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Effects of Fermented *Sargassum binderi* Meal on Productivity and Egg Quality of Laying Hens

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ABSTRACT

Brown algae, such as *Sargassum binderi* (*S. binderi*), are abundantly present in marine ecosystems and constitute a valuable natural resource. The present study aimed to investigate the effect of fermented *S. binderi* meal on the performance of laying hens and egg quality, as well as to establish the safe limits of its usage in the diet of laying hens. The 200 Isa Brown laying hens were randomly assigned to five different treatment groups based on fermented *S. binderi* levels, including the control group, which received 0% fermented *S. binderi*, while the other treatment groups received 4%, 8%, 12%, and 16% fermented *S. binderi*, respectively, over six weeks. At the end of the experiment, 80 eggs were assessed for quality. The variables included feed intake, hen day egg production, egg weight, egg mass, and feed conversion ratio, as well as external egg quality measures such as eggshell weight, percentage of eggshell, eggshell thickness, eggshell strength, egg length, egg width, and egg shape index and internal egg quality parameters including yolk weight, percentage of yolk, albumin weight, percentage of albumin, yolk color index, and haugh unit (HU). The current results indicated that the inclusion of fermented *S. binderi* at levels of 0, 4, 8, 12, and 16% in laying hens' diets had no significant effect on performance or external and internal egg quality. The utilization of fermented *S. binderi* is considered safe when incorporated at levels up to 16% within diets for laying hens.

Keywords: Egg quality, Fermented *Sargassum binderi*, Laying hen, Performance, *Sargassum binderi*

INTRODUCTION

Algae, encompassing brown, red, and green varieties, serve as biomass sources that grow and develop naturally and are readily cultivated. Consequently, the availability of this biomass is substantial (Cabrita et al., 2016). The Food and Agriculture Organization of the United Nations reported that global algae production amounted to 35.8 million tons, contributed by 54 countries, with 97% of the production derived from cultivation (FAO, 2021). The leading nations in algae production are Norway, China, and Chile for wild brown and red algae; China, Indonesia, the Philippines, and South Korea engage in the cultivation of *Undaria pinnatifida*, Euchema, Gracilaria, and Japanese kelp (FAO, 2018).

The rising cost of conventional poultry feed ingredients has prompted increasing interest in exploring alternative, sustainable feed resources. Marine macroalgae, such as brown algae, have emerged as promising candidates due to their rich nutritional profiles and bioactive compounds (Michalak and Chojnacka, 2015). Brown algae consisting of *Ascophyllum* spp., *Fucus* sp., *Hizikia* spp., *Laminaria* spp., *Macrocystis* spp., *Padina* sp., *Sargassum* spp., *Turbinaria* spp., and *Undaria* spp. (Michalak and Mahrose, 2020). *Sargassum binderi* (*S. binderi*) is a species of brown algae that has been investigated for its potential application as poultry feed (Dewi et al., 2024).

The recent studies demonstrated that the nutritional content and polyphenolic compounds of algae have beneficial effects on health, which has increased scientific interest in exploring the potential applications of algae (Li et al., 2025). Algae contain different components, including polysaccharides such as alginate, fucoidan,

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laminarin, and cellulose in brown algae, carrageenan, agar, xylan, cellulose, and porphyran in red algae, and xylan, ulvan, cellulose, inulin, and pectin in green algae. Algae also have proteins, essential amino acids, minerals, vitamins, lipids, polyunsaturated fatty acids, pigments such as carotenoids, chlorophyll, phycobilins, and fucoxanthin, as well as antioxidant compounds, particularly polyphenols (Michalak and Chojnacka, 2015; Øverland et al., 2018). Polyphenols, which are found in algae, have antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, and immunomodulatory properties (Michalak and Chojnacka, 2015; Corino et al., 2019). These compounds have hypocholesterolemic, antithrombotic, anticoagulant, antilipidemic, hypo-cholesterolemic, and antitoxic activities (Kumar et al., 2021). In addition to algae's abundant availability, the unique and diverse nutritional compositions of different algae species make them attractive and potentially advantageous for inclusion in poultry feed formulations.

