MASTER THESIS

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Macroalga-derived alginate as a feed additive for Atlantic salmon

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ABSTRACT

Alginates are fibres that beneficially affect the host by selectively enhancing the growth and the activities of certain beneficial organisms in the intestine. They are mainly derived from brown algae. The aim of the present study was to understand the effect of an oligo alginate on the growth, feed efficiency, whole body proximate composition and intestinal morphology of Atlantic salmon. Three groups of Atlantic salmon were fed with either a basal feed or oligo alginate additive-incorporated (0.5% and 2.5%) feeds for 63 days.

At the end of the study, the assessed parameters were not significantly altered, except for the increased feed intake in the alginate-fed fish. Apparent weight gain, higher organosomatic indices, higher protein retention, lower lipid and energy retention were associated with the alginate-fed fish. Furthermore, histomorphological observations of the distal intestine revealed that the alginate fed fish had more supranuclear vacuoles, suggesting better macronutrient absorption.

The result suggests that seaweed-derived oligo alginate can be used as an additive in Atlantic salmon feeds. The prebiotic effect of the additive should be evaluated by examining relevant parameters including changes in the intestinal microbiota and its metabolites.

ACKNOWLEDGEMENTS

1 INTRODUCTION

1.1 Aquaculture

Aquaculture is one of the fastest growing industries, and it provides protein-rich food for humans. According to Lare-Flores et al. (2003), aquaculture is an increasingly important source of animal protein. Fish and its products have contributed to human nutrition; they are sources of protein and essential micronutrients that play important roles in human health. In the last decade, it has supplied one-third of seafood consumed worldwide (Reverter et al., 2014). But, the industry has been bedeviled by infectious diseases-related losses.

In the past, antibiotics have been employed in the field of aquaculture in preventing infectious diseases. This led to the emergence of antibiotic-resistant bacterial strains, accumulation of chemical residues in edible tissues, and immunosuppression in farmed fish (Tu et al., 2008, Bricknell and Dalmo, 2005). Therefore, the sector has reduced the use of antibiotics in preventing infectious diseases. Now, stringent rules are in place to regulate the application of antibiotics in the United States, Europe and other countries.

Aquaculture sector has been focusing on feeding the farmed animals with a balanced and immune system-enhancing diet; carefully avoiding the negative impacts on the host organism, human health, and environment as a whole. Two main additives that are used in aquafeeds, to fulfill this role, are probiotics and prebiotics (Gatesoupe, 1999, Torrecillas et al., 2007). Apart from serving the purpose of disease prevention via boosting of the fish immune system or status, the additives enhance feeding efficiency, growth and nutrient utilization in fish (Faggio et al., 2015a). Effects of prebiotics on growth, feed conversion, gut microbiota, innate immune parameters such as alternative complement activity, lysozyme activity, natural haemagglutination activity, respiratory burst, superoxide dismutase activity and phagocytic activity, cell damage, and resistance against pathogenic bacteria in fish and shellfish were investigated previously (Kiron, 2012). Prebiotics and probiotics, applied either singly or in combination, are known to induce health responses in fish and shellfish. Furthermore, they have the potential to increase aquaculture production efficiency and ensure sustainable growth.

Thus, the use of prebiotics and probiotics is an alternate way or measure, adopted in the aquaculture sector, to prevent diseases and to maximize the output.

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1.2 Probiotics vs prebiotics

Gastrointestinal tract (GIT) is the site that accommodates micro organisms, both pathogenic and non pathogenic organisms. The GIT is a diverse, complex system and provides an ecosystem for the co-existence of these organisms. The human colon is the most densely colonized region of the gastrointestinal tract with up to 10^{12} bacteria per gram of intestinal contents (O'Sullivan et al., 2010b). Fish intestine may hold up to 10^{5-8} bacteria per gram as reported in the review of Austin (2002).

In human nutrition, probiotics are health-promoting live micro organisms incorporated into diet as additives, aimed to benefit the host. Oral probiotics can be defined as viable microbial culture that benefit the host by improving its intestinal microbial balance, health and nutritional status (Schaafsma, 1996, Havenaar and Veld, 1992, Salminen, 1996, Fuller, 1989). Havenaar (1992) further broadened the definition of probiotic in reference to the host and habitat of the microflora as follows: "A viable mono- or mixed culture of microorganisms which applied to animals or man, beneficially affects the host by improving the properties of the indigenous microflora." The benefits of these probiotic supplements include feed value improvement, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activity, growth-promoting factors, and increased immune response (Verschuere et al., 2000).

On the other hand, prebiotics in diet serve as food sources for the health-promoting micro organisms (Faggio et al., 2015b). Definition of prebiotics, as given by Gibson et al. (2004) is "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confer benefits." Several carbohydrate compounds or indigestible fibers can be considered as prebiotics, and oligosaccharides are one among them. Bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium* are known to act on/ferment oligosaccharides (Slavin, 2013a). These health-promoting bacteria in the gut are considered as probiotics. According to Roberfroid et al. (2008) these bacteria are beneficial to the health and growth of the host–they reduce the intestinal pathogens and/or change the production of health-related bacterial metabolites.

As per Slavin "All prebiotics are fibers, but all fibers are not prebiotics" (Slavin, 2013b) The Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of medicine, USA has proposed definitions for dietary fiber, added fiber and total fiber as follows: "Dietary fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants. Added or functional fiber consists of isolated,

nondigestible carbohydrates that have beneficial physiological effects in humans. Total fiber is the sum of dietary fiber and added fiber (Medicine, 2001). These fibers are not hydrolysed by endogenous enzymes of humans/animals, and promote one or more of the following beneficial physiological effects: (i) laxation, (ii) reduction in blood cholesterol, (iii) modulation of blood glucose. Added fibers, which are able to demostrate additional physiological effects or exert additional benefits or function on the host is termed as functional fibers.

