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DIETARY SODIUM ALGINATE EFFECT ON GROWTH, DIGESTION, BODY COMPOSITION, ANTIOXIDANT CAPACITY, AND MUCOUS IMMUNE RESPONSE IN YELLOWFIN SEA BREAM, *ACANTHOPAGRUS LATUS*

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Abstract

In the present study, the effects of dietary supplementation of low molecular weight sodium alginate on growth, body composition, antioxidant enzymes, digestive enzymes, and immune response in yellowfin sea bream juvenile (*Acanthopagrus latus*) were investigated. Fish (n=180) were divided into 3 groups with 3 replicates and received dietary treatments including basal diet without sodium alginate (control treatment), basal diet containing 5 g sodium alginate per kg diet (0.5% treatment), and 10 g sodium alginate per kg diet (1% treatment) for 8 weeks. The results showed that the final weight, weight gain, specific growth rate, condition factor, feed conversion, and protein efficiency ratios did not reveal a significant improvement compared to the control treatment (P>0.05). The obtained results indicated that dietary sodium alginate did not affect body composition (P>0.05). The activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in the groups fed on 0.5 and 1% sodium alginate showed a significant increase (P<0.05) compared to the control group. But, the activity of lipid peroxidation (MDA) in the groups fed on 0.5 and 1% sodium alginate showed a significant decrease (P<0.05) compared to the control group. The digestive enzymes increased significantly (P<0.05) by supplementation of sodium alginate, compared to the control group. Mucus lysozyme and complement activity were not significantly different (P>0.05). Overall, it can be concluded that feeding of yellowfin sea bream on the diet supplemented with 0.5 and 1 percent sodium alginate for a period of 8 weeks does not enhance the non-specific immune response and growth. Nonetheless, sodium alginate improved the activity of the antioxidant and digestive enzymes, indicating the positive effects of sodium alginate on enzymatic responses.

Key words: dietary supplementation, growth and feeding performance, sea bream juvenile, physiological response

In the past decade, the aquaculture sector has played key role in providing essential sources of lipid and protein for human consumption (Naylor et al., 2021). This sector continues to rapidly increase to fulfill the requirements for edible fish for the human population (Costello et al., 2020).

Yellowfin sea bream (*Acanthopagrus latus*) belongs to carnivorous sparid species and is regarded to be a promising marine species because of its great economic value, ability to spawn in captivity, ability to adapt to different culture stressors such as salinity and temperature (Hu et al., 2007; Zarei et al., 2021). Recently, many studies have investigated the effects of different natural extracts on *A. latus*'s growth, health, and performance (Esmaeili et al., 2019; Dehghani et al., 2020; Sangari et al., 2021).

Among the medical herbs that could be used in aquaculture for different beneficiary activities, seaweeds are as a noble source of natural bioactive compounds known for their antibacterial, anti-viral, antioxidative, and anti-inflammatory properties (Ashouri et al., 2020). Low molecular weight sodium alginate (LMWSA) is a derivative of the sodium alginate and obtained through extraction from brown seaweed. It has been reported to enhance the non-specific immune response and disease resistance in various fish species (Harikrishnan et al., 2011; Van Doan et al., 2014). Sodium alginate possesses immunostimulatory properties and promotes the immunocompetence of orange-spotted grouper, *Epinephelus coicoides* when the fish were injected intraperitoneally with 20 mg kg^{-1} sodium alginate after 24 and 72 h and increases resistance from *Vibrio alginolyticus* infection (Cheng et al., 2007). Moreover, previous studies have reported beneficial effects of sodium alginate on growth performance and physiological responses in some fish species such as Nile tilapia, *Oreochromis niloticus* (Van Doan et al., 2016 a, b), grouper (Lee et al., 2017), Malaysian mahseer, *Tor tambroides* (Asaduzzaman et al., 2019) and African sharptooth catfish, *Clarias gariepinus* (Neamat-Allah et al., 2019).

There are few studies investigating the beneficial role of sodium alginate on yellowfin sea bream. Therefore, the main aim of the study is to evaluate the potential impacts of sodium alginate on growth, feeding efficiency, digestive performance, antioxidant trait, and skin mucus immunity in *A. latus*.

