PROTEIN FROM FERMENTED PRAWN WASTE SILAGE AS AN INGREDIENT IN THE DIET OF SEABASS

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ABSTRACT

Prawn processing industries remain as one of the main marine activities in the world as well as in Malaysia and it normally generates large volumes of prawn waste. This waste product which contains high amounts of chitin (14 – 30 %) and protein (15 – 40 %) has been exploited to obtain chitin through chemical processes. However, this process renders the valuable protein useless. In this work, a lactic acid fermentation process was used to ferment the prawn waste and the performance of using the fermented prawn waste liquor (FPWL) as a protein source in seabass's diet was investigated. Initially, prawn waste with an addition of 10 % (w/w) of glucose and 10 % (v/w) starter culture was fermented for 60 days at 37°C. The pH value decreased from 7.51 to 4.00 within 24 hours and remained stable with no signs of spoilage up to 60 days. The fermentation produced solid chitin and protein liquor component, the latter of which contains 50 and 56 % protein after 3 and 7 days of incubation period respectively and increased only slightly to 58 % on day 60. After 3 days of fermentation period, 78 % of the protein from the waste can be recovered as FPWL and 97 % of chitin remained in the solid prawn waste. It was also observed that as the glucose concentration decreased, the lactic acid concentration and the bacterial growth increased. Similar fermentation was also successfully conducted at ambient temperatures (27 to 33°C). Attempts were made to increase protein content of liquor by freeze drying into a powdered form and also by removing the calcium lactate through storage at -20^{o}C. However, both processes despite producing a product which can be easily handled, is time and energy consuming while not improving the protein content. Thus, the usage of protein liquor in liquid form was more suitable. A feeding trial was conducted for 49 days to evaluate the effect of using FPWL as a substitute for fishmeal in seabass diet. The inclusions of FPWL were at 10, 20 and 30 %. Growth performance of seabass with initial weight of 16.2g ± 0.4, fed with FPWL-based diet was not significantly different ($P>0.05$) compared to control diet which contain fishmeal. The most effective diet was 30 % inclusion of FPWL in seabass diet, with weight gain, FCR and PER of 180 %, 1.78 and 1.2 respectively. Overall, FPWL is feasible to be used as a protein source to partially substitute fishmeal in diet of seabass.

ABSTRAK

Industri pemprosesan udang merupakan salah satu aktiviti marin utama dunia dan juga di Malaysia di mana industri ini biasanya menghasilkan sisa udang yang tinggi. Sisa udang ini yang mengandungi kandungan kitin (14 – 30 %) dan protein (15 – 40 %) yang tinggi telah dieksploitasikan untuk mendapatkan kitin melalui proses tindakan kimia. Walaubagaimanapun proses ini menjadikan protein tersebut rosak. Dalam kajian ini, fermentasi asid laktik telah digunakan untuk memproses sisa udang dan keberkesanan penggunanan cecair sisa udang terfermentasi (CSUT) sebagai sumber protein alternatif dalam diet ikan siakap dikaji. Sebagai permulaan, sisa udang difermentasi bersama 10 % (g/g) glukosa dan 10 % (mL/g) kultur pemula selama 60 hari pada suhu 37^{o}C. Nilai pH menurun dari 7.51 hingga 4.00 dalam masa 24 jam pengeraman dan berterusan stabil tanpa tanda kerosakan sehingga 60 hari. Proses fermentasi ini menghasilkan pepejal kitin dan komponen protein cecair. Komponen protein cecair mengandungi 50 dan 56 % protein selepas 3 dan 7 hari masa pengeraman masing-masing dan meningkat sedikit sahaja kepada 58 % pada hari ke-60. Selepas 3 hari fermentasi, 78 % protein boleh diekstrak daripada sisa udang ke dalam cecair protein manakala 97 % kitin masih kekal pada sisa pepejal udang. Hasil kajian juga menunjukkan apabila kandungan glukosa menurun, kepekatan asid laktik dan pertumbuhan bakteria meningkat. Fermentasi yang sama juga berjaya dijalankan pada suhu bilik (27 sehingga 33°C). Usaha dijalahkan untuk meningkatkan kandungan protein dalam CSUT dengan cara pengeringan sejukbeku menjadi bentuk debu dan juga mengeluarkan pepejal kalsium laktat melalui penyimpanan pada suhu -20^{o}C. Walaubagaimanapun, kedua-dua kaedah ini walaupun dapat menghasilkan produk yang mudah diselenggarakan, ia memakan masa yang lama dan menggunakan tenaga yang tinggi tetapi tidak dapat meningkatkan kandungan protein. Oleh itu penggunaan CSUT dalam bentuk cecair adalah lebih sesuai. Ujikaji pemberian makanan telah dijalankan selama 49 hari untuk mengkaji keberkesanan CSUT sebagai pengganti tepung ikan dalam diet ikan siakap. Diet kawalan dan 3 diet lain yang mengandungi CSUT pada peratusan 10, 20 dan 30 % telah dihasilkan. Ikan siakap yang mempunyai berat asal 16.2g ±0.4 dan diberi makanan berasaskan CSUT menunjukkan prestasi pertumbuhan yang tidak berbeza ($P>0.05$) dengan diet kawalan yang mengandungi tepung ikan. Diet yang paling efektif adalah diet yang mempunyai 30 % CSUT, yang merekodkan pertambahan berat, FCR dan PER sebanyak 180 %, 1.78 dan 1.2. Kesimpulannya, CSUT boleh digunakan sebagai sumber protein bagi menggantikan sebahagian tepung ikan dalam diet ikan siakap.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Nutrition and feeding strategies play a central and essential role in the development of an aquaculture sector. At present, commercial diets are the most commonly used diets for aquaculture purposes to maintain fish health and growth. However, development of aquaculture sector is hampered by inadequate supply of cheap and nutritional feedstuffs in particular the protein source. Demand for fish diet raw materials is expected to increase in the near future as aquaculture industry has been identified as one of the potential sector to be expanded especially in Malaysia (Malaysian Fisheries Department, 2004). Since major items such as maize, rice bran, meat meal, and fishmeal are imported, dependency on these expensive imported materials is viewed as one of the major constraints for aquaculture augmentation (Food and Agriculture Organization, 2007).

