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Effects of mannan oligosaccharide supplementation in the diet on growth performance and physiology of juvenile lobster, *Panulirus polyphagus*

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Abstract
The present study demonstrates the effects of dietary mannan oligosaccharide (MOS) on growth performance and physiology of lobster, *Panulirus polyphagus* (initial carapace length, CL ~6 mm). An experiment was executed with four treatments supplemented with increasing quantities of MOS, including 0% (control), 0.10%, 0.30% and 0.50% and fed the lobster for 8 weeks. Results revealed that after Eight Weeks of diet feeding, the lobster fed 0.30% MOS had the highest growth (average carapace length gain, ALG = 1.25 ± 0.07 mm wk^{-1}), followed by the growth rate of lobster fed 0.50% and 0.10% MOS (ALG = 1.22 ± 0.07 mm wk^{-1} and ALG = 1.20 ± 0.05 mm wk^{-1}, respectively). The lowest growth rate belonged to the lobster served the control diet (ALG = 1.09 ± 0.04 mm wk^{-1}) ($P< 0.05$). The highest survival rates were groups of lobster fed 0.30% and 0.10% of MOS and were greater than the survival of the lobster fed the basal ($P< 0.05$). Perimeter ratios (PR) more developed in lobster fed MOS than PR value of losbters fed the basal diet ($P< 0.05$). Also, a level of 0.30% dietary MOS should be supplemented for lobster.

Keywords: *Panulirus polyphagus*, lobster, growth, body composition, mannan oligosaccharide

1. Introduction
The lobster, *Panulirus polyphagus* is one of the economic aquaculture species in Vietnam. Lobster cultured is relied on post-larval puerulus collected from the wild, and are grown in sea-cages to marketable size. Lobster aquaculture industry meet several problems such as inappropriate diet and health issues, which have caused considerable losses in yield and in some cases mass death happened [1, 2]. In fact, farmers use antibiotics to cure lobster diseases. Its has been known that antibiotics could boost growth, survival of cultured animals [3]. However, it was reported that the risk of antibiotic includes toxicity, resistance, as well as harm public health [4]. Research also revealed that antibiotic utilisation to animal production may cause economic loss [5]. In aquaculture, it has been proven that using prebiotic and probiotic supplementation in the diet could bring a range of beneficial effects. Besides vaccine, probiotic and prebiotic, e.g. mannan oligosaccharides has received high attention to alternate the use of antibiotic in aquaculture [6].
Prebiotics are defined as non-digested substance, but it brings advantages to the host including stimulating the growth of host's intestinal beneficial bacteria [6]. In practice, studies proved that prebiotics administration can promote growth rate in a lot of aquaculture animals [7-11], boosting Innae immune, disease tolerance [12] and improving the function and health of the gut [7, 8]. Dietary mannan oligosaccharide (MOS) is widely utilised in fish and crustacean aquaculture [13, 14]. Publications revealed that dietary MOS could enhance growth in many aquaculture species including clownfish, *Amphiprion ocellaris* [15, 16], white leg prawn, *Litopenaeus vannamei* [17], spiny lobster, *Panulirus homarus* [7], European lobster, *Homarus gammarus* [18], western king shrimp, *Penaeus latisulcatus* [19], green tiger shrimp, *Penaeus semisulcatus* [20].
In addition, gut surface is one of the values the estimate the health of aquaculture species. Many researches have been revealed that MOS could boost gut surface in rainbow trout, *Oncorhynchus mykis* [21], lobster, *Panulirus homarus* [7] or *Homarus gammarus* [18]. Moreover, dietary MOS supplementation can improve intestinal micro villi length of white sea bream [22], Pacific white prawn, *Litopenaeus vannamei* [17], lobster, *H. gammarus* [18], rainbow trout,

Oncorhynchus mykiss [23] and gilthead sea bream [24]. Nevertheless, Pryor, Royes [25] stated that MOS inclusion in the diet did not impact the growth, gut structure, micro villi length and micro villi density in sturgeon, *Acipenser oxyrinchus desotoi.*

Many studies has been carried out to examine the effects of MOS on cultured animals, yet, the benefit of MOS supplementation on lobster, *Panulirus polyphagus* is still not understood. Thus, the pupose of this research is to examine the optimal levels of mannan oligosaccharide (MOS) supplemented in diet on growth rate, survival and intestinal morphology of *Panulirus polyphagus.*

