Research Article

Enzymatic Production of Alginate Oligosaccharides from *Turbinaria murayana* Seaweed for Use as a Poultry Feed Additive

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Abstract | This research was carried out to investigate the impact of enzymatically degraded alginate derived from *Turbinaria murayana* seaweed, namely alginate oligosaccharide *Turbinaria murayana* (AOS*Tm*), as an ingredient in poultry feed. The study employed an experimental design featuring a completely randomised approach with five treatments of alginate lyase enzyme at varying concentrations to degrade alginate into AOS*Tm*, specifically 1, 2, 3, 4, and 5 mL per 100 mL of a 1% alginate solution. Each treatment was repeated 4 times. The parameters examined in this study were the percentage yield, viscosity, molecular weight, and diameter of the inhibition zones against *Escherichia coli, Salmonella* sp., and *Staphylococcus aureus*. The research data were analysed through analysis of variance (ANOVA) followed by the Duncan Multiple Range Test. The findings from the variance analysis indicated that the degradation of alginate derived from the seaweed *T. murayana* into AOS*Tm* using different doses of the enzyme alginate lyase exhibited a highly significant impact (P<0.01) on the yield percentage, but not had significant effect (P>0.05) on viscosity, molecular weight, and the diameter of the inhibition zone against the bacteria *E. coli, Salmonella* sp., and *S. aureus*. It can be concluded that the degradation of alginate from the seaweed *T. murayana* into AOS*Tm* using the enzyme alginate lyase at a dose of 3 mL/100 mL alginate solution is the best dose for producing AOS*Tm* yield, which is 74.90% with a viscosity of 5.93 cPs, molecular weight of 28,659.54 g/mol, and inhibition zone diameters of 17.76, 23.63 and 21.10 mm against *E. coli*, *Salmonella* sp. and *S. aureus* respectively.

Keywords | Alginate, Alginate Lyase, Feed additive, *Turbinaria murayana*, Poultry

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INTRODUCTION

The poultry industry in Indonesia is currently facing serious challenges since the prohibition on the use of antibiotic growth promoters (AGP). In Indonesia, the ban on the use of AGP began on January 1, 2018, with the issuance of the Indonesian Ministry of Agriculture Regulation Number 14 of 2017. This policy is not only in Indonesia but has already been widely implemented by various countries such as China, Korea, and America (Salim *et al*., 2018). The use of AGP in livestock can have negative impacts, such as leaving harmful residues in animal products and contributing to antimicrobial resistance in livestock. This necessitates the development of alternative materials to replace AGP that do not leave harmful residues in animal products and can also enhance livestock growth. Natural materials used as feed additives to replace AGP in livestock usually come from plants and herbs, both from land and sea (Rusli et al., 2024a; 2024b).

One material with potential for use as a feed additive to replace AGP is oligosaccharide alginate (AOS). According to Liu *et al.* (2019), AOS is known to have antimicrobial, antioxidant, and immunomodulatory properties and also acts as a prebiotic. Furthermore, Zhu *et al.* (2023) also reported that AOS utilized as a feed supplement in broiler diets at a concentration of 200 mg/kg of ration can improve broiler growth and enhance the population of beneficial bacteria, such as bacteria of lactic acid, as well as decrease the population of pathogenic microbes, including *Escherichia coli* and *Salmonella* species in the gastrointestinal system. In addition, Hu *et al.* (2005) also reported on in vitro experiments with AOS on pathogenic microorganisms, including *E. coli* and *Salmonella paratyphi*, which showed that AOS can inhibit the growth of these bacteria. AOS represents a degradation product of alginate extracted from brown seaweed. One type of brown seaweed that produces alginate is *Turbinaria murayana* seaweed (Reski *et al.*, 2021).

Turbinaria murayana seaweed is widely found in the Nipah River Coastal Area of South Pesisir Regency, West Sumatra Province (Reski *et al.*, 2022, 2023). This seaweed grows naturally and has not been utilised by the surrounding community as food or feed (Reski *et al.*, 2024). Reski *et al.* (2025) indicate that alginate derived from *T. murayana* seaweed through the acid pathway method complies with the quality standards for alginate. It was further stated that the resulting alginate yield was 26.93% with a moisture percentage of 13.44%, a percentage of ash 23.74%, a viscosity of 15.75 cPs, and an average molecular weight of 76,786.97 g/mol.