In the year 2000, Bangkok Declaration and Strategy Conference was held in Bangkok discussing issues pertaining to the interest of aquaculture industries. This conference has emphasized the importance of using agriculture and fisheries by-products and non-food grade feed materials in order to reduce fishmeal consumption in fish diet (Food and Agriculture Organization, 2000). This will also reduce the cost of fish diet and avoid competition between fishmeal exporters. One of the potential protein sources available widely in Malaysia is prawn waste. Due to the urgent priority in developing a cost-effective diet parallel with the

tremendous tonnage of prawn waste being produced each year, taking this issue seriously will definitely be beneficial for fisheries industries both in solving waste dumping problems and also to generate extra income.

The prawn waste or processing leftover contains 35-55% calcium, 15-40% protein, and 14-30% chitin (Ornum, 1992; Legaretta *et al.*, 1996). Chitin, which was discovered by Braconot in 1821, is now a well-manufactured and commercialized product since 1970's (Brzeski, 1987). Chitin is conventionally recovered from prawn waste using strong chemicals at extreme pH and temperature. However, this method generates large volume of aqueous waste as well as discarding many useful components including the precious protein (Brzeski, 1987; Zakaria *et al.*, 1998). In order to overcome the environmental problems as well as to recover the valuable protein, a biotechnological approach has been developed on the treatment of prawn waste using lactic acid fermentation. Fermentation involving lactic acid bacteria (LAB) for chitin recovery is seen to be promising whereby added advantages are protein recovery and a reduced chemical consumption (Zakaria *et al.*, 1998). It is interesting to note that the liquor from fermentation process is rich in protein and is suitable for animal feed (Hall and Silva, 1992).

Fermentation method can be easily adopted by local small-scale industries as the fermentation is generally accepted as a safe and low cost process (Soomro *et al*., 2002). From the economic point of view, the production of nutritional protein locally will relieve the local aquaculture industries of using the expensive imported protein. The problem of using local protein ingredient is not the lack of resources, but how to get it as nutritious as imported ones and at a cheaper price.

1.2 Problem Statement

Protein source have been gaining much attention, as it is generally the most expensive ingredient and important ingredient in preparing adequate nutrition for

fish (Steven and Helfrich, 2002). The most commonly used of the animal protein source is fishmeal, which is high in protein, highly digestible and a feed attractant. However, the availability of fishmeal that is largely dependent upon weather phenomenon such as El Nino or Tsunami, and added with over exploitation of fish has caused projection of higher future prices (Pontecorro, 2001; Delgado *et* al., 2002). Besides that, based on the recent scrutiny, fishmeal also been verified as contaminated with polychlorinated biphenyl (PCBs) (Jacobs *et al,* 2002; Easton *et al.*, 2002). All of these issues have necessitated efforts to find for alternative protein source (Naylor *et al.*,2000).

At present, small-scale aquaculture industry makes an effort to save cost of preparing fish feed diets by using the whole prawn waste as feed meal. The prawn wastes are sun dried and ground before it could be used as fish diet. Disadvantages of using dried prawn waste meal includes its high fibre and chitin content and unhygienic preparation, which might lead to feed meal with low protein content and high microbial loading (Meyers, 1986). The alternative method of using lactic acid fermentation on prawn waste is gaining interest worldwide, as the process and its products are safe and cheap. Nwanna (2003) reported that the liquid portion from the fermentation of Nigerian prawn head for 14 days using 15% of cane molasses and 5% lactic acid bacterium culture was suitable as an ingredient in feeds for African Catfish, *Clarias gariepinus.* The high protein and amino acid profile in the liquor were comparable to the usual fishmeal. Although it was evident that the research in Nigeria had been successful, the process variable needs to be improved in order to be applied in Malaysia using available raw material. Similar study by Zakaria *et al.* (1998) has shown that more than 90% protein from the prawn waste could be recovered within a shorter period of only 72 hours. Shirai *et al.* (2001) also concluded that the best result in fermentation of prawn waste was obtained using 10% of carbon source (glucose) and 5% inoculation levels for 72 days. As prawn wastes contents were varied depending on the handling and processing method, a new formulation of fish feed involving our own tropical prawn waste need to be studied. So far, no study on lactic acid fermentation of prawn waste for aquaculture purposes has been done in Malaysia especially for use in seabass diet.

1.3 Research Objectives

Based on the research background and problem statement mentioned above, the objective of this study is to investigate the feasibility of using protein obtained from fermented tropical tiger prawn (*Penaeus monodon*) waste as a substitute for fishmeal in the diet of seabass (*Lates calcarifer*).

1.4 Research Scopes

In order to achieve the above objective, the following scopes of study have been drawn:

1. Characterization of tiger prawn waste.

2. Lactic acid fermentation study on tiger prawn waste using previously isolated lactic acid bacteria and in particular to optimize production of the protein fraction as it will be used in the diet formulations. Characterization of fermentation products namely chitin and protein liquor.

3. Fish feed formulation using protein from fermented prawn waste and lab-scale juvenile seabass feeding trial study.

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