2. Materials and Methods

2.1 Cultured system and experimental lobster

There were 12 cylindrical cages size of 30 x 50 cm. The mesh size of the net was ~4 mm, The cages were hung at ~ 4 m water depth. The seed of the lobster (puerulus), *Panulirus polyphagus* were bought from buyers and transported into the sea cages and acclimated there for one week before the experiment. The experimental lobsters were offered bycatch at ~5% of body weight two times a day during acclimation period. In each experimental cage, the puerulus lobsters (n =10) were erratically stocked. The average carapace length of lobster at commencement was 5.90 ± 0.18 mm (SE).

2.2 Experimental diets and feeding

A swimming crab flesh was employed as basal diet (un-supplement, NT1). Four levels (0.10% (NT2), 0.30% (NT3) and 0.50% (NT4)) of mannan oligosaccharide (Bio-MOS®, Alltech, USA) were added to the basal (NT1). To make other the other diet treatments (NT2, NT3 and NT4), the MOS was weighed and stirred in squid oil and then mixed with basal diet. Lobsters were fed two times per day (25% at 07:00 - 8:00 and the rest 75% at 17:00 - 18:00). The uneaten feed was observed every morning in to adjust the feed. Also, the health of puerulus lobster was watched in the morning, then removed remaining feed, faecal subtances and death individual and shells.

2.3 Design of the experiment

The diet experiment was conducted to compare the growth, survival and intestinal morphology of lobster, *Panulirus polyphagus* fed different levels of dietary MOS inclusion. There were four levels of MOS supplementation making four experimental diets consist of the basal (without MOS supplementation), and three grading concentrations of MOS supplemented to the basal. Five replicates were in each treatment. Post-acclimation period, the puerulus lobsters were measured and started feeding the experimental diets (day 0). There were a total of 16 cages and 160 puerulus were used for the experiment. The experiment was conducted for 8 weeks.

2.4 Data and sample collection

The carapace lengths of all lobsters in each cage were measured by using callipers. The measurement was conducted at the beginning and at week 8 (end). At day 56 (end) two lobsters per cage (10 lobsters per treatment) were sacrificed to examine the intestinal morphology.

2.5 Gut morphology of lobster fed dietary mannan oligosaccharide

The 2nd abdominal segment of lobster was dissected to obtain the intestine. The intestine was preserved in 10% formaldehyde. Afterwards, the gut tissue was dehydrated by using 70% ethanol, followed by equilibration using xylene before paraffin embedding. The samples of lobster gut were then cut into cross sections of eight micrometer. Next, the sections were then get rid of paraffin, followed by hydrated and dyed in haematoxylin and eosin (H&E). Later, photos of the gut were taken under Olympus microscope at a magnification of 400x. Images were then measured internal and external perimeter of the gut by ImageJ v.1.8.0. The intestinal perimeter ratio (PR, arbitrary units) was computed as the following formula: PR = IP/EP, where IP and EP were internal and external perimeters, respectively. It was proved that a high PR indicates the gut has longer villi, higher mucosal fold, or both [7, 18, 23].

2.6 Protein and lipid content in the flesh of lobster fed MOS

At week 8, two lobsters from each diet treatment were randomly collected and sacrificed for body composition analysis. Protein, lipid contents in the body of experimental lobsters were measured by using the laboratory standard methods [26, 27].

2.7 Data calculation and statistical analysis

The average growth rate (ALG mm wk^{-1}), survival rate and coefficient of variation [7] were analysed by the equations as follows: (ALG (mm wk^{-1}) = (CL_t – CL_o)/t, survival rate = (Nt)/No. 100%; where CL_o (mm) and CL_t (mm) are carapace length of lobster at initial and at time t correspondingly, No and Nt are the numbers of puerulus lobsters at commencement and at time t, respectively. Variation in carapace length was estimated by the variation coefficient, CV (%) = 100 × SD / $\bar{X}$. Where $\bar{X}$ and SD were the average of carapace length and the standard deviation, respectively [28].

