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Development of Protein-Rich Fish Feed by Using *Gammarus sp.* and Chitosan

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Abstract: Aquaculture is the cultivation of aquatic animals and plants under controlled or semi-controlled conditions. Aquaculture meets most of the world's fish needs. Due to the high nutritional value of fish and meeting the nutritional needs, interest in aquaculture has increased. In aquaculture, the most spending is made on fish feed. By reducing this cost, an increase in fish production can be achieved and incentives for fish farming can be supported. The main protein source used in fish farming is fish meal. However, it has been getting harder to supply fishmeal recently and its price is increasing rapidly. It is of great importance to investigate high nutritional and protein-rich sources in fish feed. The target with quality and balanced feed is to ensure that the fish reach the market weight as soon as possible. In this study, Gammarus and chitosan were used as feed additives. The effect of gammarus, which is rich in protein, on growth and development performance of rainbow trout juveniles was investigated by using it as a feed additive together with commercial feed. It was aimed to achieve this goal by coating with non-toxic, biocompatible chitosan in order to ensure that the feed composition has a high nutritional value, sufficient and balanced, as well as providing resistance against diseases. As a result, it was determined that the coating of fish feeds with Gammarus and chitosan have a growth-supporting effect and, together with this, they provide resistance against diseases in fish. In this regard, we believe that our work has the potential to bring innovation to the fish feed industry.

Keywords: Gammarus, chitosan, fish feed.

Introduction

In aquaculture, it is a wide and important field of production and science that includes the principles of aquaculture, by taking the development of aquatic organisms under the most suitable environmental conditions, without disturbing the ecological structure of water resources, by taking the natural environment and natural game stocks under control. In the field of production, technological and scientific developments have made significant contributions to aquaculture in the last 60 years (Bostock, 2011).

One of the main problems of the developing world and increasing population is nutrition. The animal protein needs of the rapidly growing population cannot be met. On the other hand, the need for protein is constantly increasing. In order to meet this need, aquaculture fishing and cultivation are carried out. However, it is not sufficient for animal protein needs. In our country, especially in landlocked inland fish consumption is well

below the average in Turkey with red meat are to meet the demand for animal protein. Considering the difficulties in terrestrial animal husbandry and the increasing costs to meet the animal nutritional needs, it should be considered that we should give due importance to aquaculture. Aquaculture has been started to meet the animal protein needs of fishery products, which have important nutritional value for human health (Korkut et al., 2002).

In addition to the continuous development in aquaculture, it has brought various problems. Intensive production in aquaculture has caused a significant increase in fish diseases. Diseases spread and huge economic losses were experienced due to wrong drug use and wrong information. The use of chemical drugs in the fight against diseases has increased costs and caused immunity to drugs over time. A solution was sought against this problem with the additives of natural products, not chemicals, in fish diseases. It is aimed to increase resistance to diseases, strengthen the immune system and accelerate growth by adding natural products to fish meal. The fact that natural additives do not leave residues on fish and do not threaten human health has made it inevitable to carry out different studies in aquaculture (Kabak, 2009).

In this study, we worked on two feed additive materials to solve the problem and contribute to healthy and efficient fish production activities. In our study, the protein-rich *Gammarus sp.* and we will describe the immunostimulating and immune-supportive chitosan.

Gammarus sp.

Gammarids are commonly used organisms for risk assessment of freshwater quality criteria (Rinderhagen et al., 2000; Serdar et al., 2018). Generally, they are found in the source parts of rivers and are an important food source for fish, birds, and amphibians (MacNeil et al., 2002), and leaf litter plays an important role in the breakdown process (Forrow & Maltby, 2000).

Table 1. Chemical composition of Gammarus sp.

%	Taleb et al, 2020-Abo	Harlıoğlu & Farhadi, 2018
Protein	40	40-45
Lipid	5,5	5-10
Carbohydrate	27,4	6-15
Ash	21,4	25-35

It has been reported in previous studies that *Gammarus sp.* consists of 40-45% protein and chitosan consists of 12% protein.