1.3 Properties of a functional prebiotic

If a prebiotic is able to perform the following functions it can be termed an ideal functional prebiotic. These are the criteria given by Gibson and Roberfroid (1995) :

1. Resistance to digestive processes in the upper part of the GIT.

2. Fermentation by intestinal microbiota.

3. Selective stimulation of health-promoting bacteria.

4. It must induce luminal or systemic effects that are beneficial to host health.

Fermentation of the non-digestible food components in the gut by the health-promoting bacteria releases byproducts such as short-chain fatty acids (SCFAs), which are believed to have significant effects on the colonic health. SCFAs such as acetate, butyrate and propionates reduce or lower the pH of the large intestine contents (Reyed, 2007), increases solubility of calicum (and other minerals) in the large intestine contents and also increase the passive concentration-dependent calicum absorption in the colon especially in babies (Griffin and Abrams, 2008, Lobo, 2004). SCFAs have trophic effect on the intestinal epithelia, thereby increasing the absorptive surface area (Scheppach, 1994). SFCAs produced as a result of prebiotic inclusion make the colon area unsuitable for pathogenic invasion or dwelling, suppress putrefactive bacteria and reduce the release of toxic compounds (Kolida et al., 2002, Yokoyama and Carlson, 1979). The modes of action of the prebiotic in human and animals are summarized in the adopted table (**Fig. 1**).

Prebiotics and probiotics are employed in aquaculture. However, both additives have to be used with caution to exploit their potential. The viability of probiotics during processing and storage can alter their beneficial effect. An ideal prebiotic should be stable–the beneficial microbe-stimulating property of prebiotics can be lost during food processing i.e. either when chemically altered or converted to mono- or disaccharides (Patel and Goyal, 2011, Wang, 2009)
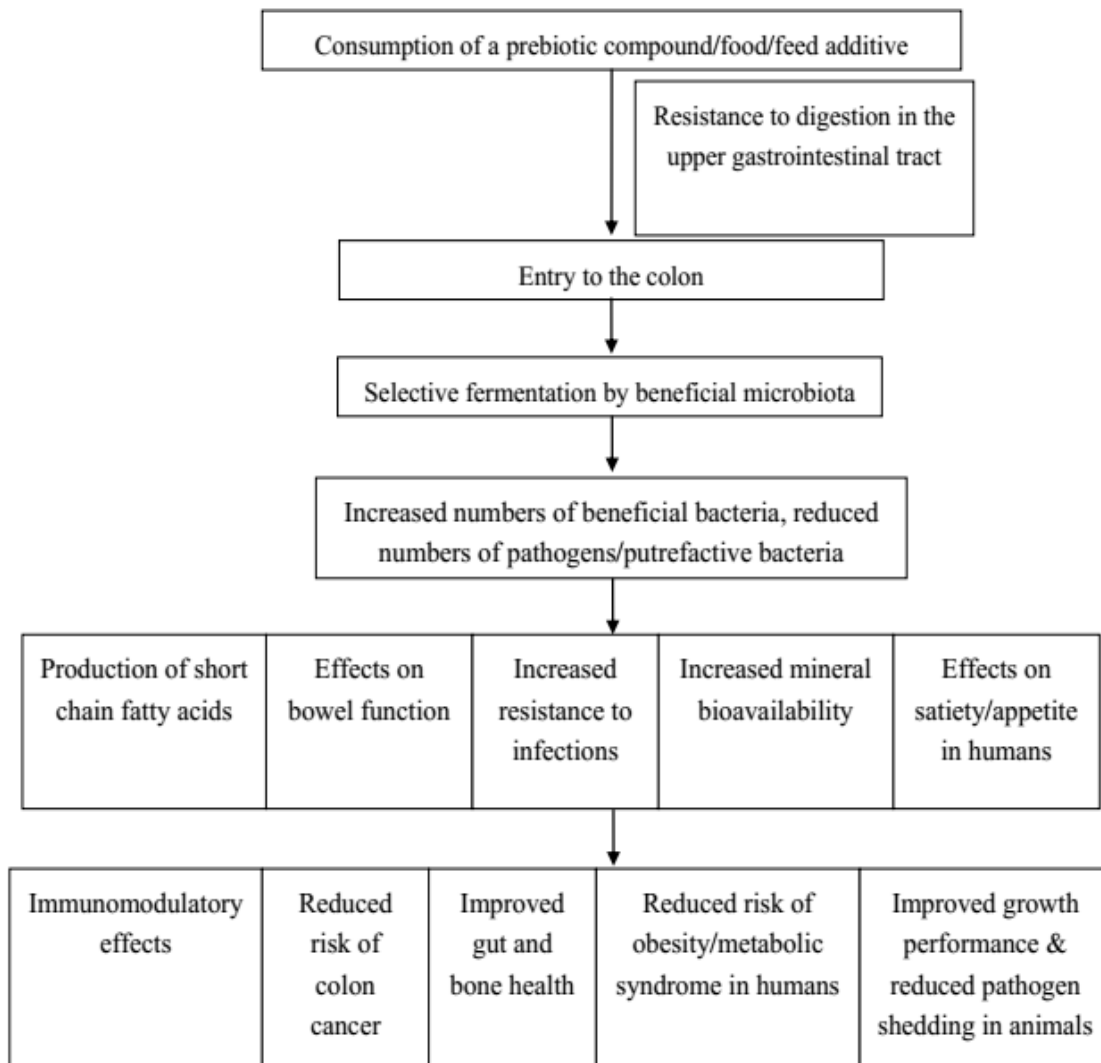
Figure 1. Mode of action of prebiotic and its benefit on the host animal and human (O'Sullivan et al., 2010a)

1.4 Functional oligosaccharides

Oligosaccharides are present in plants, fruits, vegetables, algae etc.–prebiotic oligosaccharides exist as trehalose (a disaccharide), xylo-oligosaccharides, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), pectic-oligosaccharides, raffinose and alginate oligosaccharides (Courtois, 2009). The beneficial effects of inulin (fructans), FOS, GOS and lactulose (synthetic disaccharide) are well documented.