Alginate compounds can be degraded into AOS using enzymatic, chemical, and physical methods. Degrading alginate into AOS using the enzymatic method is better than chemical and physical methods because it can produce AOS with good structure and bioactivity (Liu *et al.*, 2019). According to Xing *et al.* (2020), degrading alginate into AOS using the chemical method requires high costs due to the extensive use of chemicals and the production of waste products that can harm the environment. Meanwhile, degrading alginate into AOS using the physical method requires strict control and a long degradation time (Wang *et al.*, 2024). According to Subaryono *et al.* (2017), AOS produces enzymatically results in a more regular structure and bioactivity compared to chemical and physical methods. Then, Addina *et al.* (2020) reported that enzymatic degradation of alginate into AOS using alginate lyase resulted in AOS of superior quality and structure.

There has been no research on the enzymatic degradation of alginate extracted from the brown seaweed of the *T. murayana* species into alginate oligosaccharides *T. murayana* (AOS*Tm*), which will be used as a feed additive to replace AGP in poultry. Therefore, research has been conducted on the enzymatic degradation of alginate from *T. murayana* seaweed into AOS*Tm* using the enzyme alginate lyase at different doses, with respect to yield, viscosity, molecular weight, and the diameter of the inhibitory zone against pathogenic microbes (*Salmonella, E. coli,* and *S. aureus*).

MATERIALS AND METHODS

Materials

The primary material utilised in this research was alginate extracted from *T. murayana* seaweed using the acid extraction method, as described by Reski *et al.* (2025). Other materials used included alginate lyase enzyme (Sigma-Aldrich), distilled water, phosphate buffer (pH 7), nutrient agar (NA), and nutrient broth (NB) media, as well as pathogenic microbes (*E. coli*, *Salmonella*, and *S. aureus*). In addition, the equipment used to support this research included an analytical balance, blender, water bath, shaker incubator, oven, jar, funnel, stirring rod, measuring cylinder, petri dish, test tube, autoclave, hot plate, micropipette, caliper, inoculating needle, spirit lamp, filter paper, aluminum foil, gauze, gloves, mask, labeling paper, stationery, and camera.

Methods

The research method employed was an experimental design featuring a Completely Randomised Design, comprising of five treatments and four replications. The treatments consisted of alginate lyase enzymes at different doses, specifically 1, 2, 3, 4, and 5 mL per 100 mL of a 1% alginate solution. The activity of the alginate lyase enzyme used was 1 Unit/mL.

Experimental procedure

Dissolved 1 gram of sodium alginate in 100 mL of heated distilled water at a temperature of 40–50°C. Next, diluted the alginate lyase enzyme with an activity of 1 Unit/mL by dissolving 1 mg of enzyme in 10 mL of phosphate buffer solution with a pH of 7. The prepared 1% alginate solution was then treated with the alginate lyase enzyme according to the following treatments: 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL. The solutions of alginate with added alginate lyase enzyme according to each treatment were thereafter incubated in a shaker incubator at 37°C for 8 hours. After the incubation process was completed, the mixture was heated with boiling water for 10 minutes to inactivate the alginate lyase enzyme. The solution was then cooled, and the remaining macromolecules, which had not been degraded by the enzyme, were precipitated using 96% ethanol at a 1:1 (v/v) ratio. The alginate oligosaccharide (AOS) solution was separated from the precipitate by filtration. The separated AOS solution was then dried

using a dehydrator at 50°C to obtain *T. murayana* alginate oligosaccharides (AOS*Tm*). The dried AOS*Tm* was then measured for yield percentage and analyzed for viscosity, molecular weight, and in vitro antimicrobial activity by observing the inhibitory zone diameter of pathogenic bacteria (*E. coli*, *Salmonella*, and *S. aureus*).

