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FULL PAPER

Oligochitosan for mutual paddy and tilapia growth performance in aquaponics system

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Abstract

This study was conducted to evaluate the influence of oligochitosan added to the fish tank to benefit both tilapia and paddy in the aquaponics system. Chitosan was irradiated at 25 kGy to produce low molecular weight chitosan (oligochitosan). The characteristics of chitosan such as molecular weight, solubility, viscosity, and colour were investigated. The low molecular weight chitosan (Mw = 5 kDa), obtained from the radiation degradation by gamma-ray was further used as a dietary supplement for tilapia. Nitrogen, one of the most important macro-nutrients for plant growth and crop productivity was supplied by fish faeces and food waste containing oligochitosan in fish tanks. The addition of oligochitosan could improve growth, and immune responses and alter the bacterial flora in the intestine of tilapia, so as to play a role in promoting the growth of paddy in the aquaponics system.

1. Introduction

Oligochitosan obtained from gamma-irradiated chitosan is a potential product to enhance the growth of plants such as chilli, mulberry, banana tissue culture and pineapple. A recent study (Idris *et al*., 2020) showed that it also has the potential for growth performance and feed digestibility of tilapia. The addition of oligochitosan in the dietary supplement of tilapia shows the best result on final weight, growth rate, feed conversion ratio, protein efficiency ratio and survival rate.

Oligochitosan or low molecular weight chitosan are the hydrolytic products of chitosan, which possess, high water solubility and reduced viscosity, thus making these polymers suitable for various applications (Du *et al*., 2010). Low molecular weight chitosan can effectively reduce the number of harmful bacteria such as *Escherichia coli* in fish intestines. It can increase the number of *bifidobacteria* and *lactobacilli* hence, improving the growth performance of aquaculture animals. Low molecular weight chitosan can also improve the immune function of fish. The physiological activities such as the absorption and metabolism of nutrients and the immune response were improved by affecting the beneficial microbial flora of the animal intestine. Studies have shown that oligochitosan can reduce the concentration of plasma, triglycerides and total cholesterol, thereby improving lipid metabolism, increasing protein deposition rate, and reducing urea deposition in the kidney, thereby helping to reduce the possibility of kidney urea poisoning. In addition, the addition of oligosaccharides also has a certain effect on immunoglobulins (IgG, IgM, etc.), growth hormone (GH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the blood (Marine-oligo, 2019).

The performance of oligochitosan as a plant growth promoter and in aquaculture can be explored in a combination system of aquaculture and hydroponic (aquaponics). Hydroponics requires expensive nutrients to feed the plants as well as periodic flushing of the systems which can lead to waste disposal issues. Re-circulating aquaculture needs to have excess nutrients removed from the system which can ordinarily mean that a percentage of the water is removed on a daily basis. That nutrient-rich water then needs to be disposed of and replaced with clean fresh water. Aquaponics will produce fish and plants in one system with a large reduction in water use. Microorganisms convert fish waste and faeces into nitrogen for plant growth while plant roots purify water for fish.

2. Materials and methods

2.1 Preparation of oligo chitosan

Chitosan powder with a molecular weight of 37 kDa and a degree of deacetylation of >85% was obtained

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from the USA. The chitosan powder was mixed with distilled water and irradiated under gamma irradiation (SINAGAMA) at 25 kGy to produce low molecular weight chitosan. The irradiated solution was then dissolved with mild acid and homogenized for 24 hours. Low molecular weight chitosan (5 kDa) obtained from the gamma irradiation process was used as a dietary food supplement (oligochitosan).

2.2 Molecular weight and chemical bonding determination

The molecular weight of chitosan before and after irradiation by gamma-ray was measured by Size Exclusion Chromatography (SEC) equipped with MALLS (multi-angle laser light scattering) detector brand Wyatt. The chemical bonding of chitosan was analysed by Fourier-Transformed Infrared Spectroscopy (Bruker -Tensor II).

2.3 Experimental procedure and conditions

Young tilapias were obtained from a local supplier and transferred into a 500 L tank. The tank was connected to the aerator and filter system to support the required condition. After acclimation for two weeks, the weight of each tilapia was taken and randomly distributed to a fish tank with a diameter of 100 cm and 65 cm in height. The tilapia was fed with food pellet with addition of irradiated chitosan (oligochitosan). The control tank was served with a conventional food pellet as a control parameter. The fishes were fed at the rate of 5% of their body weight thrice per day. The effective microorganism was also added to act as a bio-stimulant increasing the resident nitrogen fixation capacity directly through the stimulation of N-fixing bacteria. The basic parameters of water such as pH, temperature, dissolved oxygen and ammonia were checked regularly to maintain the optimal level. The fish were reared for 60 days and weighed every ten days.