Functional oligosaccharides are obtained from various sources and they have been extensively used as food ingredients, prebiotic supplements in aquafeeds, for drug delivery, as immunostimulators, in cosmetics, animal feeds and agrochemicals (Qiang X et al., 2009).

Oligosaccharides are seen to play many functional roles such as, as mentioned before, acting as nutrients for the intestinal microbiota, in boosting or enhancing the growth of probiotic organism such as *Bifidobacteria and Lactobacilli*, and helping in expulsion of pathogenic organisms in the gut (Yokoyama and Carlson, 1979). Functional oligosaccharides are reported to affect the host positively, by influencing the microbial population–isomaltooligosaccharides-incorporated diet has increased cecal *Bifidobacterium* population, and soybean meal oligosaccharides (SMO) promote competitive exclusion of potential pathogens (Chen et al., 2000). Production of oligosacharride is achieved either by extraction from natural sources or synthesized by physical, chemical or enzymatic methods.

1.4.1   Alginate oligosaccharides

Alginates are fibers, and they are polysaccharides derived from microalgae, brown seaweeds and certain bacteria (Yuguchi et al., 2000). Alginate which is also called alginic acid or algin is the most abundant cell wall polysaccharides in the brown algae (Davis et al., 2003).

Bacterial alginates are those alginates that are extracted from some bacteria, and bacteria belonging to the genera *Pseudomonas* and *Azotobacter* have the capacity to produce alginate (Cote and Krull, 1988, Gorin and Spencer, 1966, Govan et al., 1981, Linker and Jones, 1966).

Commercially or industrially, the extraction of alginate is heavily based on the brown seaweed or brown algae (Pereira et al., 2009) and the extraction includes the following processes:

1. Large quantity of the harvested brown algae are washed, then macerated and extracted with sodium carbonate and filtered.
2. Sodium/calcium is added to the filterate which result in a fibrous precipitation of sodium/calcium alginate formation.
3. Hydrochloric acid is then used to treat the sodium/calcium alginate formation or alginate salt to transform into alginic acid.
4. The alginate is purified, dried and converted to a powder.

The marketed alginates are usually obtained from *Laminaria hyperborea*, *L. digitata, L. japonica*, *Ascophyllum nodosum* and *Macrocystis pyrifera* (Lee and Mooney, 2012*)*, all belonging to the family Phaeophyceae of brown algae. The biochemical and biophysical properties of aliginate are dependent on the molecular weight, M:G ratios, sequence and the G-block lengths (Brownlee et al., 2005). Alginates belong to a family of unbranched or linear polysacharrides and non-repeating copolymers. They contain variable amounts of (1,4)-linked β-D-mannuronate and α-L-guluronate residues. The monomers are distributed as continuous

blocks of mannuronate residues (M-blocks), guluronate residues (G-blocks), or alternating residues (MG-blocks) as shown in **Fig. 2** (Rehm and Valla, 1997). Alginates from different sources will have different guluronate to mannuronate ratios, G-block lengths, molecular weight etc. For example, alginate from *Pseudomonas* lack the G-blocks, but that from *Azotobacter* has high level of G-blocks. Alginates from *L. hyperborean* has high G-block content and the range in marketed alginates is 14.0–44.0% (Qin, 2008, Lee and Mooney, 2012).
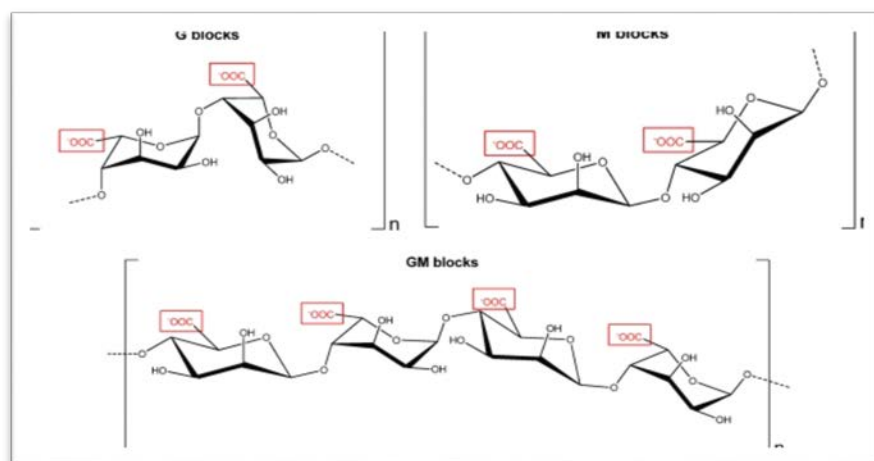
Figure 2. The structure of alginate in brown algae showing the various blocks (Vera et al., 2011)

Alginates are used to deliver drugs, protein, in wound dressing and in cell culture (Lee and Mooney, 2012, Corporation).

1.4.2 The effects of functional oligosaccharides

1.4.2.1 Fish growth, weight gain, feed efficiency, digestion and nutrient utilization

Prebiotic inclusion in aquafeeds enhances the activities of certain beneficial organisms that are associated with dietary nutrient and energy digestion in the gastrointestinal tract of some species of fish (Gatlin and Peredo, 2012). When nutrient utilization is enhanced by these beneficial organisms, fish growth, in terms of the size and weight, within certain period of time will be significantly high.