Supplementation of algae in poultry diets has been widely investigated and developed. Including algae in poultry feeding has several positive impacts on different performances, including increased egg-laying rate, improved egg composition and quality parameters, enhanced growth performance, and acting as a prebiotic. Additionally, supplementation of algae enhances immune activities, improves intestinal villi, and boosts useful bacteria (Michalak and Mahrose, 2020). Alga is a new, natural, environmentally friendly, and healthy feed ingredient that can produce eggs enriched with active compounds such as fatty acids, amino acids, vitamins, and pigments, along with functional properties such as antioxidant, anti-inflammatory, and antimicrobial effects (Michalak and Chojnacka, 2015; Qadri et al., 2019).

However, despite these nutritional and functional benefits, the direct application of algae in poultry diets encounters several limitations. Algae contain anti-nutritional factors such as high sodium chloride (NaCl) (Dewi et al., 2018), as well as heavy metals, tannins, and mannitols (Stengel et al., 2011), which may interfere with nutrient absorption. In addition, algae have low energy content (Dewi et al., 2024) and indigestible nutrients that reduce feed efficiency (Holdt and Kraan, 2011; Dewi et al., 2019). High NaCl and alginate levels limit the use of *S. binderi* as poultry feed (Dewi et al., 2018; 2019). According to Dewi et al. (2018), it has been found that the alginate and NaCl contents of *S. binderi* were 20.68% and 17.20%, respectively. Laying rations contain NaCl at a concentration of 0.33% (NRC, 1994). Previous studies have demonstrated that elevated levels of NaCl in poultry feed can induce diarrhea, mortality, and ascites (Zhang et al., 2013; Dewi et al., 2018). Adding algae-derived polysaccharides to poultry feed may cause a decrease in performance due to their ability to bind and reduce nutrient absorption in the digestive tract (Jacob, 2025).

To address the limiting factors associated with the utilization of *S. binderi*, processing procedures are necessary. The NaCl content can be reduced by immersing the seaweed in flowing water for a duration of 15 hours, resulting in a reduction of the salt content from 16.86% to 0.94% (Dewi et al., 2018). Meanwhile, the alginate content can be reduced through fermentation. Fermentation can enhance the nutritional quality of seaweed and extend its shelf life (Choi et al., 2018; Dewi et al., 2019). In brown seaweed, alginate can be depolymerized using the alginate lyase enzyme (Guarino et al., 2015). *Bacillus megaterium* (*B. megaterium*) S245 is one of the bacterial strains capable of producing alginate lyase enzyme (Subaryono et al., 2016). Fermentation with *B. megaterium* S245 can reduce the alginate content of *S. binderi* from 37.10% to 31.77%, representing a 14.37% decrease (Dewi et al., 2019). The present study aimed to determine the safe usage limits of fermented *S. binderi* meal within the diet of laying hens and to assess its effects on egg quality as well as the performance of the laying hens.

MATERIALS AND METHODS

Ethical approval

The present study was conducted in compliance with the regulations established by the Official Ethics Committee for Animal Care and Welfare of Andalas University, Padang, Indonesia (577/KEP/FK/2019).

Collecting and processing of *Sargassum binderi*

The collection of *S. binderi* was carried out using a random sampling method at Sungai Nipah Beach (coordinates: -1.371664, 1.371664,100.579617/1o22'18.0" S 100o34'46.6" E), West Sumatra, Indonesia. The present study used *S. binderi* individual parts, such as the talus, bladder, and holdfast. The alga was then submerged in the flowing water of the River Gunung Nago, located in the Pauh District of Padang, West Sumatra, Indonesia, with a flow rate of 0.6745 m³ per second and a depth of 1.3 meters for 15 hours (Dewi et al., 2018). Following immersion, the alga was harvested and dehydrated in an oven at 60℃ until the moisture content reached around 14%, then milled into a powder with a particle size of 0.5 mm. Furthermore, the alga flour was fermented with