Material and methods

Experimental design and fish maintenance

This study was carried out in accordance with the principle of the Basel Declaration and recommendations of the Faculty of Veterinary Medicine at University of Tabriz, the FVM.REC.1396.939. The protocol was approved by the FVM.REC.1396.939.

This study was carried out at the Mariculture Research Station of the South Iranian Aquaculture Research Center (SIARC), Sarbandar, Iran. One hundred and thirty five juveniles of yellowfin sea bream were randomly distributed into 9 cylindrical polyethylene tanks of 250-L of volume, and each tank stocked with 20 fish (initial body weight 0.5±0.002 g, mean ± standard error). Fish were fed and acclimated for 2 weeks before the onset of the nutritional trial with a commercial diet (Biza, Iran, containing 47% crude protein, 17% crude fat, 2% crude fiber and 14% ash). Tanks were supplied with filtered running seawater; salinity ranged between 47 and 49‰ (48.0±0.5‰) and temperature between 25 and 31°C (28.9±1.5ºC) during the experimental period (56 days). Average values for dissolved oxygen and pH were 6.8±0.4 mg l^{-1} and 7.7±0.2, respectively. The photoperiod was left under natural conditions (30°32′N, 49°20′E; 14 L: 10 D). Low molecular weight sodium alginate (LMWSA) was used as a supplement in this study. The practical diets were supplemented with different levels of LMWSA (0 [control], 5, 10 g kg^{-1}) according to the method suggested by (Merrifield et al., 2011). The dietary ingredients were first ground to a uniform particle size (<1 mm), then all the ingredients were thoroughly mixed with fish oil and soybean oil. Also, LMWSA was thoroughly mixed with the ingredients and water was added to produce stiff dough. The dough was then extruded by a pellet feed maker through a 1-mm-diameter die (Table 1). The moist pellets were dried in a forced air oven at 60°C for about 12 h and then stored at −20°C until used. Diets were tested by triplicate; fish were fed by hand to visual satiation two times per day (09:00h and 15:00h) for 56 days.

Growth measurement

At the end of the growth trial, the fish in each of the 9 tanks were individually weighed and growth performance was evaluated by specific growth rate (SGR), weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF) and daily feed intake (DFI) as follows:

SGR (% day^{-1}) = 100 × [Ln (Mean final body weight) - Ln (Mean initial body weight)] / time (days); natural logarithm (Ln)

WG (%) = 100 × [(Mean final body weight - Mean initial body weight) / Mean initial body weight]

FCR = dry feed intake (g) / wet weight gain (g)

PER (%) = wet weight gain (g) / protein intake (g)

CF (%) = 100 × wet weight (g) / total length (cm) ^3

DFI (g) = dry feed intake (g) / time (days)

Table 1. Ingredient of the experimental diets (g kg^{-1} dry diet)

Ingredient[a]	0	5	10
Fish meal[b]	350	350	350
Soybean meal[c]	270	270	270
Corn gluten[b]	200	200	200
Beef gelatin[b]	20	20	20
Yeast	20	20	20
DL-methionine[d]	2	2	2
L-lysine[d]	4	4	4
Low molecular weight sodium alginate[e]	0	5	10
Agar[d]	10	10	10
Fish oil[b]	33	33	33
Canola oil[c]	33	33	33
Soy lecithin[c]	10	10	10
Vitamin and mineral premixes[f]	20	20	20
Vitamin C[e]	2	2	2
Dicalcium phosphate	10	10	10
Cellulose	16	14	12
Crude protein	51.9±1.1	53.0±0.1	52.6±0.1
Crude fat	14.9±0.0	15.2±0.1	15.1±0.3
Ash	8.2±0.0	8.6±0.1	8.6±0.0
NFE	16.3±0.1	16.5±0.2	16.3±0.1
Moisture	8.7±0.0	6.7±0.1	7.4±0.1
Energy (KJ g^{-1})	21.0±0.0	21.4±0.0	21.2±0.0

[a]Composition of ingredients as % dry-weight basis [fish meal (73.5% crude protein, 10.7% crude lipid); soybean meal (41.0% crude protein, 4.2% crude lipid); corn gluten (71.4% crude protein, 4.1% crude lipid); gelatin (85.0% crude protein, 4.2% crude lipid).