Aquafeeds supplemented with dietary prebiotic affected the length, regularity and density of mucosal folds and/or microvilli of teleosts positively (Dimitroglou et al., 2009, Dimitroglou et al., 2010b, Dimitroglou et al., 2010a, Sweeteman et al., 2008, Salze et al., 2008, Yilmaz et al., 2007). Prebiotic-incorporated aquafeeds can increase the absorptive area of the GIT; the results were based on the qualitative changes in the GIT i.e. intestinal fold height, enterocyte height, and microvilli height (Dimitroglou et al., 2010b, Zhou, 2010). This increase in the absorptive

area is one of the contributing factors to enhanced nutrient utilization and absorption. Nutrient utilization and metabolism enhancement associated with prebiotics and probiotic inclusion in diets can result in increased weight gain and feed efficiency (Gatlin and Peredo, 2012). Fish growth enhancement and survival in relationship to dietary prebiotic supplementation is connected to fish GIT morphology, microbiology, enzyme activity and immune status (Daniels and Hoseinifar, 2014). Prebiotic MOS-containing diet was found to cause weight gain in turbot larvae (Mahious et al., 2006).

1.5 Hypothesis of the study

The aim of the study was to investigate the effect of prebiotic feed additive (oligo alginate) on the growth performance, feed intake and distal intestinal morphology of Atlantic salmon.

The hypothesis of this study was that oligo alginate derived from a brown alga may have an effect on the growth, viscerosomatic indices, feed efficiency, whole body proximate composition and intestinal absorptive area.

2 MATERIALS AND METHODS

2.1 Information on the feeds and prebiotic

Three experimental feeds (C, L and H) were employed for the growth experiment. The ingredients and the composition of the feeds are given in Table 1. The C feed was the control feed and the other two feeds were supplemented, at different inclusion levels 0.5 (L) and 2.5 (H), with oligo alginate derived from the polysaccharides of a brown alga. The experimental feeds were prepared by Sparos Lda (Olhão, Portugal).

Table 1. Ingredients and proximate composition of the feeds used in the study

Ingredients (g/1000 g)	Experimental feeds		
	C	L	H
Fish meal[1]	57.50	57.50	57.5
Wheat gluten[2]	10.70	10.70	10.0
Wheat meal[3]	5.00	5.00	5.0
Corn meal[4]	9.18	9.18	9.2
Fish oil[5]	8.30	8.30	8.3
Rapeseed oil[6]	8.30	8.30	8.3

Vitamin and mineral premix[7]	1.00	1.0	1.0
Yttrium oxide	0.02	0.02	0.02
Oligo alginate*		0.5	2.5
Proximate composition (g/1000 g)			
Dry matter	93.03	90.23	95.35
In dry matter			
Crude protein	53.77	55.93	55.15
Crude lipid	15.47	16.28	15.71
Ash	7.76	8.11	8.96
Energy (MJ/1000 g)	23.11	23.47	22.63

[1] NORVIK 70, Sopropeche, France; [2] VITAL, , ROQUETTE Frères, France; [3] Wheat meal, Casa Lanchinha, Portugal; [4] Corn meal, Casa Lanchinha, Portugal ; [5] SAVINOR, UTS, Portugal; [6] Henry Lamotte Oils GmbH, Germany; [7] PREMIX Lda, Portugal.

*The estimated oligosaccharide levels in the feed, derived from wheat gluten: 0.0107, wheat meal: 0.105, corn meal: 0.092.

2.2 Fish and rearing condition

The experimental species employed for the growth experiment was Atlantic salmon (*Salmo salar*) and it was procured from Cermaq AS, Hopen, Norway. The fishes were transported to the Research station, Nord University, Bodø, 4 months before the onset of the growth experiment. The growth experiment was conducted in Hall 2 of the research station (**Fig. 3**).

A total of 660 fish were used for the experiment and were distributed into 15 fiberglass tanks of 800 litres capacity. The tanks are green coated inside, and have conical bottom which facilitate easy collection of effluent and uneaten feeds. The tanks are part of a flow-through system, and were supplied with seawater (salinity of 35ppt) pumped from a depth of 250 m from Saltenfjord. The flow rate in the tanks, during the entire period of the experiment, was maintained at 1000 L/h. The seawater was filtered by both mechanical and using ultra-violet (UV light) ray filtration methods. The experimental fish were reared under 24 h of illumination. The temperature and oxygen saturation in the rearing water ranged between 6.8-9.5°C and 69-96%, respectively.

The fish were randomly distributed into the 5 replicate tanks of the 3 study groups. They were acclimatized for 2 weeks before the commencement of the growth experiment. Prior to the experimental feeding, the initial fork lengths and weights of all fishes were registered. The guidelines of the Norwegian Animal Research Authority were followed throughout the study. All the data were entered in excel files.

Fish of mean weight 185.67 g and length 25.91 cm were randomly distributed into 15 tanks (44 fish/tank, 5 tanks/study group). The weights and lengths of 16 fish from the general stock were also noted—and kept in -20°C for evaluating the initial whole fish proximate composition.