The weight of each specimen of tilapia was recorded at the beginning and every 10 days during the experiment. Based on the weight of each fish, the weight gain was calculated as follows (Lin *et al*., 2011):

Weight Gain (WG) = Final weight (g) – Initial weight (g)

Paddy seedlings (MR 219) were first prepared in the nursery and then transplanted in the combination of film nutrient and media bed aquaponic system after about 35 days. The paddy takes up the nutrients from the fish tank containing fish excrete, food waste and oligo chitosan which was added into the tank earlier. The waste and oligochitosan within the water were supplied to the paddy as nutrients, and in turn, the paddy purified the water for the tilapia by taking up the nutrients. In most cases, no additional fertilizer was added to ensure that only fish faeces, food waste, bacteria and oligochitosan influence the performance. The growth of paddy in the aquaponic system was recorded during the experiment. The water tank quality such as pH, ammonia, nitrite and nitrate was recorded.

3. Results and discussion

3.1 Effects of irradiation dose on the molecular weight and chemical structure of oligochitosan

The effect of irradiation dose has been interpreted through molecular weight analysis (Figure 1). In this study, the molecular weight of the original chitosan (37 kDa) was reduced drastically to 5 kDa upon gamma irradiation interaction. The chain scission reaction reduced the molecular weight of the chitosan when subjected to the gamma irradiation dose at 25 kGy. The result from FTIR revealed chain scission which causes degradation in chitosan structure. The degradation mechanism is an important parameter to acquire appropriate low molecular weight hence finds commercial applications of chitosan compared to the higher molecular weight. The FTIR spectra indicate that no new chemical groups were formed by the γ-irradiation. Therefore, irradiation does not induce the observable crosslinking between chitosan molecules (Desai and Park, 2006).

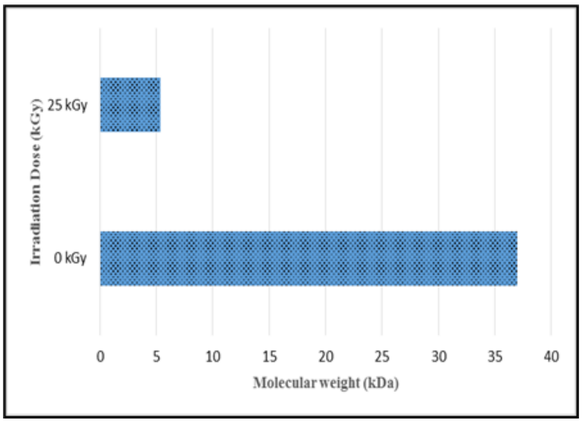

Figure 1. Molecular weight of chitosan before and after irradiated (25 kGy) by gamma ray.

The FTIR study (Figure 2) revealed the decrease in glycosidic bond linkage (relative intensity between the bands 1124 and 865 cm^{-1}) indicating the molecular chain scission of chitosan. Figure 2 indicated that only the change in relative intensity could be seen due to the overlapping of the bands corresponding to the tensions of the carboxyl groups with other bands. The intensity of the absorption peak at 1600 cm^{-1} assigned to the N–H bend vibration of $–NH_2$ was slightly increased after chitosan was irradiated. This may be due to the decrease of inter or intra-molecular hydrogen bonding between the –OH and $–NH_2$ groups (Hassan, 2015). During

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exposure to the γ-irradiation source, random polymer chain scission occurs (Faisant *et al.,* 2002), and the average macromolecular weight decreases (Choi *et al.,* 2002). This leads to a decrease in the extent of polymer chain entanglement and an increased mobility of the macromolecules.

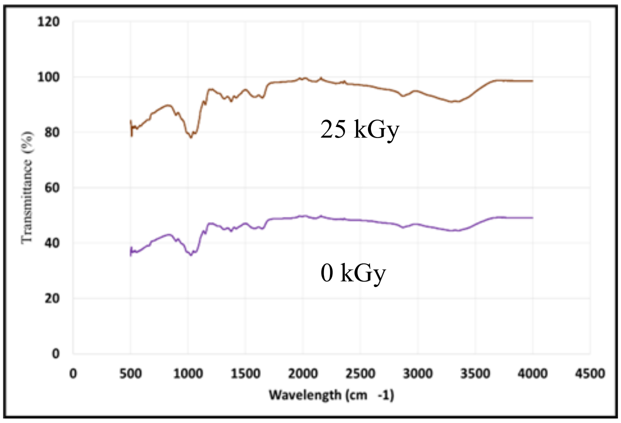

Figure 2. FTIR of chitosan before and after irradiated (25 kGy) by gamma ray.

Irradiation of chitosan causes the chain to scission into smaller fragments. This can improve chitosan molecular mobility due to its shorter chain and size and make it suitable for use in the production of oligochitosan for agriculture and aquaculture.