[b]Beyza feed mill, Shiraz, Iran.

[c]Behpak industrial company, Behshahr, Mazandaran, Iran.

[d]Merck, Germany.

[e]Sumchun Pure Chemical, South Korea.

[f]Vitamin and mineral premix U kg^{-1} of premix: vitamin A, 5,000,000 IU; vitamin D_3, 500,000 IU; vitamin E, 3000 mg; vitamin K_3, 1500 mg; vitamin B_1, 6000 mg; vitamin B_2, 24,000 mg; vitamin B_5, 52,000 mg; vitamin B_6, 18,000 mg; vitamin B_{12}, 60,000 mg; folic acid, 3000 mg; nicotinamide 180,000 mg; antioxidant, 500 mg, copper, 3000 mg; zinc, 15,000 mg; manganese, 20,000 mg; iron, 10,000 mg; potassium iodate, 300 mg, career up to 1 kg, Damloran pharmaceutical company, Broujerd, Iran.

Chemical analysis

After 8 weeks, all the fish were fasted for 24 h. Three fish from each replicate tank (nine fish per treatment) were randomly collected and carcass taken for whole body proximate analysis. The analyses of proximate composition of fish were performed using the standard methods of AOAC methods (AOAC, 2005). Briefly, dry

matter was measured gravimetrically after oven drying of homogenized samples for 24 h at 105ºC. Crude protein (N×6.25) was determined by the Kjeldahl procedure using an automatic Kjeldahl system. Crude lipid was determined by ether extraction using Soxhlet.

Mucus sampling and immune response

At the end of feeding trial, three fish per tank (nine fish per treatment) were randomly sampled and anaesthetized (2-phenoxyethanol at 0.5 mL^{-1}) and individually weighed. The mucus collection method was based on using a polyethylene bag containing 50 mM NaCl and harvested by centrifuging at 3000×g for 10 min at 4°C. All mucus samples were preserved at –20°C prior to analysis. Mucus lysozyme activity was determined by a turbidimetric assay according to the method described by Esmaeili et al. (2019) based on the lysis of the lysozyme sensitive gram positive bacterium *Micrococcus lysodeikticus* (Sigma, USA). Hen egg white (in 0.1 M phosphate citrate buffer, pH=5.8) was used for the preparation of the standard curve. The optical density was measured after 15 and 180 s, using a spectrophotometer (Hitachi 220A, Japan) at 670 nm. The results of lysozyme activity are given as U mL^{-1}. The alternative complement pathway haemolytic activity (ACH50) was estimated following the procedure of Tort et al. (1996) and the volume yielding 50% hemolysis was determined and used for calculating the complement activity of the sample.

Digestive enzymes assays

At the end of the experiment (after 24 h starvation), the fish were collected for digestive enzyme analyses. Samples of the fish intestines (three fish from each replicate tank or nine fish per treatment) were homogenized immediately in 100mM Tris–HCl buffer with 0.1 mM EDTA and 0.1% Triton X-100, pH 7.8, followed by centrifugation (30000×g; 12 min at 4°C). After centrifugation, the supernatant was collected and frozen at –80°C (Furne and Sanz, 2018). Total protease, lipase and amylase were assayed according to the methods described below. The specific activity of lipase was performed by the enzymatic photometric method using lipase kit (Bionik, Canada). It was based on 1, 2-o-dilauryl-rac-3-glutaric acid (6-methyresorufin) ester as a substrate that was broken down into 6-methyresorufin and glutaric acid 6-ethylresorufin-ester by lipase. Specific activity of amylase was measured using the enzymatic photometric method using amylase kit (Bionik, Canada). It was based on 4, 6-ethylidene-(G7)-p-nitrophenyl-(G1)-alpha-D-maltoheptaoside (EPS-G7) as a substrate. Total protease activity quantification followed according to the method published by Anson (1938). In this method, azocasein was used as substrate. The reaction mixture contained 1 mL of 1.5% casein solution, pH 7.0, was placed at 37°C and, then, 1 mL of a supernatant sample was added. The reaction was incubated for 10 min before the addition of 2 mL of 0.4 M trichloroacetic acid. The solution was filtered, and 2.5 mL of 0.4 M Na_2CO_3 and 0.5 mL of Folin reagent were added. Finally, the color density developed was determined at 660 nm. Total soluble protein was measured by Bradford (1976) method using bovine serum albumin as a standard. Enzyme activity is expressed as specific activity/mg protein.