Chitosan

Chitin, a natural polysaccharide that is the most abundant in nature after cellulose, is the main component of the exoskeletons of the cell walls of crab, insect, fungus and some fungi and green algae. Chitosan is one of the best known non-toxic polymers that can be broken down naturally, adapt to our body. Chitosan [poly-β- (1 → 4) N-acetyl-D-glucosamine] differs from the chitin molecule is the deacetylated [N-deacetylated] form of chitin (Jikakis, 1984; Chhabra, 1999).

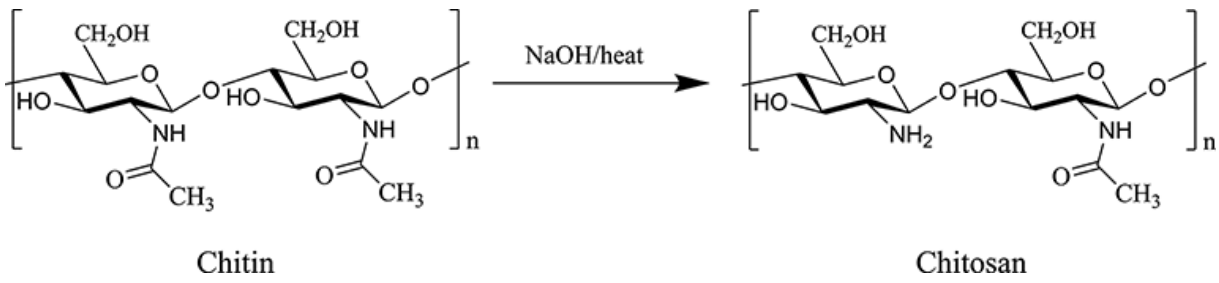


Figure 1. Chitin deacetylation process to produce chitosan.

The deacetylation degree (DD) of chitosan is calculated by the ratio of 2-acetamido-2-deoxy-D-glucopyranose units to 2-amino-2-deoxy-D-glucopyranose units. The degree of deacetylation has been found to have significant antimictrobial effects on some bacterial species (Tsai et al., 2006).

Method

Preparation of Gammarus sp.

Gammarus sp. were collected from the wetland in Gaziantep province Karkamış district. *Gammarus sp.* to be used as feed material were cleaned after being collected and shocked at -80^{o}C.
After shocking, *Gammarus sp.* were homogenized (Figure 2) and distributed in sterile sample containers with cover (Figure 3). Later, the samples were kept at -40^{o}C for 2 days and the excess water was taken and freeze dried in the lyophilizer (Figure 4).

Figure 2. Grinding of *Gammarus sp.*

Figure 3. Sharing of *Gammarus sp*. to sterile sample containers

Figure 4. Freeze drying in lyophilizer

Figure 5. *Gammarus sp.* to be used as feed covering material.

Preparation of chitosan

Commercially available crayfish *(Astacus leptodactylus)* were washed cleaned and dried (Figure 6).

Figure 6. Cleaning and drying Freshwater Lobsters *(Astacus leptodactylus)*

The dried crayfish were ground into powder (Figure 7) and stored at + 4^{o}C until use (Figure 8).

Figure 7. Grinding Freshwater Lobsters

Figure 8. Keeping Freshwater Lobsters at + 4^{o}C

Chitosan Synthesis from Chitin

The chitosan synthesis from chitin consists of 4 steps:
Deproteinization: Proteins are removed.
Demineralization: Minerals are removed.
Decoloration: Bleaching is done.
Deacetylation: Acetyl groups are removed and chitosan is synthesized from chitin.

For deprotenization, the sample was treated in 3.5% NaOH [1:10, (w / v)] solution at 65oC for 2.5 hours. At the end of the process, the sample was washed several times with distilled water and dried in the oven. For demineralization, the sample was treated in 1N HCl [1:15, (w / v)] solution at room temperature for 30 minutes under continuous stirring. At the end of the process, the sample was washed several times with distilled water and dried in the oven. For decoloration, acetone & 0.315% NaOCl [1:10, (w / v)] was used (No et al., 1989).