Parameters measured

The variables observed in this research were yield percentage, viscosity, molecular weight, and inhibition zone diameter of pathogenic bacteria (*E. coli*, *Salmonella* sp., and *S. aureus*). The yield percentage of AOS*Tm* was calculated by dividing the weight of AOS*Tm* obtained by the initial weight of alginate before treatment, followed by multiplying the result by 100% (Subaryono *et al.*, 2017). The viscosity of AOS*Tm* was determined by formulating a 1% (b/v) alginate solution, comprising 3 grams of AOS*Tm* dissolved in 300 mL of distilled water. The viscosity was measured with a viscometer, and the results were reported in centipoise (Husni *et al.*, 2012). The molecular weight of AOS*Tm* was determined by the Mark–Houwink equation, $[\eta] = KM_v^a$, with constant values $K = 2.5 \times 10^{-4}$ and $a = 0.98$ for AOS*Tm* in boiling water. In this formula, M_v signifies the viscosity-average molecular weight (g/mol) and [η] denotes the intrinsic viscosity (mL/g) (Lee and Mooney, 2012).

Furthermore, each treatment was tested for the antibacterial activity against pathogenic bacteria (*E. coli*, *Salmonella*, and *S. aureus*) using the well diffusion method (Pasaribu *et al.*, 2021). The procedure for testing antibacterial activity included preparing media and microbial cultures, sterilising the media, and inoculating the microbes into nutrient broth (NB). The microbial cultures were then spread onto Petri dishes containing nutrient agar (NA) media. After that, the produced AOS*Tm* from each enzyme dosage treatment was applied to the Petri dishes inoculated with *E. coli*, *Salmonella* sp., and *S. aureus*. The dosage of AOS*Tm* used for each treatment was 300 mg AOS*Tm* with 1 mL distilled water. The Petri plate was subsequently incubated at 37°C for 24 hours.

Data analysis

The experimental data were analyzed using analysis of variance (ANOVA) under a completely randomized design (CRD) to assess treatment effects. Whenever a significant effect of the treatment is observed, the difference among the treatment means is examined and analysed utilising Duncan's Multiple Range Test, in accordance with the methodology used by Steel and Torrie (1991).

RESULTS

The results of the degradation of alginate from *T. murayana* seaweed into Alginate Oligosaccharides of *Turbinaria murayana* (AOS*Tm*) using alginate lyase enzyme with different doses can be seen in Table 1.

Table 1: Average yield, viscosity, and molecular weight of AOSTm produced using alginate lyase enzyme at different doses.

Enzyme dosage	Yield (%)	Viscosity (cPs)	Molecular weight (g/mol)
1 mL	59.89^{c}	5.78	27,777.29
2 mL	65.77^{b}	6.13	29,847.75
3 mL	74.90^{a}	5.93	28,659.54
4 mL	72.02^{a}	6.01	29,137.33
5 mL	73.33^{a}	5.68	27,226.92
SE	1.93	0.12	0.70

SE: Standard error. Means within a column followed by different superscripts (a–c) differ significantly (P < 0.01).

The analysis of variance data indicated that the enzymatic degradation of alginate from *T. murayana* seaweed into Alginate Oligosaccharides of *Turbinaria murayana* (AOS*Tm*) with different enzyme doses significantly affected (P<0.01) the yield percentage but did not significantly impact (P>0.05) the viscosity and molecular weight of the produced AOS*Tm* among treatments.

Table 2: Inhibition zone diameter and optical density produced by each treatment against pathogenic bacteria (*Salmonella* sp., *E. coli*, and *S. aureus*).

Enzyme dosage	*Salmonella* sp.		*Escherichia coli*		*Staphylococcus aureus*	
	Inhibition zone (mm)	Optical density	Inhibition zone (mm)	Optical density	Inhibition zone (mm)	Optical density
1 mL	25.46	0.150	18.28	0.142	20.98	0.194
2 mL	26.12	0.158	17.46	0.186	19.81	0.179
3 mL	23.63	0.164	17.76	0.184	21.10	0.190
4 mL	22.71	0.163	15.87	0.164	19.35	0.185
5 mL	23.81	0.141	16,44	0.182	19.74	0.192
SE	1.17		0.95		0.57	

SE: Standard error

Furthermore, the results of the AOSTm test, which were produced using different doses of alginate lyase enzyme, are presented in Table 2, which showing the inhibition zone diameter of pathogenic bacteria (*Salmonella*, *E. coli*, and *S. aureus*) and optical density (OD).

The analysis of variance data indicated that the AOS*Tm* produced using different doses of alginate lyase enzyme had no significant impact (P > 0.05) on the inhibition zone diameter of the pathogenic microorganisms (*Salmonella* sp., *E. coli*, and *S. aureus*).