Antioxidant enzymes assays

For evaluating activity of antioxidative stress enzymes, livers of fish were washed in ice-cold salt buffer (0.9% NaCl) and were homogenized in 10 vol (w/v) ice cold 100 mM Tris–HCl buffer containing 0.1 mM EDTA and 0.1% (v/v) Triton X-100, pH 7.8 then centrifuged at 11312 g for 30 min at 4°C and the resulting supernatants were used for analyses. The MDA level was measured according to Ringwood et al. (2003) and expressed as nano-moles of MDA per gram wet weight. Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was measured according to McCord and Fridovich (1969). The activity of catalase (CAT, E.C. 1.11.1.6) was determined using the method described by Aebi (1984) using 10 mM hydrogen peroxide as substrate in 0.05 mM Tris-HCl buffer (pH 7.8) at 25°C for 10 min. The enzymatic reaction was prohibited by ammonium molybdate 4% and the absorbance read at 410 nm.

Statistical method

All data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Normality and homogeneity of variances were initially tested using the Kolmogorov–Smirnov and Levene tests, respectively. Differences between the dietary groups were tested using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Data are presented as means ± standard error (n = 3) and differences were considered to be significant at $P<0.05$.

Results

Growth performance and body composition

Growth parameters are shown in Table 2. Dietary supplementation with low molecular weight sodium alginate (LMWSA) did not show any negative effects on growth performance except for the 1% LMWSA group. However, it did not reveal a significant improvement ($P<0.05$) over the control group. The group receiving 1% LMWSA showed more significant difference ($P<0.05$) than the control group.

Same findings were recorded for body composition, where dietary sodium alginate did not show significant ($P>0.05$) differences in protein, fat and moisture body contents for LMWSA groups over the control group (Table 3).

Antioxidants enzymes

Antioxidant enzymes activities including superoxide dismutase (SOD) and catalase (CAT) showed a significant increase ($P<0.05$) in the groups that received 0.5 and 1% sodium alginate compared to the control group, with

the highest significant (P<0.05) activities for the 1% LMWSA group (Table 4). However, the activity of lipid peroxidation (MDA) in the groups fed on 0.5 and 1% LMWSA showed a significant (P<0.05) decrease compared to the control group, with the most significant (P<0.05) decrease for the 0.5% LMWSA group (Table 4).

Digestive enzymes

Activities of digestive enzymes including protease, amylase and lipase increased significantly (P<0.05) with LMWSA supplementation compared to the control group, with the most significant increase for the 1% LMWSA group (Table 5).

Immune response

The dietary incorporation of LMWSA revealed that mucus lysozyme and complement activity did not show any negative effect or significant differences (P>0.05) compared to the control group (Table 6).

Table 2. Growth and feeding performance of *Acanthopagrus latus* juvenile fed the different levels of low molecular weight sodium alginate (LMWSA) for 8 weeks (mean ± SE)

Parameters	Levels of LMWSA (mg kg^{-1})		
	control	0.5% LMWSA	1% LMWSA
Initial weight (g)	0.55±000.0 a	0.53±001.0 a	0.53±005.0 a
Final weight (g)	2.13±03.0 a	1.95±12.0 ab	1.69±10.0 b
Specific growth rate[1] (% day^{-1})	2.61±03.0 a	2.30±12.0 ab	2.05±12.0 b
Weight gain[2] (%)	333.62±95.8 a	265.47±93.24 ab	217.08±15.22 b
Feed conversion ratio[3]	1.08±06.0 b	1.64±12.0 a	1.34±01.0 ab
Protein efficiency ratio[4] (%)	1.85±11.0 a	1.22±09.0 b	1.48±01.0 ab
Condition factor[5] (%)	1.87±06.0 a	1.57±11.0 ab	1.47±07.0 b
Daily feed intake[6] (g)	0.97±032.0 a	1.06±025.0 a	0.79±048.0 b

[1]SGR, [2]WG, [3]FCR, [4]PER, [5]CF, [6]DFI.
In each row, groups with different letters are statistically different (P<0.05).