The chemical production of chitosan from chitin is based on the removal of acetyl groups in chitin using high alkaline solutions. For deacetylation, Koçer (2015) performed the deacetylation process in an autoclave (121^{o}C; 15 minutes) in his master's thesis. He stated that the use of autoclaves increased the deacetylation efficiency (18%). In our study, we performed the deacetylation process in an autoclave. At the end of the process, the sample was washed with distilled water and dried in an oven.

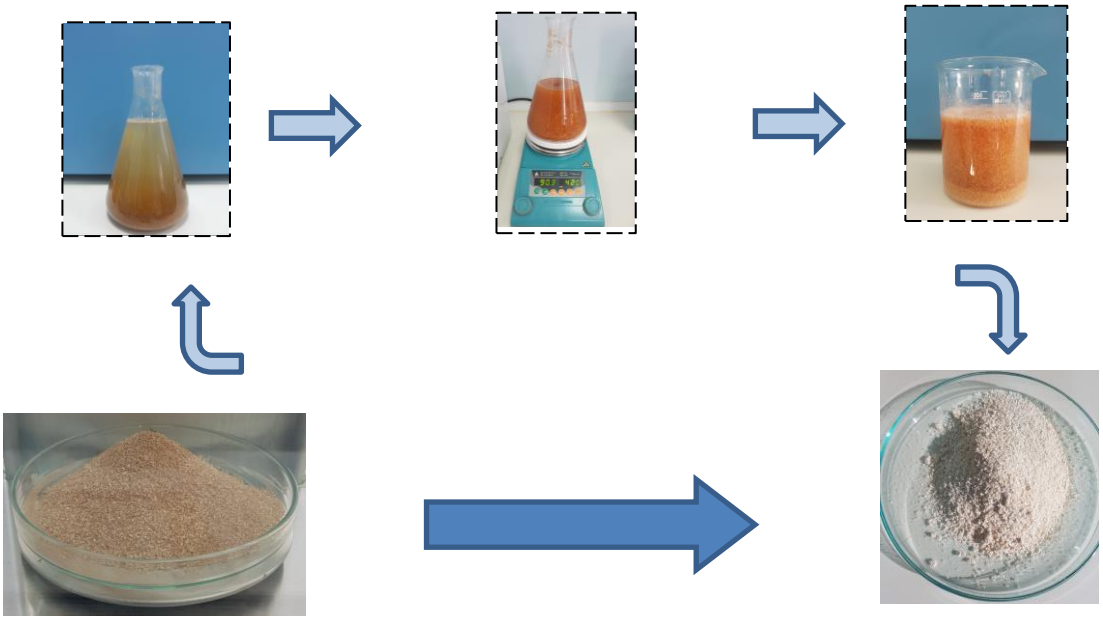


Figure 9. Chitin

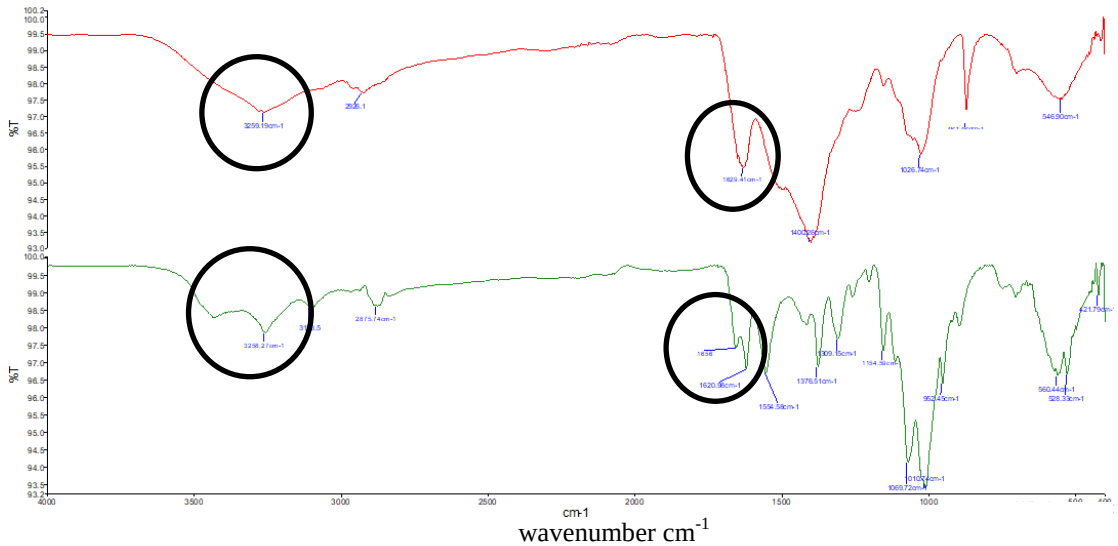
Figure 10. Chitosan

Figure 11. Scheme showing chitosan synthesis from chitin

Findings

FT-IR Analysis and Determination of Deacetylation Degree

FT-IR peaks of chitin and chitosan are given in figure 12. chitin is shown in red and chitosan in green. Here, it is seen that chitin in the spectrum of 3550-3230 (OH band) gives a peak at 3259.19 cm^{-1}. Chitosan is observed to give two sharp peaks in the OH band interval. In the spectrum of 1660-1600 (amide I band), it is seen that chitin gives a peak at 1629.41 cm^{-1}. Chitosan is observed to give two sharp peaks in the amide I band range. This indicates the presence of bonds and purification in chitosan.



wavenumber cm^{-1}

Figure 12: FT-IR analysis result

$$*DA\ (\%)=[(A_{1655}/A_{3450})x100/1{,}33]$$

Kitosan DA= **%85,705**

*Domszy & Roberts, 1985

FT-IR analysis results were used to calculate the degree of deacetylation. The calculation was made using the equation suggested by Domszy & Roberts (1985). The deacetylation degree of chitosan that we purified was found to be 85.705%. The protein amounts of Chitosan and *Gammarus sp.