DISCUSSION

The percentage yield of AOS*Tm* produced using alginate lyase enzyme degradation with different doses ranged from 59.89% to 74.90%. The highest AOS*Tm* yield was obtained at an alginate lyase enzyme dose of 3 mL, which was 74.90%. The high AOS*Tm* yield obtained at the 3 mL enzyme dose was due to the optimal activity of the alginate lyase enzyme at this concentration, which breaks the polysaccharide bonds of alginate into alginate oligosaccharides. According to Xing et al. (2020), an appropriate enzyme dose allows alginate lyase to work optimally in cleaving the β-(1→4) linkages between mannuronate and guluronate units, resulting in an increased production of alginate oligosaccharides. Furthermore, Liu *et al*. (2019) reported that the use of an alginate lyase enzyme at a dose that is too low cannot effectively degrade alginate polysaccharides into alginate oligosaccharides, leading to a lower yield. This finding is supported by Subaryono *et al*. (2017), who stated that using alginate lyase enzyme at excessively high doses may degrade alginate into very small alginate oligosaccharides that dissolve easily and are challenging to precipitate, thus lowering the yield (Subaryono *et al*., 2017). The AOS*Tm* yield percentage in this study was similar to the alginate oligosaccharide yield reported by Addina *et al*. (2020), which ranged from 77.29% to 85.46% using commercial alginate lyase enzyme (Sigma-Aldrich product). However, the AOS*Tm* yield in this study was higher than that reported by Afni *et al*. (2017), which was 38.33% using alginate lyase enzyme derived from *Bacillus megaterium*.

The viscosity level of AOS*Tm* produced in this study ranged from 5.68 to 6.13 cPs. The viscosity level of AOS*Tm* obtained in this study was lower than that of alginate polysaccharides from *T. murayana* before degradation using the alginate lyase enzyme, which was 15.75 cP (Reski *et al*., 2025). The expected viscosity of AOS*Tm* in this study should be lower than that of alginate polysaccharides. Lower viscosity facilitates easier dissolution in water, thereby allowing its application as a feed additive for poultry through drinking water. The lower viscosity level in this study was attributed to the AOS*Tm* produced having been hydrolysed by the alginate lyase enzyme into shorter alginate oligosaccharides, resulting in lower viscosity and higher solubility compared to alginate polysaccharides. According to Lee and Mooney (2012), the viscosity level of a material is directly proportional to the length of its molecular chain; the shorter the alginate oligosaccharide chain formed due to degradation, the lower the material's viscosity. Furthermore, Zimoch-Korzycka *et al*. (2021) also reported that the degradation process of alginate into alginate oligosaccharides can reduce its viscosity compared to alginate polysaccharides. Liu *et al*. (2019) also stated that the enzymatic degradation of alginate produces alginate oligosaccharides with smaller molecular sizes and lower viscosities.

The molecular weight of AOS*Tm* produced in this study ranged from 27,226 to 29,847 g/mol. The molecular weight of AOS*Tm* in this study was lower than that of alginate polysaccharides before enzymatic degradation, which was 76,786 g/mol (Reski *et al*., 2025). The lower molecular weight of AOS*Tm* among the treatments in this study was due to its lower viscosity level compared to that of alginate polysaccharides. The viscosity level of a material is closely related to its molecular weight; the higher the viscosity, the higher the molecular weight, and vice versa (Subaryono *et al*., 2017). This result is supported by Lu *et al*. (2022), who stated that the molecular weight of alginate oligosaccharides is generally lower than that of alginate polysaccharides. The low molecular weight of AOS*Tm* produced in this study resulted from the degradation of alginate polysaccharides into simpler forms by specific enzymes, such as alginate lyase. According to Liu *et al*. (2019), the enzymatic degradation of alginate produces alginate oligosaccharides with low molecular weight, which exhibit higher bioactivity compared to alginate polysaccharides. Furthermore, Xing *et al*. (2020) also reported that alginate oligosaccharides with low molecular weight are more effective as antimicrobial, antioxidant, and immunomodulatory agents because they are more easily absorbed and can interact more readily with target cells. Alginate oligosaccharides resulting from enzymatic degradation have a lower molecular weight and exhibit better bioactive properties as antimicrobial agents against pathogenic bacteria (Zimoch-Korzycka *et al*., 2021).