Table 3. Body composition of *Acanthopagrus latus* juvenile fed the different levels of low molecular weight sodium alginate (LMWSA) for 8 weeks (percent of wet weight, mean ± SE)

Parameters	Levels of LMWSA (mg kg^{-1})		
	control	0.5% LMWSA	1% LMWSA
Protein (%)	19.52±0.22 a	19.46±0.21 a	18.53±0.74 a
Lipid (%)	4.91±0.036 a	4.89±0.13 a	5.5±0.25 a
Moisture (%)	71.91±0.098 a	69.09±0.099 a	71.21±1.22 a

No significant changes were observed (P<0.05).

Table 4. Antioxidant enzyme activity of *Acanthopagrus latus* juvenile fed the different levels of low molecular weight sodium alginate (LMWSA) for 8 weeks (mean ± SE)

Parameters	Levels of LMWSA (mg kg^{-1})		
	control	0.5% LMWSA	1% LMWSA
MDA (nmol/g wet liver)	6.10±0.11 a	4.40±0.10 c	5.10±0.01 b
SOD (mmol/mg protein)	1.36±0.06 b	1.55±0.12 ab	1.88±0.02 a
CAT (mmol/mg protein/min)	1.69±0.00 c	2.88±0.15 b	4.61±0.17 a

In each row, groups with different letters are statistically different (P<0.05).

Table 5. Digestive enzymes activity of *Acanthopagrus latus* juvenile fed the different levels of low molecular weight sodium alginate (LMWSA) for 8 weeks (mean ± SE)

Parameters	Levels of LMWSA (mg kg^{-1})		
	control	0.5% LMWSA	1% LMWSA
Alkaline protease (U/mg protein)	0.29±0.000 c	0.45±0.020 a	0.37±0.000 b
Amylase (µmole/mg protein)	0.53±0.000 c	1.26±0.018 a	0.917±0.004 b
Lipase (µmole/g/h)	0.019±0.000 c	0.115±0.005 a	0.058±0.005 b

In each row, groups with different letters are statistically different (P<0.05).

Table 6. Mucus immune response of *Acanthopagrus latus* juvenile fed the different levels of low molecular weight sodium alginate (LMWSA) for 8 weeks (mean ± SE)

Parameters	Levels of LMWSA (mg kg^{-1})		
	control	0.5% LMWSA	1% LMWSA
Lysozyme (mg/ml)	12.00±0.57 a	10.35±1.01 a	9.95±0.14 a
ACH50 (mU/ml)	118.5±1.44 a	124.0±3.46 a	121.0±0.57 a

No significant changes were observed ($P<0.05$).

Discussion

Currently, herbals and medicinal plants are capturing attention globally and highly recommended by the aquaculture industry as cheap available alternatives for maintaining fish health, and consequently sustaining aquaculture. Recent reports tackle the significance of various natural extracts on fish performance (Elabd et al., 2021; Mahboub et al., 2022) and on fish skin mucosal immunity (Rashidian et al., 2022). The incorporation of nutraceuticals in aquafeed is effective strategy in aquaculture industry. There are no studies assessing the efficiency of sodium alginate as a nutraceutical to investigate its impacts on growth, digestion, and immune-antioxidant traits in *A. latus*. Hence, the present perspective is a pioneer attempt to assess the potential role of sodium alginate on growth, feeding efficiency, digestion, physiological, antioxidant response, and skin mucosal immunity of *A. latus*.

Assessment of the growth-related parameters is crucial to assess the fish performance in response to a dietary plant supplement (Ahmed et al., 2022). In the current investigation, dietary supplementation with LMWSA did not show any negative effects on growth performance parameters or body composition. However, it did not reveal a significant improvement over the control group. The group receiving 1% LMWSA showed more significant difference than the 0.5% LMWSA group. Same findings were reported by (Ashouri et al., 2020), who recorded that Asian sea bass (*Lates calcarifer*) diets only incorporated with a combination of LMWSA and *Pediococcus acidilactici* (PA) significantly improved growth performance parameters, while groups incorporated with only LMWSA did not reveal significant improvement. Similar to our results, the whole body proximate composition did not show significant differences by any dose of sodium alginate supplementation in Malaysian mahseer *Tor tambroides* after 60 days of feeding trial (Asaduzzaman et al., 2019). This confirms our concluded recommended usage to combine with a pre/probiotic with growth promoting properties to get benefited from the synergistic actions, supported with Van Doan et al. (2017) report where LMWSA in the synbiotic form with *Lactobacillus plantarum* significantly increased growth in Nile tilapia, *Oreochromis niloticus*. Concurrent with another study, Van Doan et al. (2016 a) verified the potent ability of LMWSA as a growth promoter in *Oreochromis niloticus*.

