	0,27
Alanine	0,15
Tyrosine	0,29
Methionine	0,48
Valine	0,15
Phenylalanine	0,28
I-Leucine	0,36
Leucine	0,30
Lysine	2,45
Amino acid Total	7,32

Yolk Cholesterol: Yolk cholesterol was analyzed by the Liebermann Burchard method (Kleiner and Dotti, 1962). The principle of this method is that the cholesterol in the chloroform extract will react with acetic anhídride and concentrated sulfuric acid, giving the absorption color reaction which a spectrophotometer can measure.

The yolk is separated from albumin, then dried in an oven at 60^{o}C until the yolk is dry with a moisture content of ± 14%. After that, dried yolk from each treatment was mashed with a mortar until it became flour. Yolk sample was weighed 0.05 g, then added acetone and ethanol in a ratio of 1: 1, with the volume of the mixture reaching 5 ml, then homogenized and let stand for 30 minutes. Then let it stand in a water bath until it boils, then cools it to room temperature. Furthermore, centrifuge (Heraeus Multifuge X3R Centrifuge) at a speed of 1500 rpm for 15 minutes. The supernatant (clear part) or the resulting extraction is inserted into a new test tube, then evaporated and heated in a water bath until it dries in the form of a paste. Next, the extraction of yolk cholesterol was dissolved with 3 ml of chloroform. After that, it was reacted with a solution of a mixture of sulfuric acid and acetic anhydrite as much as 1.05 ml, then shaken with a vortex until it formed a green color and kept in the darkroom for 15 minutes (this process was carried out in a dark room). The absorption of cholesterol samples was measured using a spectrophotometer (Shimadzu UV-1800) at a wavelength (λ) of 680 nm. The absorbance obtained was entered into the linear equation of the standard curve, namely $y = 3.1055x + 0.0299$, the concentration of yolk cholesterol (mg/ml) was obtained.

To get the yolk cholesterol value in fresh ingredients (mg / 100g), here is the calculation:

$$\frac{\text{(y/b) (mg/ml) x the dilution factor (ml) x \% dry weight yolk x 100}}{\text{sample weight (g) x 100}}$$

y : absorbans b : slope

Table 3. Composition of the experimental diets containing different levels of fermented *Sargassum binderi* (%).

Ingredients (%)	Control	4%	8%	12%	16%
Concentrate K 38 Royal	30.10	30.10	30.10	30.10	30.10
Maize	45.35	46.36	46.36	46.36	46.36
Rice bran	20	13.94	9.14	4.34	0
Palm oil	0	0.60	1.40	2.20	2.54
Limestone	4.55	5	5	5	5
Fermented *S.binderi*	0	4	8	12	16
Calculated or determined analysis	100	100	100	100	100
Crude protein(%)	16.08	16.16	16.25	16.34	16.47
Crude lipid (%)	2.67	3.16	3.86	4.51	4.82
Crude fiber (%)	6.32	5.94	5.74	5.54	5.42
Ca (%)	3.33	3.44	3.45	3.47	3.48
P (%)	0.36	0.35	0.34	0.34	0.33
ME (kg/kkl)	2603.69	2614.92	2628.91	2642.91	2624.43
Methionine (%)	1.03	1.03	1.04	1.04	1.05
Lysine (%)	1.42	1.48	1.55	1.45	1.70
Alginate (%)	0	1.17	2.33	3.50	4.66

Statistical analysis: Data were analyzed statistically with ANOVA One-way with a completely randomized design. The difference among treatment means was determined using the Duncan Multiple Range Test (DMRT) ($P<0.05$) (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Plasma Lipid Profile: The effect of fermented *Sargassum binderi* levels in laying hens' diet on total cholesterol, LDL (Low-Density Lipoprotein), and blood serum triglycerides of laying hens is shown in Table 4.

Table 4. The Content of Cholesterol Total, LDL, and Triglyceride in Plasma Lipid of Laying Hens.