* are shown in the table (Table 2). BCA method was used for protein determination. Two solvents are used; PBS, ammonium bicarbonate. While the protein amount of *Gammarus sp.* was found to be 800,217 µg/mL in PBS, it was found 738,673 µg/mL in ammonium bi carbonate. The amount of protein was determined mostly in PBS. While the protein PBS of chitosan was found to be 577,143 µg/mL, it was found as 632,063 µg/mL in ammonium bicarbonate. The amount of protein was determined mostly in ammonium bicarbonate. When the data obtained are examined, it is seen that high amounts of protein are detected.

Table 2. Gammarus sp. and chitosan crude protein amounts

	µg/mL
Gammarus sp. (PBS)	800,217
Gammarus sp. (Ammonium bicarbonate)	738,673
Kitosan (PBS)	577,143
Kitosan (Ammonium bicarbonate)	632,063

Conclusion

In this study, synthesis was carried out using the method recommended in the literature for chitosan extraction from chitin (No et al., Koçer, 2015). In previous studies, the deacetylation degree of chitosan was reported as 69.4-77%. The deacetylation degree of chitosan we obtained in our study is 86%. We used the same method in our study and were able to obtain purer chitosan. This indicates that chitosan may be more effective in our study.

Note: We shared the work we did from Kadriye GUNBATTI's ongoing Master Thesis in this study.

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This study was approved by Gaziantep University Animal Experiments Local Ethics Committee. GAÜN-HADHEK approval was obtained. In the continuation of our work, Fırat Su Ürünleri Ltd. We will do it in Şti. Thank you to this business for giving us the opportunity.