2.3 Experimental setup
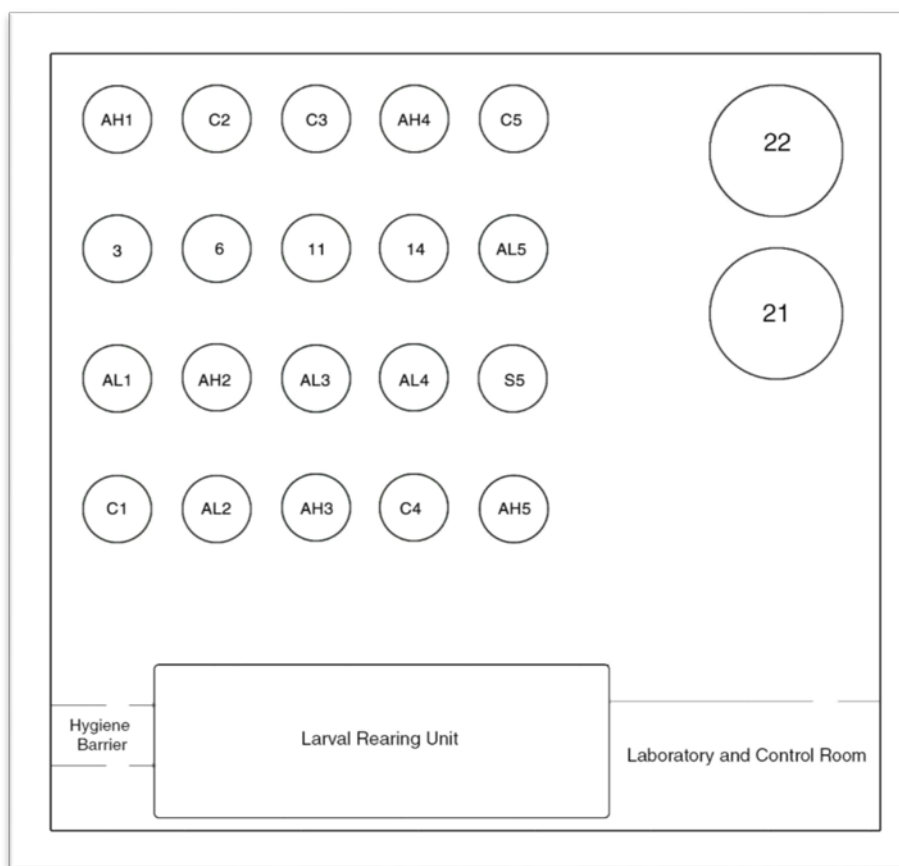
Figure 3. Layout of the experimental tanks in hall 2

2.4 Fish feeding

Fish were fed twice daily, between 8-9 am and 2-3 pm, to satiation. All experimental tanks are fitted with automatic feeders (Arvo Teck, Finland). These feeders are connected to computer simulation programme which trigger the release of feeds at the above-mentioned time periods and at a desired feeding rate. The computer simulation programme allows a user to adjust the

feeding rate and record the daily feed delivered to each tank. Weekly evaluation of the feed consumption was computed from the daily reports generated by the computer.

Thirty minutes prior to daily feeding, feed waste traps attached to every tank were flushed to remove the fecal matter. In order to collect the uneaten feed, the feed waste traps were flushed 1 h after feeding. The matter collected on the wire meshes were examined and the uneaten feeds alone were separated from the faecal material, and placed in metallic container assigned to each tank and stored at -20°C. At the end of every week, the collected uneaten feeds were placed in an oven at 110 °C to determine the dry matter (DM) content and the dry feed consumption. The feed consumption Excel data sheet was used to assess the amount of feed consumed by the fish; this was done for each tank after the weekly computing of the dry matter of the uneaten feeds.

2.5 Final sampling and data collection

Prior to weighing and fork length measurement, the fish were anaesthetized with MS 222 (Tricaine methanesulfonate, Argent Chemical Laboratories, Redmond, USA; 80 mg/l). Individual fish were weighed and the respective fork lengths were recorded at the termination of the growth experiment.

Fish per tank were collected for whole body proximate analysis, six fish per tank. Samples were kept in well labelled transparent bags and kept at -20°C, and later stored at -40°C. Rest of the samples were collected from another set of 10 fish/tank. Blood was drawn with a syringe from the caudal region of anaesthetized fish and a small amount of blood was allowed to enter the micro haematocrit capillary tubes for haematocrit determination. The tubes were sealed with a sealant and kept aside for centrifugation. Fish were dissected and liver and viscera were collected to measure their weights; to assess the organosomatic indices. The gut was separated from the viscera and distal intestinal samples meant for histology were taken. The distal intestinal pH was taken with a pH meter (Meterlab PHM201, Radiometer Aanalytical SAS, Villeurbanne, France).

2.6 Biochemical analyses

Proximate composition of the experimental feeds and whole fish (both initial and final samples) were determined adopting standard methods. All the chemical analyses were done using triplicate samples. Determination of energy was based on duplicate samples.

The fish samples were thawed, sliced into small pieces, and thoroughly homogenised. The homogenized samples (initial - pool of 16 fish; final - pool of 6 fish/tank) were used for the determination of crude protein, crude fat, moisture and ash contents.

For analysing the experimental feeds, 100 g of each feed was thoroughly ground in a mixer (Retsch, Grindomix GM 200, Germany). The ground samples were then kept in labelled bags for analysis of moisture, protein, lipid, ash and bulk energy.

Prior to determination of energy content in the whole body of fish, the samples were dried in a freeze drier (VirTis Benchtop K, SP Industries, Stone Ridge, NY, USA).

2.6.1 Moisture

Moisture content of the samples were determined by oven drying of approximately 5 g of the samples, both whole fish and feeds, for 24 h at 105ºC. The samples were weighed in aluminium foil cup before oven drying. The percentage moisture content was then calculated as the difference in the weight before and after oven drying.

2.6.2 Protein

Protein content in the homogenised samples (both the whole fish and feeds) were analysed employing the Kjeldahl automated method. Approximately 1 g and 0.5 g of whole fish and experimental feed, respectively were weighed on a paper boat. The samples were then placed in a glass tube and two Kjeldahl tablets were added. The tubes were then placed in a fume hood and 15 ml of concentrated sulphuric acid was added. The glass tubes were then placed on a digester at 420 ℃ for 1 h, afterwhich they were allowed to cool down for 30 min. Distilled water (75 ml) was then added to the digested sample. Samples were then automatically titrated in the distillation unit of the Kjeldahl machine (auto analysis), which calculated the nitrogen level. The level of nitrogen was used to calculate the protein percentage in the samples. The factor of 6.25 was used to calculate the protein from the determined nitrogen content.