Level of Fermented *S. binderi* (%)	Cholesterol Total (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)
0	211.60^{a}±29.75	95.55^{a}±23.81	616.13±2.86
4	152.49^{b}±1.36	49.05^{b}±5.81	615.77±0.97
8	155.24^{b}±30.71	47.92^{b}±5.65	585.85±64.04
12	151.50^{b}±17.25	45.55^{b}±15.84	575.00±40.30
16	135.65^{b}±21.00	38.07^{b}±8.79	588.83±50.50

Means followed by different letters in superscript in same column differ significantly ($p < 0.05$)
LDL = *Low Density Lipoprotein*

The results of variance analysis showed that different levels of fermented *S. binderi* in the laying hen's diet had a significant effect ($p < 0.01$) on the total cholesterol and LDL content of laying hens' blood serum but had no significant impact ($p > 0.05$) on triglycerides. DMRT further test results showed total cholesterol, and LDL serum blood of laying hens in the control treatment (0% fermented *S. binderi*) was significantly different ($p < 0.01$) with 4, 8, 12, and 16% fermented *S. binderi* in the diet. However, treatments 4, 8, 12, and 16% fermented *S. binderi* showed no significant difference ($p > 0.05$) between treatments. The mean total cholesterol, LDL, and blood serum triglycerides of laying hens can be observed in Table 4.

Total cholesterol and LDL in blood serum, which decreased by using fermented S. binderi in the laying hen's diet, were influenced by the polysaccharides contained in fermented *S. binderi*, especially alginate. Brown seaweed polysaccharides, such as alginate, fucoidan, mannitol, and laminaran, are soluble fibers (Yuan, 2008; Casas, 2009; El-Said and El-Sikaily, 2013). Jensen *et al*. (2013) reported that cholesterol decreased because calcium alginate could bind to bile salts. Furthermore, Idota *et al*. (2016) described that calcium

alginate binds bile salts to inhibit the reabsorption of bile salts in the digestive tract. Thus, bile salts are excreted with feces because this increases the metabolism of cholesterol to bile salts in the liver, thereby reducing cholesterol levels. This opinion also follows Al-Harthi and El-Deek (2012), the soluble fiber from S. dentifebium influences the decreased cholesterol and LDL in blood ser*um*.

Alginate content of each diet containing fermented *S. binderi* 4, 8, 12, and 16% in this study were respectively 1.17; 2,33; 3.50; and 4.66%. The increased fermented *S. binderi* in the diet, the higher alginate content (1.17–4.66%), but this alginate had a no different effect on total cholesterol and LDL of blood serum in layer hens. The alginate causes the issue in fermented *S. binderi*, which is still in the form of alginate mixed with other elements (crude alginate), which led to the difference in the alginate dose between these treatments having the same effect on total cholesterol and LDL.

The decrease in total blood serum cholesterol in this study follows the research of Al-Harthi and El-Deek (2012). Adding 3 and 6% *S. dentifebium* can reduce blood cholesterol, with an average blood serum cholesterol of 103–166. mg/dl, but this different concentration of seaweed does not affect blood cholesterol. Furthermore, it was explained that the treatment of 0, 3, and 6% addition of *S. dentifebium* s in the diet of laying hens reduced LDL in blood serum by an average of 41.3–70.9 mg/ml.

Blood serum triglycerides which are not affected by using fermented *S. binderi* with different levels in the diet, can be caused by the synthesis of triglycerides in the livers of laying hens that cannot be inhibited by fermented *S. binderi* polysaccharides, such as alginates because alginates work in the digestive tract to inhibit fat entry. Following El-Said and El-Sikaily (2013) opinion, seaweed polysaccharides, such as alginate, fucoidan, mannitol, and laminaran, are soluble fibers. These compounds have a hypercholesterolemic effect which forms the formation of viscosity system in the small intestine, which causes a decrease in the rate of nutrients absorption, such as glucose and fat in the blood, then forms an ionized colloid, after which it is excreted with feces (Kiriyama *et al*., 1968; Lamela *et al*., 1989; and Panlasigui *et al*., 2003). Also, although fermented S. binderi polysaccharides can inhibit fat absorption from the diet, the liver will carry out lipogenesis to meet the needs of laying hens for fat to form yolk. Following the opinion of Hermier (1997), if the poultry diet contains low fat (>50 g/kg), then the liver will carry out lipogenesis to convert glucose into triglycerides that all tissues can use. Klasing (1998) states that poultry liver cells can synthesize saturated fatty acids from non-lipid substrates, for example, de novo synthesis, and oxidize them to unsaturated single or double chain fatty acids. The high synthesis of triglycerides in the liver in laying hens during the production period is because triglycerides are a component of fat that is very important for the formation of yolk. Following the opinion of Sato *et al*. (2004) stated that high serum lipids in laying hens were closely related to the yolk lipid requirement for oocyte growth. Oocyte development has been identified as a characteristic for yolk lipid storage for laying hens (Schneider *et al*., 1998 and Schneider, 2016). In chickens that enter the early egg-laying phase, the lipid content, including free fatty acids, triglycerides, and phospholipids, is significantly increased in blood plasma to synthesize yolk precursors in oocytes (Moon, 2018). According to Alvarenga *et al*. (2011), during the egg production phase, the size of the chicken liver increases due to the intensity of the synthesis of triglyceride-rich lipoproteins by the liver to meet this need. Hermier (1997) found that lipogenesis in chicken livers was high and was particularly active in laying hens that produced eggs because it was influenced by increased estrogen secretion.