Results are demonstrated in average ± standard error (S.E.). Growth, survival and variation coefficient considered in cage unit. Growth rate of lobsters among treatments were compared by using ANOVA F-test. Least significant difference (LSD) post hoc test was applied, if F-test result was significant. Prior to each analsysis, data were tested for normal distribution and converted if needed. The difference survival data among treatments was analysed by using non-parametric test. When $P< 0.05$, differences between means were considered significant. All statistics were executed by SPSS 19 (IBM).

3. Results

3.1 Water quality in culture place

Water temperature ranged 27.5 – 28.5 ^oC, pH was from 8.1 – 8.3, salinity was 34 – 35 ‰, NH_4/NH_3 0 – 0.05 mg L^{-1} and dissolved oxygen (DO) ranged 5.5 – 6.6 mg L^{-1}.

3.2 Growth rate of lobster fed dietary mannan oligosaccharide

The growth rate of lobster was shown in Figure 1. Initial carapace lengths were not different among treatments. After 8 weeks of diet feeding, the highest mean carapace length (CL) was in the lobsters fed NT3, with significantly greater than the lobsters in the basal (NT1). The CL of the two groups of lobsters fed diets NT2 and NT3 were significantly greater than the CL of lobster fed the basal (NT1) ($P< 0.042$). CL of lobsters fed diet NT4 and NT1 (basal) did not differ significantly ($P = 0.121$).

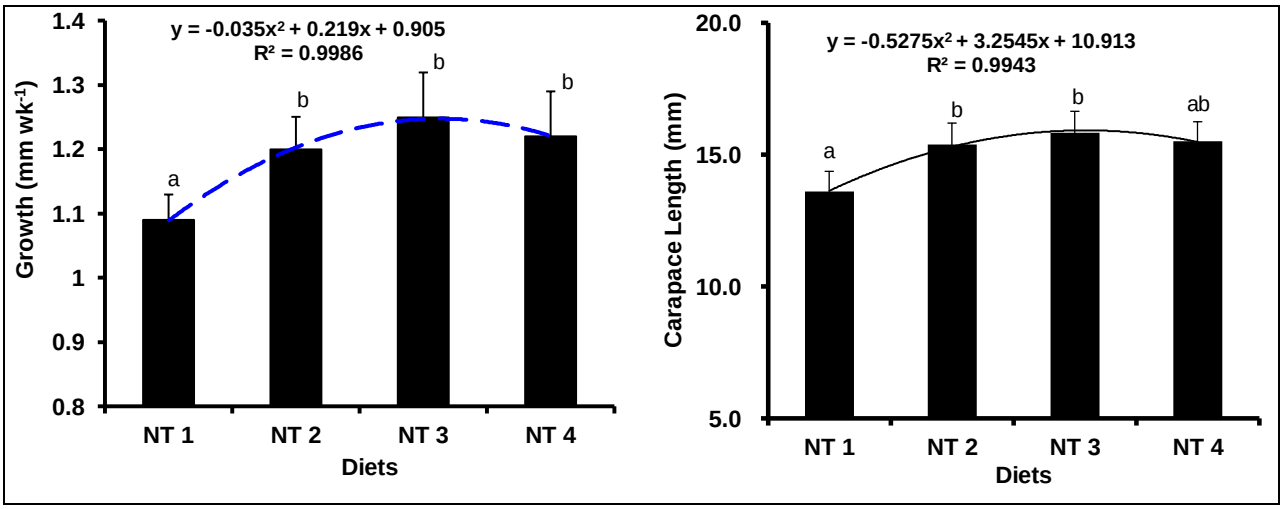

Fig 1: Growth rate of lobster (mm wk^{-1}) fed different concentration of dietary MOS (week 8). NT 1: Control, NT 2: 0.10% MOS. NT 3: 0.30% MOS. NT 4: 0.50% MOS. Data are shown as average ± SE. Data is significant difference if the letter are different (P<0.05).

The average length gain (ALG, %) was highest in lobster fed diet NT 3 (1.25 mm wk^{-1}), followed by the growth rate of lobster fed diet NT4 and NT2 (1.22 mm wk^{-1} and 1.20 mm wk^{-1}, respectively). The lobster fed the basal (NT1) had the lowest length gain, which was significantly lesser than all the lobsters fed the diets with MOS supplementations ($P\leq$ 0.036) (Figure 1). In addition, the length gain (ALG) of lobsters fed MOS supplemented in the diet did not significantly differ from each other (P ≥ 0.472). Growth rates were highly correlated with the levels of MOS supplemented in the diet of lobster (R^2 = 0.99).