The inhibition zone diameter formed by the administration of AOS*Tm* produced through degradation using alginate lyase enzyme with different doses against *Salmonella* sp. ranged from 22.71 to 26.12 mm. These results showed no significant difference among treatments in the inhibition zone of *Salmonella* sp. However, all treatments were able to inhibit the growth of *Salmonella* sp., as indicated by the formation of inhibition zones in each treatment. The inhibition zone results were also supported by the optical density (OD) values among treatments, which ranged from

0.141 to 0.194. Based on these results, it can be concluded that the administration of AOS*Tm* can suppress the growth of Salmonella sp. According to Hu *et al.* (2005), alginate oligosaccharides can disrupt the permeability of bacterial cell membranes, thereby inhibiting their growth. Furthermore, Xing *et al.* (2020) also stated that alginate oligosaccharides with low molecular weights have higher antibacterial activity because they can interact more easily with the bacterial cell surface.

The inhibition zone diameter formed by the administration of AOS*Tm* against *E. coli* ranged from 15.87 to 18.28 mm. These results showed that administering AOS*Tm* produced through degradation using an alginate lyase enzyme with different enzyme doses did not cause significant differences among treatments. However, all treatments were able to inhibit the growth of *E. coli*, as indicated by the formation of inhibition zones in all treatments. According to Lu *et al.* (2022), alginate oligosaccharides with low molecular weight characteristics have a higher ability to diffuse and interact with bacterial cell membranes, thereby disrupting and inhibiting bacterial growth. The inhibition zone test results, which suppressed *E. coli* growth, were also supported by optical density (OD) test results, which ranged from 0.142 to 0.186. According to Zhu La *et al.* (2023), alginate oligosaccharides can suppress the growth of *E. coli* not only through direct interaction but also by modifying the intestinal microbial environment and stimulating the growth of lactic acid bacteria that act antagonistically against pathogenic bacteria.

The inhibition zone diameter formed by the administration of AOS*Tm* against the growth of *S. aureus* ranged from 19.35 to 21.10 mm, with optical density (OD) values ranging from 0.179 to 0.194. These results showed that the administration of AOS*Tm* produced through degradation using alginate lyase enzyme with different enzyme doses did not cause significant differences among treatments; however, all treatments were able to inhibit the growth of *S. aureus*, as indicated by the formation of inhibition zones in all treatments. This was due to the AOS*Tm* produced in each treatment having low molecular weight and low viscosity, making it easier to diffuse and interact with bacterial cells, thereby inhibiting the growth of *S. aureus*. Addina *et al.* (2020) stated that alginate oligosaccharides can act as antibacterial agents through mechanisms of metal ion binding and interaction with bacterial cell membrane proteins. The results of this study were also supported by the relatively low OD values obtained in each treatment, indicating that the presence of AOS*Tm* inhibited the growth of *S. aureus*.

CONCLUSION

The alginate lyase enzyme dose of 3 mL per 100 mL alginate solution was the best dose for degrading alginate into AOS*Tm*, as indicated by the yield percentage of 74.90%, viscosity of 5.93 cPs, and molecular weight of 28,659.54 g/mol. In addition, the AOS*Tm* produced at the dose of 3 mL per 100 mL alginate solution was also able to inhibit the growth of pathogenic bacteria, as shown by the inhibition zone diameters of each pathogenic bacterium: 17.76 mm for *Escherichia coli*, 23.63 mm for *Salmonella* sp., and 21.10 mm for *Staphylococcus aureus*.

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NOVELTY STATEMENT

The novelty of this study lies in determining the optimal dose of the alginate lyase enzyme for degrading alginate extracted from the specific brown seaweed species *Turbinaria murayana* into AOS*Tm*, which are proposed as a potential alternative to antibiotic growth promoters (AGPs) in poultry.

AUTHOR'S CONTRIBUTION

The contribution of all authors to the preparation of the manuscript and the research was substantial. MEM, AY, YR, and SR collaborated on the research preparation, conceptualisation, experimental design, data collection, and data analysis. All authors have reviewed and approved the final version of the manuscript, consenting to its submission to the Animal Health and Production Journal.

Generative AI and AI-assisted technology statement

During the preparation and writing of this manuscript, no artificial intelligence (AI) tools were used

Conflict of interest

The authors have declared no conflict of interest related to the publication of this manuscript.

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