This study follows the research of Al-Harthi and El Deek (2012), giving *S. dentifebium* with concentrations of 0, 3, and 6% did not affect blood triglycerides of laying hens. However, different research results were found in the study by Choi *et al*. (2018), giving brown seaweed by-product and fermented brown seaweed by-product and fusiforme seaweed and fermentation of fusiforme seaweed each of 0.5% in the diet of laying hens can increase blood triglycerides while control treatment (without seaweed) can't afford.

Crude Fat and Cholesterol of Yolk: The effect of fermented *Sargassum binderi* levels in layer hens diet on crude fat and cholesterol in yolk is shown in Table 5.

Table 5. The Content of Yolk Lipid and Cholesterol.

Level of Fermented *S. binderi* (%)	Yolk Lipid (g/hen/d)	Yolk Cholesterol mg/100 g
Control	54.04±0.78	1279.54^{a}±49.60
4	53.82±1.17	1202.06^{ab}±118.11
8	53.46±0.58	1163.42^{ab}±120.02
12	53.56±0.79	1084.98^{b}±98.65
16	54.07±0.79	1074.30^{b}±79.26

Means followed by different letters in superscript in same column differ significantly ($p < 0.05$)

The results of the variance analysis showed that different levels of fermented *S. binderi* in the diet of laying hens had no significant effect ($p > 0.05$) on yolk fat and had a significant impact ($p < 0.05$) on yolk cholesterol. DMRT further test results showed that yolk cholesterol at 0% PORL treatment was significantly different ($p < 0.05$) with treatment 12 and 16% PORL, and not meaningfully different ($p > 0.05$) with treatment 4 and 8% fermented *S. binderi* in the treatment diet. Treatments 8, 12, and 16% fermented *S. binderi* showed no significant difference ($p > 0.05$) with one another.

In this study, yolk fat treated with fermented *S. binderi* 4, 8, 12, and 16% did not differ from the control diet (0% fermented *S. binderi*). The fermented is to be influenced by the high synthesis of triglycerides in the liver of laying hens which cannot be inhibited by fermented *S. binderi* polysaccharides, such as alginates, because alginates work in the digestive tract to inhibit fat entry. Also, although PORL polysaccharides can hinder fat absorption from the ration, the liver will carry out lipogenesis to meet the needs of laying hens for fat to form yolks. Following the opinion of Hermier (1997), if the poultry diet contains low fat (> 50 g / kg), then the liver will carry out lipogenesis to convert glucose into triglycerides that all tissues can use. Alvarenga *et al*. (2011) stated that during the egg production phase, the size of the chicken liver increases due to the intensity of the synthesis of triglyceride-rich lipoproteins by the liver to meet the needs yolk formation. He also stated that a large amount of fat deposition is required to form yolk to produce several eggs. The yolk contains around 50% solids (Puertas and Vázquez, 2018), most of these solids are fat by 65–67% (Laca *et al*., 2010). Therefore, the composition of egg lipid consists of 70% triglycerides, 25% phospholipids, and 5% cholesterol (Scanes *et al*., 2004).