2.6.3 Lipid

Lipid content of the homogenised samples were measured by extracting crude fat using ethyl acetate. Approximately 10 g and 5 g of homogenised whole fish sample and feeds, respectively were weighed and transferred into porcelain cups. Twenty grams of water free sodium sulphate was added to the sample. The contents were thoroughly mixed and ground to a point where the sodium sulphate absorbed all the moisture. The water free samples were then transferred to 100 ml glass bottle. Approximately 50 ml of the ethyl acetate was added to the water-free sample and then the bottle was capped. The bottle was then placed on a shaker for 1 h to extract the crude fat from the sample. Thereafter, the sample content was filtered through Whatman (41, CAT NO.1441-150) filter paper into 50 ml graduated cylinder, kept in a fume hood. Twenty milliliters of the filtered solution was transferred into a pre-weighed evaporation cup, using a glass pipette. The pre-weighed evaporation cups with their contents were then placed on a steam

bath to allow evaporation of the ethyl acetate and water. The evaporation cup was further kept in an oven, at 105ºC, for an additional 15-20 min before it was allowed to cool down in a desiccator. Fat content was calculated as the difference in weight of the empty evaporation cup and the evaporation cup with fat.

2.6.4 Ash analysis
Ash content of the homogenized samples for both the whole fish and feeds was determined by combusting the pre-weighed sample in a ceramic crucible in a muffle furnace at a temperature of 550ºC for 12 h. Approximately 5 g of the homogenised samples was used in the combustion and the difference in the weight of the sample plus the crucible and oven dried sample plus the crucible was used in the calculation of percentage ash.

2.6.5 Energy
Energy was measured with a Bomb calorimeter (C200, IKA-Werke GmbH & Co. KG, Staufen, Germany). Approximately 0.5 g and 1 g of pelleted freeze dried sample of whole fish and pelleted feed, respectively were used for the energy analysis. The pelleted sample was placed in the calorimeter and combusted following the instruction manual. The result was then recorded.

2.7 Histological analysis
Distal intestinal samples from 5 fish (n=5) were retained for histological analysis, adopting the processing and sectioning techniques of the distal intestinal samples as described in Vasanth et al.(2015). This part was performed by a histology specialist. Approximately 5 μm of distal intestinal samples were sectioned and fixed for the histological examination. The sectioned samples were stained with Alcian Blue-Periodic Acid Schiff's reagent (AB-PAS, pH 1.0) (Bancroft and Gamble, 2007). The photomicrographs of the stained samples were magnified and captured by employing an Olympus BX61/Camera Color View IIIu (Olympus Europa GmbH, Hamburg, Germany) and processed using the imaging system, photo program Cell P (Soft Imaging System GmbH, Munster, Germany).

2.8 Formulae
The growth and feed performance were assessed based on the following parameters. The equations used for the calculations are given below:

The weights of the fishes were determined at the start and at the end of the 63-day growth study.

Survival rate (%) was calculated as:

$$= \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

Condition factor was calculated as:

$$= \frac{\text{Body weight (g)}}{\text{Body length}^3 \text{(g)}} \times 100$$

Hepatosomatic index was calculated as:

$$= \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100$$

Viscerosomatic index was calculated as:

$$= \frac{\text{Visceral weight (g)}}{\text{Body weight (g)}} \times 100$$

Feed conversion ratio (FCR) was calculated as:

$$= \frac{\text{Dry weight of feed consumed (g)}}{\text{Wet weight gain(g)}} \times 100$$

Specific growth rate was calculated as:

$$= \frac{(\ln(\text{final weight}) - \ln(\text{ initial weight}))(g)}{\text{Days}} \times 100$$

Days=Duration of the feeding trial

Thermal growth coefficient was calculated as

$$= \frac{\text{Final weight}^{1/3} - \text{Initial weight}^{1/3}}{(T \times t)} \times 100$$

where T is temperature in °C and t is time in days.

Protein efficiency ratio was calculated as:

$$= \frac{\text{Wet weight gain (g)}}{\text{Protein consumed (dry weight basis, g)}}$$

Retention values (%) were calculated as:

$$= \frac{(\text{Finalbiomass x nutrient or energy in final fish}) - (\text{Initial biomass x nutrient or energy in initial fish})}{\text{Feed intake x nutrient or energy in feed}} \times 100$$

2.9 Statistical analyses

Statistical analyses were performed using GraphPad Prism 6 (Version 6.03). Assumptions of Anova, namely normal distribution and equal variance were checked using Kolmogorov–Smirnov normality test and Bartlett's test, respectively. Transformations were performed for certain data. Parametric Anova, followed by Tukey's multiple comparisons test was employed to find the differences between the means of interest. For non-parametric data, Kruskal-Wallis test and Dunn's multiple comparisons test were performed. The differences are reported as significant when $p < 0.05$.

3 RESULTS

3.1 Effect of oligo alginate on the fish growth performance, survival and feed utilization

The effect of the oligo alginate on the growth performance of the fish is summarized in Table 2. The fish in the 63-day growth experiment were in good health. Hundred percent survival was observed in the C and L groups. The 99.5 survival recorded in the H group was due to the one mortality recorded in that group. The average body weight gain in the C group was 87.9%, those in L and H were 90.2% and 89.90%, respectively. The specific growth rate, feed conversion ratio, thermal growth coefficient and protein efficiency ratio of the three groups did not differ significantly. Feed intake values of the alginate fed groups were significantly different. However, these values when compared to that of C were not different.

Table 2. Survival, growth and feed utilization of Atlantic salmon

	C	L	H
Survival (%)	100	100	99.50
Initial weight (g)	184.5 ± 1.3	185.6 ± 1.6	186.9 ± 1.6
Final weight (g)	346.6 ± 4.3	353.0 ± 4.3	355.7 ± 3.7
SGR (% day^{-1})	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
TGC	2.7 ± 0.1	2.9 ± 0.1	2.9 ± 0.0
FI (% BW day^{-1})*	0.7 (0.67, 0.67)[ab]	0.7 (0.66, 0.67)[a]	0.7 (0.67, 0.69)[b]
FCR	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
PER	2.0 ± 0.0	2.1 ± 0.0	2.0 ± 0.0

Specific growth rate – SGR, Thermal growth coefficient – TGC, Feed intake - FI. * median, with 25th and 75th percentiles for the non-parametric data, Feed conversion ratio – FCR, Protein efficiency ratio - PER.
Different superscripts in a row indicate statistically significant differences (P < 0.05) among groups.
n=5 tanks, values from 44 fish/tank and presented as mean ± SEM.