Bacillus megaterium S245. The fermentation process involved liquid fermentation. *S. binderi* flour, palm sugar, and water were used as the substrate. The ratio of alga and water was 1:5, with palm sugar added at 3% of the total volume of substrate. Before the fermentation process began, the substrate was sterilized using an autoclave at 121°C for 15 minutes. Then, inoculated with *B. megaterium* S245 at a concentration of 27×10^7 CFU/mL. The fermentation process was conducted under anaerobic conditions for nine days at ambient temperature (25-35°C). After the fermentation process finished, the fermented alga was removed from the plastic and then aerated. Additionally, the fermented alga was dried, placed into a plastic bag, and stored at room temperature. The following process was carried out on proximate, metabolic energy, alginate, and fucoxanthin (Table 1), based on Sibbald (1986), AOAC (1990), Hegazi et al. (1998), and Dewi et al. (2019), respectively. Amino acid content determination was carried out based on the method of IK.LP-04.7-LT1.0 (HPLC; Table 2).

Animals and diets

Two hundred Isa Brown laying hens (body weight: 1571-1586 g; age: 62 weeks old) were-randomly assigned to five treatments with four replications. Each replication consisted of ten layers for each group. The treatment groups were based on fermented *S. binderi* levels, including the control group, which received 0% fermented S. *binderi*, while the other treatment groups received 4%, 8%, 12%, and 16% fermented *S. binderi*, respectively, over six weeks as-fed in daily rations. The diet calculation was conducted based on NRC (1994), and water was prepared *ad libitum* during the study period (Table 3).

Performance and egg qualities

Each day, eggs were collected, and their production was recorded. Weekly summaries were provided for feed intake, conversion ratio, production, and egg weight. Eighty eggs were randomly selected (Four eggs from each replicate of treatment) on the last two days of the study and used to evaluate egg quality. Internal egg quality was measured by yolk weight, albumin weight, and albumin percentage, while external egg quality was assessed by the weight, thickness, haugh unit (HU), strength, length, and width of the eggshell. Egg length and width were measured using a caliper. The percentage of egg yolk, shell, and albumin was obtained by dividing the weight of the yolk, shell, and albumin by the weight of the egg and multiplying by 100%. Yolk color was evaluated using a Roche yolk color fan. Eggshell strength was assessed using an EGG Shell Force Gauge EFG-0502, while eggshell thickness was determined with a micrometer accurate to 0.01 mm. The shape index (SI) was calculated using the following formula. Width/length × 100 (Anderson et al., 2004). The HU was calculated using the following formula.

$$HU = 100 \times \log (H + 7.57 - 1.7 \times W^{0.37})$$

H means the height of the thick albumen in millimetres, and W is the egg weight in grams (Silversides and Villeneuve, 1993).

Statistical analysis

The data was evaluated by variance analysis using the WPS Excel-Statistics 2022 version 11.2.0.11254 software in a properly randomized design. To normalize data distribution, the hen-day egg production (HDEP) and eggshell percentages were logarithmically adjusted using log10 (x + 1). The Duncan Multiple Range Test (DMRT) was performed to compare mean values for each treatment, and $p < 0.05$ indicated a significant difference (Steel et al., 1997).

Table 1. The nutrient content of fermented *Sargassum binderi* meal

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 (Ca) (%)	1.19
Phosphor (P) (%)	0.26
Natrium (Na) (%)	0.37
Metabolisable energy (kcal/ kg)	477.87
Alginate (%)	29.13
Fucoxanthin (µg/g)	2.3458

Table 2. Amino acid composition of fermented *Sargassum binderi* meal

Amino acid	Percentage
Aspartic acid	0.56
Glutamic acid	0.32
Serine	0.28
Histidine	0.74
Glycine	0.31
Threonine	0.37
Arginine	0.27
Alanine	0.15
Tyrosine	0.29
Methionine	0.48
Valine	0.15
Phenylalanine	0.28
Isoleucine	0.36
Leucine	0.30
Lysine	2.45
Total amino acid	7.32

Table 3. Different fermented *Sargassum binderi* meal (%) levels in the experimental diet compositions of laying hens

Ingredients (%)	**Control**	**4 (%)**	**8 (%)**	**12 (%)**	**16 (%)**
Concentrate K 38 Royal Maize	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* meal	0	4	8	12	16
Chemical 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
Metabolisable Energy (kcal/kg)	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