Yolk cholesterol that decreases with the provision of fermented *S. binderi* in the laying hen's diet is influenced by the decrease in cholesterol and LDL in the blood serum of layer hens. The reduction is because the supply of cholesterol to the yolk is reduced. The chemical compounds influence the decreasing cholesterol level in fermented *S. binderi*, namely seaweed polysaccharides, especially alginates. This opinion follows Al-Harthi and El-Deek (2012). In this study, the reduction in yolk cholesterol was influenced by soluble fiber and PUFA (Poly Unsaturated Fatty Acid) from *S. dentifebium* seaweed. Seaweed polysaccharides, such as alginate, fucoidan, mannitol, and laminaran, are soluble fibers (El-Said and El-Sikaily, 2013). These compounds have a hypercholesterolemic effect which causes the formation of a viscosity system in the small intestine, which causes a decrease in the rate of absorption of nutrients, such as glucose and lipid in the blood, then forms an ionized colloid, after which it is excreted with feces (Kiriyama *et al*., 1968; Lamela *et al*., 1989; and Panlasigui *et al*., 2003). According to Wolever *et al*. (1997), there are four mechanisms of cholesterol reduction by soluble fiber, namely: 1) Bile acid-binding in the small intestine, which causes increased excretion of fecal bile acids, 2) Decreased absorption of fat and cholesterol, 3) Decreased absorption rate of carbohydrates which leads to decreased levels serum insulin to reduce the stimulation of cholesterol and lipoprotein synthesis, and 4) Inhibition of cholesterol synthesis by short-chain fatty acids resulting from soluble fiber fermentation in the colon.

The alginate content of each diet containing fermented *S. binderi* 4, 8, 12, and 16% in this study were respectively 1.17; 2.33; 3.50; and 4.66%. The increase in fermented *S. binderi* in the diet, the higher the alginate content interval (1.17–4.66%), but this alginate interval did not have a different effect on chicken yolk cholesterol. The yolk cholesterol did not affect caused by the alginate in fermented *S. binderi,* which is still in the form of alginate mixed with other elements (crude alginate), so that the different alginate dose intervals between these treatments have the same effect on yolk cholesterol content.

Yolk cholesterol obtained in this study was 1,074.30 ± 79.26–1,279.54 ± 49.60 mg/100g is in the range of yolk cholesterol content based on the Animal Research Institute for 1100–1230 mg/100g (Ariani, 2006). The yolk cholesterol in this study was below the yolk cholesterol obtained by Al-Harthi and El-Deek (2011), amounting to 1280–1550 mg/100g, and Attia *et al*. (2014), yolk cholesterol from eggs on the market is 1310–1606 mg/100g. According to Ariani (2006), the cholesterol content of eggs can differ from one another due to several factors, including feed intake, age and varieties of chickens, environmental temperature, and production rate.

The cholesterol reduction in this study was following Al-Harthi and El-Deek (2012) research. The addition of *S. dentifebium* by 3 and 6% could reduce yolk cholesterol by 11.61, 17.42%, respectively, but this different concentration of seaweed did not affect yolk cholesterol. Furthermore, research by Carrillo *et al*. (2012), the addition of *Sargassum* spp. as much as 4, 6, and 8% can reduce egg cholesterol by 13, 26, and 19%, respectively, while the addition of 2% seaweed has not been able to reduce egg cholesterol. The occasion also experienced by the research of Ginberg *et al*. (2000), egg cholesterol decreased significantly (control: 12.5 mg/g vs. seaweed treatment: 9.5 and 10 mg/g yolk cholesterol) with the addition of *Porphyridium* spp. as much as 5 and 10%. Based on the results of this study, it can be seen that the addition of 16% fermented *S. binderi* in the diet of laying hens affects the cholesterol content of yolk from 1,279.54 to 1,074.30 mg/100 g of yolk with a decrease in cholesterol by 16.04%.

Conclusion: The provision of fermented *S. binderi* to 16% in the diet of laying hens can reduce total blood serum cholesterol from 211.60 to 152.49 mg/dl and LDL from 95.55 to 49.05 mg/dl, with a decrease of 27.93% and 48.66%, respectively, and decreased yolk cholesterol from 1,279.54 to 1.074.30 mg/100g with a decline of 16.04%.

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