3.3 Survival of lobster fed dietary mannan oligosaccharide

Survival rate of lobster among diet treatments ranged between 67% and 78% at day 56. The highest survival rate was in the lobster fed diet with 0.30% MOS inclusion (NT3), with the survival rate was 78%. The survival rate of this group of lobster was significantly greater than survival rates of lobster fed diets NT1 (control) (P< 0.05). Nevertheless, survival rates did not significantly differ between lobster fed diets NT2, NT4 and lobster fed the basal (NT 1) (P≥0.182). (Figure 2).

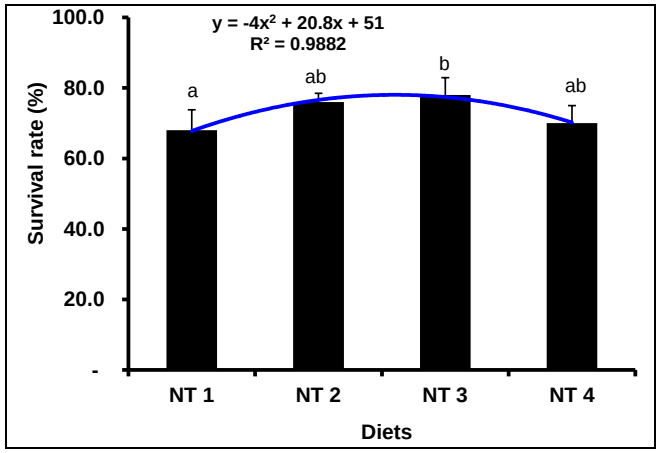

Fig 2: Survival rate of lobster after 8 weeks of diet feeding. NT 1: Control, NT 2: 0.10% MOS. NT 3: 0.30% MOS. NT 4: 0.50% MOS. Data are shown as average ± SE. Data is significant difference if the letter are different (P<0.05).

3.4 The coefficient of variance (CV, %) in size of lobster (Week 8)

At the commencement of the experiment, lobster of homogeneous sizes were used, which the coefficient of variance (CV) was less than 0.2%. CV increased to over 7% at the end od the experiment (Week 8). Coefficient of variations (CV, %) was the highest in lobster fed the control diet (10.33%) and were lowest in lobster fed diets NT3 followed by NT2 with CV values were 7.11% and 8.76%, respectively. The CV values in lobster fed diet NT3 was significantly reduced in comparison to the CV of lobster fed the basal (NT1) ($P\leq$0.021). However, there were not significant differences between CV values of lobster fed diets NT1, NT2 and NT4 (P ≥ 0.170) (Figure 3).

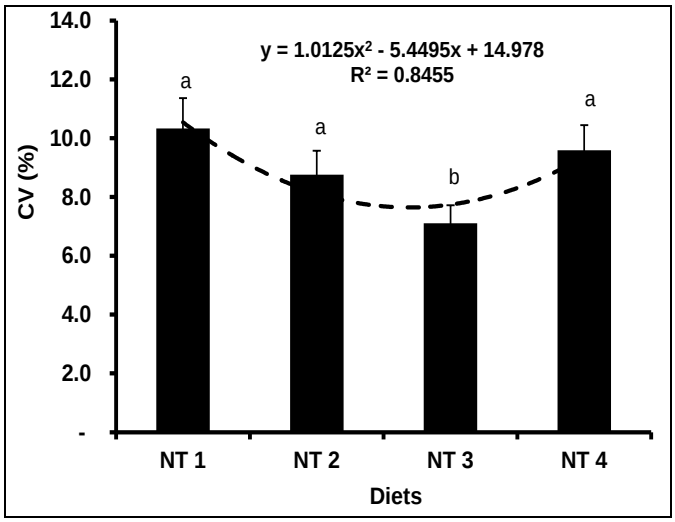

Fig 3: Coefficient of variation (CV, %) in length of lobster fed different levels of MOS (Week 8). NT 1: Control, NT 2: 0.10% MOS. NT 3: 0.30% MOS. NT 4: 0.50% MOS. Data are shown as average ± SE. Data is significant difference if the letter are different (P<0.05).