Note: Concentrate K 38 Royal is a commercial concentrate feed for laying hens from PT Leong Hup Jayapindo; Ca: Calcium; P: Phosphorus

Table 4. Laying hen productive performance of laying hens

Level of fermented *Sargassum binderi* (%)	**Feed intake (g/hen/d)**	**HDEP (%)**	**Egg weight (g/egg)**	**Egg mass (g/hen/d)**	**Feed conversion ratio**
Control	116.59	84.11	60.29	50.76	2.30
4	114.26	85.48	60.88	52.12	2.20
8	116.65	85.71	60.01	51.30	2.28
12	115.16	81.55	60.73	49.50	2.33
16	114.39	81.41	60.74	49.41	2.32
SEM	1.28	1.77	0.93	0.89	0.04
P-value	0.69	1.19	0.11	1.64	1.95

Note: HDEP: Hen day eggs production, SEM: Standard error of the mean

Table 5. Egg external quality criteria of laying hens

Egg external quality	**Level of fermented *Sargassum binderi* in Diet (%)**					**SEM**	**P-value**
	Control	**4**	**8**	**12**	**16**		
Eggshell weight (g)	6.60	6.96	6.66	6.84	6.84	0.12	1.54
Eggshell (%)	10.95	11.49	10.93	11.25	10.88	0.17	2.45
Eggshell thickness (mm)	0.34	0.33	0.35	0.37	0.35	0.01	1.54
Eggshell strength (kg/cm^2)	3.00	3.68	3.51	3.55	3.37	0.25	1.08
Egg length (cm)	5.65	5.60	5.68	5.63	5.75	0.05	1.21
Egg width (cm)	4.27	4.29	4.28	4.31	4.30	0.03	0.27
Egg shape index/SI	75.66	76.56	75.37	76.67	74.93	0.71	1.14

Note: SEM: Standard error of the mean

Table 6. Egg internal quality criteria of laying hens

Egg internal quality	**Level of fermented *Sargassum binderi* in diet (%)**					**SEM**	**P-value**
	Control	**4**	**8**	**12**	**16**		
Yolk weight (g)	14.63	14.84	14.67	14.51	14.81	0.34	0.15
Yolk (%)	24.24	24.51	24.06	23.87	23.53	0.37	1.00
Albumin weight (g)	39.13	38.76	39.65	39.45	41.21	0.78	1.46
Albumin (%)	64.81	64.00	65.01	64.89	65.58	0.45	1.63
Yolk color index	7.95	8.41	8.13	8.48	8.37	0.17	1.61
Haugh unit (HU)	96.56	94.87	98.04	94.55	94.41	0.66	1.46

Note: SEM: Standard error of the mean

RESULT AND DISCUSSION

Laying hen performance

Table 4 shows the effect of fermented *S. binderi* meal in laying hens' diets on production performance. The current results revealed that including fermented *S. binderi* meal in the laying hen feed had no significant effect on production performance ($p > 0.05$).

Feed intake

The inclusion of fermented *S. binderi* in the diet of laying hens up to a level of 16% did not influence feed intake. This outcome can be attributed to the fermented *S. binderi* utilized in the present study, which contained a low sodium chloride (NaCl) content, as a processing method was employed to decrease the NaCl concentration in flowing water. Consequently, the NaCl level was reduced to 0.94%, with 0.37% sodium (Na). The inclusion of fermented *S. binderi* up to 16% in the diet of laying hens, with a Na concentration of 0.13%, was considered safe and did not negatively impact their feed consumption. The recommended Na levels in poultry diets generally range from 0.17% to 0.19% (Baloš et al., 2016). Furthermore, the fermentation process utilizing *B. megaterium* S245 facilitated the depolymerization of alginate in the seaweed, thereby enhancing its potential as a feed ingredient (Dewi et al., 2019).