Also, the present findings showed a noticeable enhancement in digestion evidenced by elevated levels of digestive enzymes (total protease activity; alkaline protease, amylase), indicating maintenance of the digestive system capacity for protein digestion. In the same manner, African catfish (*Clarias gariepinus*) fed dietary fermented soy pulp significantly improved growth performance (Kari et al., 2022). In addition, Ashouri et al. (2020) reported marked increase in protease activity in fish fed LMWSA with the highest results for the synbiotic groups.

Reactive oxygen species (ROSs) are transformed into bland metabolites by the action of antioxidant enzymes (SOD and CAT) (Mansour et al., 2022). The elevated level of lipid peroxidation is expressed by detecting malondialdehyde (MDA) levels (Woo et al., 2006). Herein, supplementation of sodium alginate extract in the *A. latus* diet acted as a potent antioxidant. It induced an enhancement in the activities of the antioxidant enzymes (CAT and SOD) levels via removal of excess ROSs to counteract oxidative stress. Meanwhile, MDA was reduced reflecting oxidative protection. These positive influences could be returned to the content of antioxidant phenolic compounds detected in sodium alginate.

Fish skin mucus is the first line of defense mechanism that plays a major role for regulating innate immune response (Lazado and Skov, 2019; Somamoto and Nakanishi, 2020). It consists of a broad range of biological substances, such as lysozyme, proteins, immunoglobulin, and lectins that are responsible for boosting immune system (Reverter et al., 2018). Our findings indicated that *A. latus* received sodium mucus lysozyme and complement activity did not show any negative effect or significant differences compared to the control group. Similar to our results, the result of lysozyme activity of serum was not significantly influenced by any dose of sodium alginate supplementation in Malaysian mahseer (Asaduzzaman et al., 2019). In addition, Ashouri et al. (2020) reported a significant improvement in both intestinal c-type and g-type lysozyme (LYZ*)* genes expression only in synbiotic groups supplemented with LMWSA in combination with PA and not in LMWSA only supplemented groups, proving our recommendation to combine with a natural compound with growth and immune stimulating functions. On the contrary, a noticeable enhancement of skin mucosal immunity has been reported in gilthead seabream, *Sparus aurata* (García Beltrán et al., 2020), Atlantic salmon, *Salmo salar* (Leclercq et al., 2020) and Nile tilapia, *O. niloticus* (Van Doan et al., 2021) upon dietary supplement with *Origanum vulgare*, mannan-rich yeast parietal fraction, and pineapple peel powder. Likewise, African sharptooth catfish*, Clarias gariepinus* receiving 3% LMWSA showed significantly enhanced lysozyme and respiratory burst activities (Neamat-Allah et al., 2019). However, some studies confirmed the potential role of sodium alginate as immunomodulatory agent in different fish species (Cheng et al., 2007; Harikrishnan et al., 2011; Van Doan et al., 2014; Van Doan et al., 2016 a, b; Lee et al., 2017).

Therefore, the dietary sodium alginate supplement is considered a new insight for natural antioxidants, which would effectively boost the generation of aquafeed, which is a low-cost antioxidant stimulant that could be used in combination with a natural growth and immune promoter for better effectiveness toward the production of *A. latus*.

Conclusion

Based on the results, dietary intervention with 10 g sodium alginate over a period of 8 weeks is highly recommended in aquafeed and is accurate to enhance *A. latus* digestion, and boost fish antioxidant status. It could be used in combination with a natural growth promoter for a better growth performance profile. Future works are more required to assess the efficacy of sodium alginate as an antidote against various toxicants and on different fish species.

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