Reference

A., Abo-Taleb, H., F Zeina, A., Ashour, M., M Mabrouk, M., E Sallam, A., & MM El-feky, M. (2020). Isolation and cultivation of the freshwater amphipod *Gammarus pulex* (Linnaeus, 1758), with an evaluation of its chemical and nutritional content. *Egyptian Journal of Aquatic Biology and Fisheries, 24*(2), 69-82.

Bostock, J. (2011). The application of science and technology development in shaping current and future aquaculture production systems. *Journal of Aqricultural Science*, *149*, 133-141.

Chhabra, P. (2004). Antimicrobial and antioxidant properties of chitosan. Unpublished Doctoral Dissertation, University of Georgia.

Domszy, J. G., & Roberts, G. A. (1985). Evaluation of infrared spectroscopic techniques for analysing chitosan. *Die Makromolekulare Chemie: Macromolecular Chemistry and Physics, 186*(8), 1671-1677.

Forrow, D. M., & Maltby, L. (2000). Toward a mechanistic understanding of contaminant‐ induced changes in detritus processing in streams: Direct and indirect effects on detritivore feeding. *Environmental Toxicology and Chemistry: An International Journal, 19*(8), 2100-2106.

Harlıoğlu, M. and Farhadi, A. (2018*): Importance of Gammarus in aquaculture. Aquac. Int., 26*: 1327–1338. J. P. Jikakis (Ed.) (1984), Chitin, Chitosan and Related Enzymes, *Academic, Orlando, FL, pp. XVII–XVIV.*

Kabak, T., (2009). *Gökkuşağı alabalık (Oncorhynchus mykiss)'larının spesifik olmayan immün sistemi üzerine bazı geofit bitki ekstraktlarının etkilerinin incelenmesi.* Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Su Ürünleri Yetiştiriciliği Ana Bilim Dalı Yüksek Lisans Tezi.

Koçer, İ. (2015). *Farklı yöntemlerle kitosan eldesi ve karakterizasyonu.* Hacettepe Üniversitesi Fen Bilimler Enstitüsü Kimya Mühendisliği Anabilim Dalı Yüksek Lisans Tezi.

Korkut, A. Y., Hoşsu, B., Gültepe, N., (2002). Balıklarda Beslenmeye Bağlı Hastalıklar. *E.U. Journal of Fisheries & Aquatic Sciences*. *19*, 555–564.

MacNeil, C., Dick, J. T., Bigsby, E., Elwood, R. W., Montgomery, W. I., Gibbins, C. N., & Kelly, D. W. (2002). The validity of the Gammarus: Asellus ratio as an index of organic pollution: abiotic and biotic influences. *Water Research, 36*(1), 75-84.

No, H. K., Meyers, S. P., & Lee, K. S. (1989). Isolation and characterization of chitin from crawfish shell waste. *Journal of Agricultural and Food Chemistry*, *37*(3), 575-579.

Rinderhagen, M., Ritterhoff, J., & Zauke, G. P. (2000). Crustaceans as bioindicators. *In Biomonitoring of Polluted Water-Reviews on Actual Topics. Trans Tech Publications-Scitech Publications, Environmental Research Forum, 9,*161-194.

Ruiz, G. A. M., & Corrales, H. F. Z. (2017). Chitosan, Chitosan Derivatives and their Biomedical Applications, *Biological Activities and Application of Marine Polysaccharides, Emad A. Shalaby, IntechOpen,* DOI: 10.5772/66527.

Serdar, O. (2019). The effect of dimethoate pesticide on some biochemical biomarkers in *Gammarus pulex. Environmental Science and Pollution Research, 26*, 1–10.

Tsai, G. J., Tsai, M. T., Lee, J. M. ve Zhong, M. Z. (2006). Effects of Chitosan and a Low-Molecular-Weight Chitosan on Bacillus Cereus and Application in the Preservation of Cooked Rice. J. *Food Protect. 69,* pp. 2168-2175.

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