The alginate content in diets incorporating 0%, 4%, 8%, 12%, and 16% fermented *S. binderi* was recorded as 0, 1.17, 2.33, 3.50, and 4.66%, respectively. It has been documented that the inclusion levels of alginate and its derivatives, such as polymannuronates, in poultry diets are 0.04% and 0.2%, whereas Na alginate oligosaccharides are utilized at levels ranging from 0.1% to 0.4%. (Yan et al., 2011; Zhu et al., 2015). While the alginate content in the present study exceeded the levels previously reported, the alginate in the diets was broken down into its simpler form, alginate oligosaccharides, which facilitated digestive enzymes to penetrate the laying hens' gastrointestinal tract, breaking down nutrients more effectively. As a result, nutrients can be digested and absorbed without being hindered by the alginate present in the diet. Ertesvåg (2015) reported that the alginate lyase depolymerizes alginate to form unsaturated uronic acid. In addition, low molecular weight polymannuronates, a derivative of alginate, in broiler diets are more efficient than natural seaweed fibers (alginate) with higher molecular weights (Zhu et al., 2015).

The previous study has shown that depolymerized alginate or alginate oligosaccharides are useful as a prebiotic (Wang et al., 2006). According to Kulshreshtha et al. (2014), prebiotics can increase the proliferation of beneficial bacteria, such as *Streptococcus salivarius* (4-to15-fold) and *Bifidobacterium longum* (4-to14-fold), and reduce the unfavourable bacterial population of *Clostridium perfringens* in the laying hen digestive tract. These bacteria facilitate the production of secondary metabolic enzymes to degrade undigested feed ingredients in the digestive tract (Flint et al., 2012). Wan et al. (2017) reported that utilizing alginate oligosaccharides in pork rations can improve the nutrient digestibility, including crude protein, fat, and ash.

In addition to the alginate content that did not affect the laying hen feed intake in the present study, the darker color of the ration containing fermented *S. binderi* meal, compared to the control ration (0% fermented *S. binderi* meal), did not impact the palatability of the feed for the hens. The current results supported the findings of Farghly and Abou-Kassem (2014) and Farghly and Mahrose (2017), indicating that providing colorless or colored feed (red, orange, and green) does not affect turkey feed consumption at 0-16 weeks of age. Adding fermented *S. binderi* to the diet made the color of laying hen feed darker, but it did not affect its taste and was believed to meet the poultry's nutritional requirements. Feed energy substances in the laying hen diet in the present study were in accordance with the laying hen's requirements and were given every day. According to Classen (2017), a characteristic specific to poultry is their feed intake for energy acquisition, and consequently, daily feed intake is likely correlated with feed energy content.

The feed intake of laying hens in the present study aligns with the findings of Fan et al. (2021), indicating that the inclusion of up to 5% *Sargassum* meal did not influence feed intake. However, supplementing the diet of laying hens with *S. dentifebium* 3% reduced feed intake (Al-Harthi and El-Deek, 2011), and supplementing with *Sargassum* sp. 2% decreased feed intake in laying hens (Carrillo et al., 2012).

Unaffected feed intake helped stabilize HDEP, which was possible because the digestive tract of laying hens continued to digest and absorb nutrients normally, meeting their energy and nutrient requirements for egg production. According to Jacob et al. (2014), laying hens require a balanced diet to maintain egg production. A lack of energy, essential nutrients such as protein, especially lysine and methionine, or calcium can disrupt egg production, leading to a halt in egg-laying (Alagawany et al., 2021).

Hen day egg production

The HDEP observed during the current study ranged from 81.41% to 85.71%, aligning within the HDEP interval reported by previous studies, specifically between 58.3% and 91.28%, through the administration of different types of algae at levels of 0.25% to 3% in the diet of laying hens (Halle et al., 2009; Al-Harthi and El-Deek, 2011; Carrillo et al., 2012; Kulshreshtha et al., 2014; Park et al., 2015). However, previous studies indicated a decrease of more than 3% in HDEP caused by *Sargassum* (Al-Harthi and El-Deek, 2011; Carrillo et al., 2012) and 5% in laying hens' diet (Fan et al., 2021), which could be due to the high Na content. A decrease in HDEP can also be caused by high algal tannins, which interfere with the digestion process and protein absorption. The difference in HDEP caused by adding alga to laying hen diets can be affected by chicken strain, age, algae type, algae concentration, and several environmental factors (Zhang et al., 2012).