3.2 Effect of oligo alginate on organosomatic indices and hematocrit values

The condition factor for fish in all the groups after the 63-day growth experiment was 1.3. Significance differences were not detected for the hepatosomatic and viscerosomatic indices. However, the hepatosomatic indices in the groups L and H were 1.3 compared to 1.2 in the group C (Table 3). The viscerosomatic indices in the groups C, L and H were 9.5, 10 and 9.8, respectively. The hematocrit values in all the groups did not vary significantly (48.9 (C) vs 50.1 (L), 48.6 (H)).

Table 3. Organosomatic indices and hematocrit values of Atlantic salmon

	C	**L**	**H**
Condition factor	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.0
Hepatosomatic index	1.2 ± 0.0	1.3 ± 0.0	1.3 ± 0.0
Viscerosomatic index	9.5 ± 0.3	10.0 ± 0.4	9.8 ± 0.3
Hematocrit	48.9 ± 0.5	50.1 ± 1.4	48.6 ± 1.2

n=6 tanks, values from 10 fish/tank for all parameters except CF are expressed as mean ± SEM

3.3 Effect of oligo alginate on the proximate composition of the whole fish

The effect of the oligo alginate on the proximate composition is given in Table 4. No significant differences were observed in the proximate composition values of the whole fish. However, the protein and lipid contents in the groups L and H compared to the values in the C are noteworthy.

Table 4. Proximate composition (g/100 g dry matter) of Atlantic salmon

	Initial	**Final**		
		C	**L**	**H**
Whole body				
Moisture	69.4 ± 0.1	70.6 ± 0.3	71.2 ± 0.2	70.8 ± 0.3
Protein	53.8 ± 0.7	58.3 ± 0.7	61.1 ± 0.9	61.3 ± 1.2
Lipid	37.1 ± 0.6	34.1 ± 0.9	32.5 ± 0.9	31.2 ± 1.1
Ash	7.1 ± 0.4	6.3 ± 0.2	6.9 ± 0.2	7.0 ± 0.3

Values are expressed as mean ± SEM, n=5 tanks.

3.4 Effect of the oligo alginate on the nutrient and energy retention

No significant differences were observed in the protein, lipid and energy retention values of the study groups. The protein and lipid retention values of the groups L and H reflected the protein and lipid contents in the groups L and H. The energy retention values of the alginate fed fish were 43.7 and 38.3 compared to 43.4 of the C group.

Table 5. Retention (%) of protein, lipid and energy of Atlantic salmon

	C	L	H
Protein	36. 9 ± 0.6	38.4 ± 0.6	39.0 ± 0.6
Lipid	59.4 ± 5.3	48.3 ± 5.1	44.9 ± 6.6
Energy	43.4 ± 1.5	43.7 ± 0.8	38.3 ± 2.0

Values are given as mean ± SEM; n = 5 replicate tanks. Different superscripts in a row indicate statistically significant differences ($P < 0.05$) among groups.

3.5 Effect of oligo alginate on the distal intestine pH

Table 6. pH in the distal intestine of Atlantic salmon

	C	L	H
pH	8.4 ± 0.0	8.4 ± 0.0	8.3 ± 0.0

3.6 Effect of oligo alginate on the distal intestinal morphology
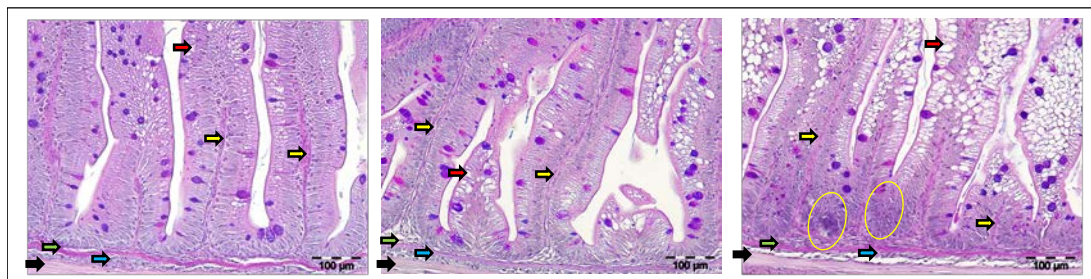
Figure 4. Photomicrographs of the distal intestine of Atlantic salmon fed oligo alginate
These are representative images from among 5 samples taken from 5 tanks. Scale bar: 100 μm. Yellow arrows: lamina propria, red arrows: supranuclear vacuoles, green arrows: stratum compactum, blue arrows: *stratum granulosum*; black arrows: *muscularis circularis*; see more stained nuclei inside the yellow circles.

Fish fed on oligo alginate had more supranuclear vacuoles; vacuolization is visible in the L group and severe vacuolization is detected in the H group (**Fig. 4**). Near the base of the mucosal folds more nuclei were detected.

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4. DISCUSSION

There is a great concern about the increased use of antibiotics and chemicals in the field of aquaculture and this worrying concerns have promoted scientific research on the alternate products that can improve fish growth, feed conversion efficiency, health, and disease resistance of the host (Daniels et al., 2006). Nevertheless, the use of the prebiotic product in aquaculture is limited (Gatlin III, 2002). The present study was conducted to investigate the effect of an oligo alginate derived from a brown seaweed on the growth performance, feed intake and intestinal morphology of Atlantic salmon.