3.5 Intestinal morphology of lobster

The perimeter ratio is shown on Figures 4. The intestinal perimeter ratio (PR) of lobsters fed diets supplemented 0.10% 0.30% and 0.50% mannan oligosaccharide were significantly greater than the PR values of lobsters fed the basal ($P\leq$ 0.011). The PR value was highest in lobster fed 0.30% MOS (NT3) and the lowest PR was in lobster fed the control (NT1). Lobsters fed diets with all levels of MOS inclusion had higher PR values than PR of lobster fed basal diet (P<0.05). (Figures 4).

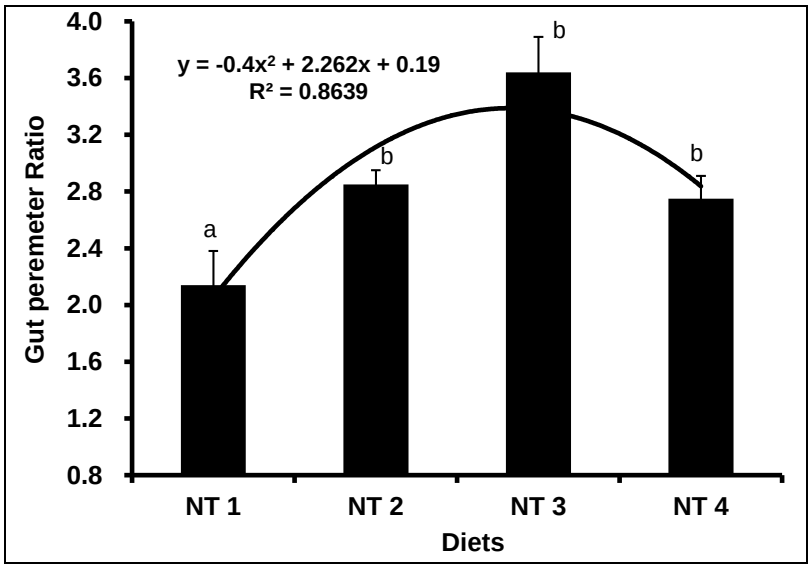

Fig 4: Gut perimeter ratio (PR) of lobster after 8 weeks of diet feeding. NT1: Control, NT2: 0.10% MOS. NT3: 0.30% MOS. NT4: 0.50% MOS. Data are shown as average ± SE. Data is significant difference if the letter are different (P<0.05).

3.6 Body composition of lobster fed dietary mannan oligosaccharide

At the end of the experiment (day 56), the muscle protein content of cultured lobster ranged from 21.93% to 22.72% in the lobster fed basal and in the lobster fed diet supplemented with 0.30% MOS, respectively. Lipid content of lobsters were from 2.2% (in lobster fed diets NT2 and NT3) to 2.5% in the lobster fed basal diet. Protein and lipid in the flesh of lobsters were not influenced by dietary mannan oligosaccharide (P > 0.05) (Figure 5).