The inclusion of fermented *S. binderi* in the diet at levels up to 16% did not influence the HDEP of laying hens, which was attributed to the observation that the feed intake of the laying hens was not inhibited by the incorporation of fermented *S. binderi* into their diet. The HDEP of laying hens is influenced by multiple factors, including the quality of the diet, the quantity of feed consumed, and its nutritional and energetic content (Jacob et al., 2014). Furthermore, the HDEP among different treatments remained consistent, given that *S. binderi* underwent processing through soaking to reduce salt content and fermentation to depolymerize alginate into simpler compounds. Consequently, the increased incorporation of fermented *S. binderi* meal into the diet of laying hens in the present study did not hinder nutrient digestion and absorption necessary for egg production.

Egg weight

In the present study, the inclusion of 4%, 8%, 12%, and 16% fermented *S. binderi* in the diet exhibited a comparable effect on egg weight. The lack of significant differences in egg weight among treatments may be attributed to the alginate presented in fermented *S. binderi*, which has depolymerized into its simpler form, namely alginate oligosaccharide. Consequently, alginate in the form of alginate oligosaccharide does not hinder the absorption of protein, fat, and minerals, including calcium and phosphorus (Wan et al., 2017), necessary for egg formation, thereby influencing egg weight. The egg weight obtained in the present study (60.01-60.88 g/egg) remained within the egg weight range reported by several previous studies, including those by Halle et al. (2009), Al-Harthi and El-Deek (2011), Carrillo et al. (2012), Kulshreshtha et al. (2014), and Park et al. (2015). These studies indicated that the addition of algae at different types and concentrations, ranging from 0.25% to 8%, did not significantly affect the egg weight of laying hens. The reported egg weight range in these studies was between 52.30 g and 66.82 g per egg. Nonetheless, Al-Harthi and El-Deek (2011) observed that dietary inclusion of 6% *Sargassum dentifebium* resulted in a significant reduction in egg weight.

Egg mass

The egg mass is influenced by HDEP and egg weight. The HDEP and egg weight measured during the present study were unaffected by adding all levels of fermented *S. binderi* (4, 8, 12, and 16%) to the laying hen diet. According to Figueiredo et al. (2012) and Cesari et al. (2014), egg mass is calculated by multiplying egg production by the average egg weight during each period. The lack of effect on HDEP and egg weight may be due to stable energy intake and nutrient availability, as the inclusion of fermented *S. binderi* in the diet did not interfere with digestive function or nutrient absorption in the hens.

The egg mass obtained in the present study (44.73-52.12 g/hen/day) was within the range reported by previous studies. According to Halle et al. (2009), Al-Harthi and El-Deek (2011), and Choi et al. (2018), adding algae at different concentrations from 0.25% to 3% did not affect the egg mass of laying hens, with reported values of 40.04-60.2 g/hen/day. Studies by Al-Harthi and El-Deek (2011) exhibited a decline in egg mass when *Sargassum* was used at 3% in the diet, and Fan et al. (2021) found a similar decrease at 5%. Egg mass levels stayed below the established level for brown laying hens during the current study.

Feed conversion ratio

There was no significant effect ($p > 0.05$) on the feed conversion ratio due to feed consumption, HDEP, and egg weight, which were unaffected by providing fermented *S. binderi* at 4, 8, 12, and 16%. Feed conversion ratio compares feed intake to egg production and the average weight of eggs laid by hens in one day (Kulshreshtha et al., 2014). The addition of fermented *S. binderi* at levels of 4%, 8%, 12%, and 16% did not influence the digestion or absorption of nutrients. Energy and essential nutrients such as protein, including amino acids (lysine and methionine), fatty acids (linoleic acid), calcium, and vitamins, are vital not only for basic physiological functions but also for optimal egg production (Leeson and Summer, 2005).