The expectation was to show a positive effect of the prebiotic because oligo alginate can be considered as a prebiotic and its inclusion may facilitate better nutrient utilization and thereby enhance growth. However, the alginate did not cause any distinct effect on fish growth, based on the SGR and TGC values. Fish fed oligo alginate had better weight gain of 89.9-90.2% compared to 87.9% of the C group. Thus, oligo alginate can apparently improve the weight gain in Atlantic salmon. This result is in agreement with the results of other studies, where dietary Ergosan (alginic acids) inclusion in feed resulted in good growth performance in beluga sturgeon, *Huso huso* (Jalali et al., 2009), rainbow trout, *Oncorhynchus mykiss* (Gioacchini et al., 2010) and shrimp *Litopenaeus vannamei* (Montero-Rocha et al., 2006). On the contrary, a study conducted by Merrifield et al. (2011) with 5g kg^{-1} inclusion of Ergosan (alginic acid) did not improve weight gain in juvenile tilapia (*Oreochromis niloticus*). Merrifield et al.(2011) and Jalali et al. (2009) have pointed out the growth promoting effect of Ergosan on great sturgeon. Other studies (Bagni et al., 2005, Raida et al., 2003, Faghani et al., 2008) have also reported insignificant differences and lack of effect on the growth performance upon feeding sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) with dietary Ergosan.

Yoo et al. (2007) observed improvement in weight gain, specific growth rate and feed efficiency ratio, when olive flounder (*Paralichthys olivaceus*) were fed for 7 days with diets containing yeast β-glucan, a polysaccharide derived from *Saccharomyces cerevisiae*. The inclusion of 0.5 and 2.5 oligo alginate in feed did not affect the FCR of the fish significantly. However, feed intake was affected by the inclusion of the additives in feed, statistically significant difference was observed; H vs L group. Note that significant differences were not detected between the C group and the alginate-fed groups. Merrifield et al. (2011) also reported no statistically significant difference in the FCR of the fish fed Ergosan.

Organosomatic indices of the study groups also did not differ significantly. However, the haematocrit values of fish fed the 0.5 % of the additive was 50.1 compared to 48.9 and 48.6 in the other groups. Even though no significant differences were observed in hepatosomatic and viscerosomatic indices of the groups, the fish group fed with low and high oligo alginate recorded high values of the indices.

Proximate body composition of the study groups was not significantly influenced by the incorporation of oligo alginate in the feed. Merrifield et al. (2011) reported that dietary alginic acid inclusion influenced the body composition of tilapia, but not the PER values. In the present study, the PER of the L group was 2.1 vs 2.0 in the other groups. The protein content in the alginate fed fish were 61.1, 61.3 vs 58.3 in the C group. The protein retention also followed a similar trend. On the other hand, the lipid content and retention of the alginate consumed fish are also noteworthy. The lipid content, lipid and energy retention were reduced (not significant) in the present study, while protein content and retention were increased (not significant) with the inclusion of alginate additive. Other authors (Brownlee et al., 2005, Jiménez-Escrig and Sánchez-Muniz, 2000) have reported, in a number of animal model studies, that the inclusion of alginate in diet and the presence of alginate in the small intestinal lumen reduce the uptake of fats and reduce plasma cholesterol. These results confirm the trend on the lipid uptake noted in the present study.

Red drum (*Sciaenops occellatus*) fed 1% alginic acid had 4.85 % lipid (wet weight) in the whole body compared to 5.39% in fish fed the basal diet (Mendoza Rodriguez et al., 2016). This study also confirms the effect of alginate on the fat uptake and the lipid deposition in the present study. They (Mendoza Rodriguez et al., 2016) suggested that alginate inclusion in fish feed as binder reduces the apparent digestibility of protein and fat. However, in the present study, the protein retention values were 38.4 and 39% for the alginate fed fish vs 36.9% for the control fish. Lipid is the densest form of energy supply, and the most efficient source of nutrient for fish, maximizing both the energy intake and storage (Bureau et al., 2002). High inclusion of oligo alginate recorded the lowest energy and lipid retention. Note that the crude lipid in the C, L and H feeds were 15.47, 16.28 and 15.71, respectively.

Histological changes related to dietary polysaccharides in the intestinal features such as *tunica muscularis* and mucus viscosity are well documented. Soluble polysaccharides and biomass of red microalga fed to rat affected the morphology of both mucosa and *tunica muscularis* layers in the jejunum (Dvir et al., 2000). Dietary fibre affected intestinal morphology in a way to

increase absorption and metabolism (Brown et al., 1979, Stark et al., 1996). Intestinal morphological changes in the structure of villi, resulting from fibre feeding in rats was reported by Cassidy et al. (1981). Soluble dietary fibre significantly ($P<0.001$) increases the intestinal length (Stark et al., 1996, Jacobs, 1983, Judd and Truswell, 1985). In the present study quantitative changes of the intestinal structure were not assessed. Fish fed on 2.5% oligo alginate had apparently more supranuclear vacuoles. This could be indicating the increase in uptake of macronutrients due to endocytosis(Bakke, 2011). Note that in the H group protein retention was higher, though not significant. Furthermore, near the base of the mucosal folds more nuclei were detected. This could be indicating the trophic effect of the oligo alginate, probably an effect of the prebiotic induced SCFAs (Scheppach, 1994).

Contrary to the present study, the additive resulted in less weight gain in rat fed red algal polysaccharides (Dvir et al., 2000), even though the feed intake was similar. This lower weight gain was linked to high viscosity intestinal content. Highly viscous chyme may prevent nutrient absorption and micelle formation, thereby reducing the overall lipid absorption and weight gain. (Schweizer and Edwards, 2013, Gallaher et al., 1993, Lairon, 1996, Schneeman and Richter, 1993). Viscosity of the intestinal chyme was not evaluated. However, the lipid content and retention were lower in the alginate fed groups.

5. CONCLUSION

The ability of oligo alginate additive, derived from a seaweed, in enhancing nutrient uptake of Atlantic salmon was evident. Even though, no statistical significant difference was realised, increased feed intake, apparent weight gain, higher protein retention and more supranuclear vacuoles were associated with the alginate fed fish. Based on the finding of this study, it can be concluded that oligo alginate can be used as sustainable alternative way and feed additive in Atlantic salmon feeds. Additional studies are necessary to ascertain the prebiotic effect of the additive.

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