3.2 Growth performance of tilapia

The addition of irradiated chitosan (oligo chitosan) into the fish tank has influenced the weight gain performance of tilapia (Figure 3). In this study, oligo chitosan has improved the growth performance of tilapia compared to the fish in the control tank. Oligo chitosan cannot be decomposed by endogenous digestive enzymes due to their intermolecular binding positions and binding types, but it can be hydrolyzed by glycosidase secreted by beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*. These bacteria generate monosaccharides, and volatile fatty acids and then be used by the body or microorganisms to indirectly achieve the growth-promoting effect.

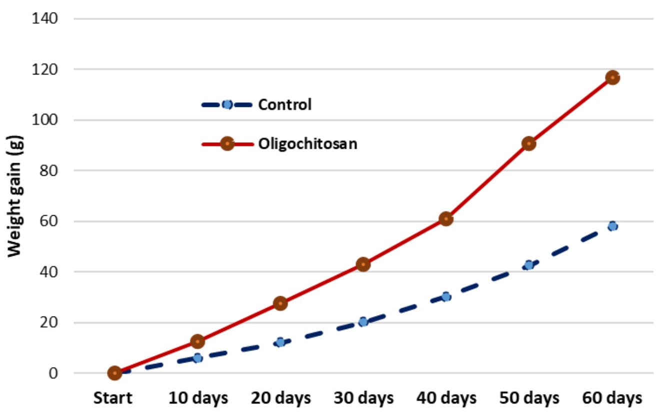

Figure 3. Weight gain of the tilapia fed with oligochitosan.

Furthermore, the addition of oligo chitosan also helps to improve the distribution of tilapia intestinal microbes. It is considered to be an important intestinal function regulator, which can improve the microflora in the digestive tract of animals. A large number of scientific studies have confirmed that oligo chitosan can effectively reduce the number of harmful bacteria such as *Escherichia coli* in fish intestines and increase the number of bifidobacteria and lactobacilli (Marine-oligo, 2019).

The effect of chitosan on the growth performance of fish has been reported by Niu *et al*. (2011), which concluded that chitosan is an active growth promoter and can be considered an essential element for the growth of aquatic animals. Improvement in the morphological structure of the small intestine can be the main effect of chitosan, which may improve nutrient absorption and growth performance as revealed by Zaki *et al*. (2015)

3.3 Performance of paddy

The paddy seedlings grew exponentially from 13 cm in height (Figure 4) until the rice was ready for harvest at about 140 days. The pH was maintained at 6.8 – 7.4, ammonia and nitrite were at 2.0 ppm. Nitrate, one of the most important elements was increased from 10 ppm to 80 ppm in two weeks and further increased to 160 ppm. It is initially indicated that the ammonia and nitrite have been decomposed by nitrifying bacteria to nitrate. The nitrifying bacteria (Pilinszky *et al.,* 2015) assisted with oligochitosan detoxifying ammonium (excreted by the fish) by converting it to the much less toxic nitrate in which the demand for nitrogen was fulfilled. Nitrogen is largely responsible for the growth of leaves on the plant. Phosphorus is largely responsible for root growth, and

Figure 4. Growth performance of paddy (MR 219) (a) 1, (b) 5 (c) 19 and (d) 24 days in aquaponics system. Although it is reported that rice crop needs seventeen essential nutrients, the most important among them are nitrogen, phosphorus and potassium which are adequately supplied from fish pallets and tilapia tanks (containing bacteria and oligochitosan).

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flower and fruit development. Phosphorus supply is naturally found in protein-rich foods such as meats, poultry, fish, nuts, beans and dairy products which are ingredients composed in tilapia pallets (32% protein). On the other hand, Potassium (K) as a nutrient that helps the overall functions of the plant perform correctly was also supplied by fish pallet which is abundant in whole foods including fish ingredients.

After 140 days, the rice grains grown in the aquaponics system were harvested (Figure 5). There are many factors that could be taken into account in producing the quality of the rice. For normal paddy plantation on fields, the level of water is to be maintained according to the growth and the fields are drained dry before the crop is harvested. Although this study has proven that paddy plantation in the aquaponic system with oligochitosan in tilapia tank served as fertilizer has successfully given good results in terms of paddy yields, the water management and oligochitosan inputs must be properly timed to obtain optimum stands, tillering, and panicle and grain formation.

Figure 5. Paddy yield in aquaponics system.

4. Conclusion

Tilapia performed well with oligochitosan in aquaponics system. In the fish tank, nitrifying bacteria convert ammonia to nitrite, and nitrite to nitrate further supplying N macronutrient for paddy. Oligochitosan as a dietary supplement containing nutrients serves as food for these bacteria and improves water quality. In the digestive system, oligochitosan improves the activity of the gastrointestinal microbiota and enhances the immune status and the growth of tilapia.

Conflict of interest

The authors declare no conflict of interest.

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