The current results on feed conversion ratio ranged from 2.20 to 2.49, which were close to the feed conversion reported by several studies. Halle et al. (2009), Carrillo et al. (2012), and Kulshreshtha et al. (2014) reported that adding algae of different types and at different concentrations from 0.25% to 8% did not affect the feed conversion ratio of laying hens' diets, with the feed conversion ratio in these studies ranging from 1.69 to 2.19. However, there was an increase in feed conversion ratio with the addition of alga at 3% and 6% (Al-Harthi and El-Deek, 2011) and *Codrus crispus* by 1% in laying hen feed (Fan et al., 2021). Changes in feed conversion ratio values can be influenced by the age and breed of chickens, species, and algae concentration, as well as differences in experimental environments (Zhang et al., 2012).

Based on previous studies, it can be observed that the inclusion of alga in the diet is ≤ 8% (Carrillo et al., 2012). However, in the present study, the utilization of fermented *S. binderi* at levels up to 16% did not adversely affect feed intake, HDEP, egg weight, egg mass, or feed conversion ratio. The processing of *S. binderi* may be impacted by fermentation with *B. megaterium* S245.

External egg quality

Table 5 illustrates the effect of fermented *S. binderi* meal supplementation on external egg quality in laying hens. The current results demonstrated that its inclusion did not have a statistically significant impact on external egg quality ($p > 0.05$).

The addition of fermented *S. binderi* at 4%, 8%, 12%, and 16% in laying hen diets indicated no difference in external egg qualities compared to a control diet (Fermented *S. binderi* 0% meal). Adding fermented *S. binderi* at these levels did not interfere with the absorption of calcium, phosphorus, vitamin D, and fat in the laying hen's digestive system tract. This was influenced by the low Na content of the diets, specifically the fermented *S. binderi* at different inclusion levels, including 0% (0.08%), 4% (0.09%), 8% (0.11%), 12% (0.12%), and 16% (0.13%). Therefore, the external egg qualities, including eggshell weight, eggshell percentage, eggshell strength, and thickness, were not disturbed. These findings align with Persson (2009), who stated that high Na content negatively impacts eggshell calcium deposition. Persson (2009) further elucidated that elevated NaCl levels significantly diminish eggshell quality due to a reduction in carbonic anhydrase activity within the glands responsible for eggshell formation. The strength of the eggshell is dependent on the mineral and vitamin composition of the feed, notably calcium, phosphorus, manganese, and vitamin D. Laying hens produce eggs with fragile or compromised shells when their feed lacks sufficient calcium (de Abreu Fernandes and Litz, 2017). Furthermore, *S. binderi* underwent fermentation to depolymerize alginate into simpler compounds. The increased inclusion of fermented *S. binderi* meal in the diet did not disrupt the digestion and absorption of nutrients within the gastrointestinal system. Consequently, it did not impact the external quality of the eggs.

The length, width, and shape index of eggs treated with fermented *S. binderi* at levels of 4%, 8%, 12%, and 16% displayed results comparable to the control diet (Fermented *S. binderi* 0%). Furthermore, the measurements of egg length and width are associated with the egg shape index (Duman et al., 2016). According to Duman et al. (2016), the egg shape index represents the ratio of the width of the egg to its length. The egg shape index of the current results indicated the criteria for regular and round eggs. The SI criteria are sharp eggs with an SI value of SI = <72, as regular, ranging from 72 to 76, and round eggs with an SI = 76 (Altuntaş and Şekeroğlu, 2008). Factors influencing the egg shape index include egg albumin quality, egg length, width, eggshell strength (Altuntaş and Şekeroğlu, 2008), albumin index, and egg HU (Duman et al., 2016).

Eggshell strength and thickness of the current results were similar to those reported by Park et al. (2015) for the addition of 0.5 and 1% microalga Schizochytrium, or for diets without microalga, which did not affect eggshell strength (4.20-4.23 kg/cm²) and eggshell thickness (0.37-0.38 mm). The current results indicated that eggshell percentage and eggshell thickness, regarding the supplementation of *S. dentifebium* 0% and 3% in the laying hen diet, did not affect eggshell percentage (13.6-13.8%) and eggshell thickness (0.43-0.44 mm), which was consistent with the result of Al-Harthi and El-Deek (2011).

Internal egg quality

Table 6 presents the impact of fermented *S. binderi* meal supplementation on internal egg quality in laying hens. The current findings indicated that its inclusion had no significant effect on internal egg quality ($p > 0.05$).