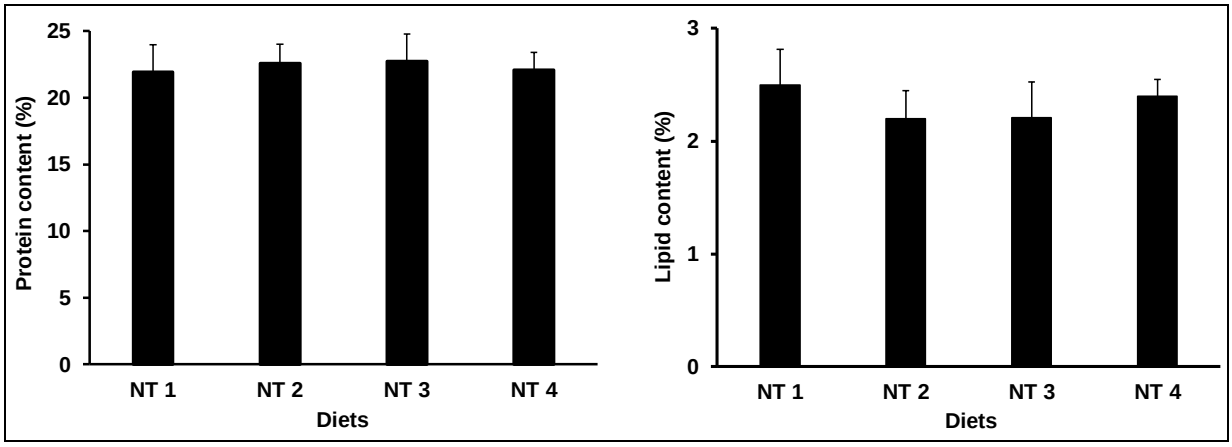

Fig 5: Protein and lipid content in the flesh of lobster fed different levels of MOS. NT1: Control, NT2: 0.10% MOS. NT3: 0.30% MOS. NT4: 0.50% MOS. Data are shown as average ± SE. Data is significant difference if the letter are different (P<0.05).

4. Discussion

This is the first findings on the benefit of MOS on the lobster, *Panulirus polyphagus*. Those fundamental results confirmed the beneficial role of dietary mannan oligosaccharide on the performance of lobster, *Panulirus polyphagus*. In accordance to our findings, other research also reported that dietary MOS supplementation could boost growth of many aquaculture species including clownfish, *Amphiprion ocellaris* [15, 16], white leg shrimp, *Litopenaeus vannamei* [17], lobster, *Panulirus homarus* [7], European lobster [18], western king prawn, *P. latisulcatus* [19], green tiger shrimp, *P. semisulcatus* [20].

The degree of size variation of cultured lobster was evaluated using a coefficient of variation. The higher the CV value is the higher variation in size of the animal. High size variation in a group of cultured animals may cause some negative impacts such as feed and ambient environment competition, in some case the small individuals can be the prey of bigger ones which will cause economic loss for the farmers. In this study, the lobster fed the diet supplemented with 0.30% MOS could reduce the size variation, with lower CV value. Also, this study indicated that the growth rate is highly correlated with gut surface area of the lobsters fed dietary mannan oligosaccharide (MOS) supplementation. Possibly, this is an indication of efficient benefits of MOS on growth and well-being of lobster.

Gut surface is the place the nutrient and energy from feed ingested by the host. The higher gut surface will enhance the digestion of animals and reduce energy for digestion [29]. The perimeter ratio is usually employed to evaluate the health of cultured animals [7, 12, 18, 21, 30-32]. In this study, the perimeter ratio of lobsters fed 0.1 - 0.5% MOS inclution in the diets significantly raised. Similarly, other researches have been revealed MOS could boost gut surface in other species for instance rainbow trout [21] and lobster [18]. Furthermore, research have indicated that dietary MOS inclusion can enhance micro villi length in cobia fish [33], sea bream [22], Pacific white prawn [17], rainbow trout [23], and lobster [18]. In

contrast, in a research has done by Pryor, Royes [25], MOS inclusion in the diet did not impact on growth, gut morphology, length and density of micro villi of sturgeon, *Acipenser oxyrinchus desotoi*. Possibly, the influence of dietary MOS might be species specific. In the present research we measured the gut morphology, but not other health index of lobster. The MOS supplementation might also boost other immune parameters of lobster. However, this should be examined experimentally.

Body composition including protein and lipid content generally reflects status of nourishment and health of aquaculture species [34]. The body composition of fish play an vital role as it impacts fish growth and survival of cultured species [35]. It was reported that dietary MOS inclusion possibly impacts protein content in the tissue of cultured animal, however the influence might species-specific [36]. In line with other study on lobster, *Panulirus homarus* [7], current findings revealed that dietary mannan oligosaccharide did not change protein and lipid content of lobsters.

5. Conclusion

This study proves that dietary mannan oligosaccharide supplementation could enhance growth, survival rate, reduce the coefficient of variation and impove gut morphology of lobster, *Panulirus polyphagus*. A levels of 3 g kg^{-1} MOS is recommended to add to lobster feed to gain better growth performance of lobster. This is only fundamental results, further studies such as the effects of mannan oligosaccharide on diverse life stages or suffer to stress or pathogens should be inspected experimentally.

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