Adding fermented *S. binderi* at 4%, 8%, 12%, and 16% in the laying hen diet exhibited no difference compared to the control diet in egg yolk weight and egg yolk percentage, which was influenced by fulfilling nutritional requirements, such as protein and fat. The yolk of the egg is primarily composed of fat and protein constituents. According to Puertas and Vázquez (2018), the egg yolk contains approximately 50% solids. The majority of these solids consist of fat (65-67%) and protein (30%; Laca et al., 2010).

The inclusion of fermented *S. binderi* at levels of 4%, 8%, 12%, and 16% in the diet did not significantly affect albumin weight or albumin percentage compared to the control group, which was influenced by the nutritional requirements of laying hens, which necessitate protein intake for albumin synthesis. According to Bouvarel et al. (2011), a reduction in protein content in feed may result in decreased levels of albumin.

Yolk weight and albumin weight of the present study are consistent with Ozaki et al. (2013), who indicated that *Gracilaria vermiculophylla* 2% supplementation did not affect yolk and albumin weight. The current results align with Al-Harthi and El-Deek (2011), who found that supplementing laying hen diets with 0%, 3%, and 6% *S. dentifebium* did not significantly influence yolk and albumin percentages.

Adding fermented *S. binderi* at concentrations of 4%, 8%, 12%, and 16% to the laying hen diet did not affect the yolk color index compared to the control diet, which was caused by the damage to fucoxanthin in fermented *S. binderi* due to processing methods such as immersion in flowing water to reduce NaCl content, sterilisation using heating during fermentation, and drying. Fucoxanthin is highly sensitive to UV light and high temperatures (Baek et al., 2021).

The yolk color index in the present study aligns with the findings of Al-Harthi and El-Deek (2011), showing that supplementation with *S. dentifebium* at levels of 0%, 3%, and 6% does not affect the yolk yellow index. Furthermore, Park et al. (2015) reported that using 0.5% and 1% microalga Schizochytrium or not including microalga in the laying hen diet did not increase yolk color. A significant increase in yolk color was observed with the inclusion of *Sargassum* spp., at levels of 4%, 6%, and 8% which is inconsistent with the findings of Carrillo et al. (2012). Halle et al. (2009) reported that microalga *Chlorella vulgaris* supplementation at 2.5, 5, and 7.5 g/kg, treated with spray drying, had a significant effect on increasing the yolk color index. Differences in yolk color index among studies may result from several variables, including whether algae were processed or not, the concentration and type of algae used, the carotenoid content in the basal diet, and the addition of extra carotenoids in the experimental feed.

The unaffected HU of eggs resulting from the addition of fermented *S. binderi* to the laying hen diet suggested that the nutritional requirements of the hens were satisfied. Several studies have reported a decrease in HU with increasing dietary energy (Pérez-Bonilla et al., 2012). Additionally, Valkonen et al. (2006) documented elevated HU levels in laying hens subjected to a low-protein diet (14%) in comparison to a high-protein diet (19%). Conversely, HU appears unaffected by the nutrient content of the ration (Wall et al., 2010; Ribeiro et al., 2014; Khatibi et al., 2021; Scappaticcio et al., 2021).

CONCLUSION

The inclusion of fermented *Sargassum binderi* meal at a level of 16% in the diet of laying hens did not adversely impact their performance, external or internal qualities of eggs. Algae have nutrients and bioactive compounds that can meet poultry's nutritional needs, boost their health, and have a positive impact on the environment. Further studies on algae need to be conducted to investigate their potential as a functional feed in poultry diet.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Authors' contributions

Mahata contributed to the conceptualization and project administration. Dewi conducted data collection and drafted the manuscript. Sofyan reviewed and approved the final edition of the manuscript. All authors provided their informed consent to participate in the study, read and approved the final edition of the manuscript.

Competing interests

The authors declared that there are no conflicts of interest related to this research.

Ethical considerations

No ethical issues such as plagiarism, informed consent violations, misconduct, data falsification, duplicate submission or publication, or redundancy were found in this article before submission to the journal.

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