Holding capability of different coating material on protease and cellulase in shrimp feed preparation

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Abstract

The aquaculture becomes more potential for the world food supply chain; therefore, the main ingredients like fishmeal are necessary for feed manufacturing. Nonetheless, there is IUU law to control the usage of fishmeal so that fishmeal replacement materials introduced to the market. The plant protein-based is the most well-known raw materials. However, there is contain a high level of fiber (cellulose) and un-usable peptide, which was bound in phytate; thereby, the enzyme becomes a vital feed additive. On the other hand, there is a limitation of aquaculture feed production, which normally applied with high temperature, and the heat can diminish enzyme ability. The study is designed to find the solution of using an enzyme in aquaculture feed by coating materials. The abilities of individual coating materials, including chitosan, Seal 4 (commercial product), pullulan, and CMC, are observed on protease and cellulase leaching. The result shows the less leaching of protease are coated by pullulan, and CMC and with 0.200% and 0.210%, respectively. CMC is not a suitable material coating for cellulase because cellulose-based materials were destroyed. As a consequence, Seal 4 and pullulan are more efficient for cellulase.

Keywords: *Protease, Cellulase, Feed, Aquaculture, Chitosan, Pullulan, CMC, Seal 4, Coating*

1. Introduction

The aquaculture business has regularly been growing in the past decades, especially the shrimp industry, which continues to be the most vital import and export product in terms of value (Jantarathin, Borompichaichartkul & Sanguandeekul, 2017). However, there is a problem lately since the amount of the main ingredient like fishmeal decreased. The number of fishmeal affected by Illegal, unreported, and unregulated (IUU) fishing and price. As a consequence, most feed formulators developed the new feed formulation by fishmeal replacement with others such as soybean meal, worm meal, and algae powder. Plant-based protein feed seriously influences aquaculture animal digestibility as soybean meal contains antinutritional factors, for example, phytate, cellulose, and other fibers (Qiu & Davis, 2016). Hence, the enzyme was introduced to solve these problems, and the common enzyme that can break down nutrients from the plant protein are cellulase, phytase, xylanase, and also protease.

Cellulose, which is a polymer of glucose residues with β-1,4 linkages, is commonly found in the plant cell wall. As more plant protein source materials are applied in aquaculture feed ingredients, the feed contains higher composite forms of high microfibrils among amorphous matrixes, which un-accessed by hydrolyzing enzymes. As a consequence, cellulase is an important enzyme to break β-1,4 glycosidic bonds in the polymer to release glucose units leading to higher digestibility and higher energy source for aquaculture (Péreza & Samain, 2010). Cellulase was produced from bacteria and fungi that can actively convert insoluble cellulosic and cellulose substrates into soluble saccharides (Zhou *et al.*, 2013)

Besides, the supplement enzyme in aquafeed today does not get high responsibility from the feedmill. Shrimp and fish feed production has a limitation due to the high temperature of the process, which can destroy the enzyme in feed materials; thereby, the enzyme can mix with coating materials for post-pelleted. Nowadays, feeds are coated with chitosan and other feed additives on the farm. This study focused on the efficiency of different coating materials for protease and cellulase enzyme by highlighting the leaching test. The various coating materials were compared, namely, chitosan, pullulan, CMC, and Seal 4, which is a mixture of gum and starch.

2. Objectives

To compare the ability of coating materials in shrimp feed preparation, including chitosan, pullulan, CMC, and Seal4 on protease and cellulase holding capability by the percentage of enzyme leaching after soaking in water.

3. Materials and Methods

3.1 Preparation of coating materials

Coating materials were 5% chitosan (VIV Interchem Co.,LTD), 12.5% Seal 4 (Pathway Intermediates Thailand), 3% Pullulan (MyskinRecipe) and only 2% Carboxymethyl cellulose or CMC (Chemipan) soluted in 3% acetic acid. For the coating sample, 50 g of shrimp feed were weighed and mixed with enzyme and 1 ml of coating materials in different treatments. The study was divided into eight treatments, namely,
(1) feed+chitosan, (2) feed+Seal 4, (3) feed+pullulan, (4) feed+CMC, (5) feed+enzyme+chitosan, (6) feed+enzyme+Seal 4, (7) feed+enzyme+pullulan, and (8) feed+enzyme+CMC. Two different enzymes, protease in powder form (AB vista, UK) and cellulase in liquid form (Jefo Nutrition Inc., Canada), were used in the experiment. After that feeds are dried for 30 min for the leaching test, 10 g of feed are soaked in 30 ml of distilled water. The solutions are taken from each treatment at 30 min. Protease concentration is 5,000 units/kg, while cellulase concentration is 20 units/kg, which was recommended by the commercial company.

3.2 Protease activity assay

To determine the activity of protease, casein was used as a substrate which digested by protease and then liberated amino acid tyrosine was measured by using Folin's reagent. In brief, 100 µl of the solution was added to 170 µl of substrate casein (2% casein in 1 M Tris-HCl, pH 7). After incubating at room temperature (25˚C) for 10 min, 1 ml of 1.2 M TCA was added. Centrifuge the sample at 10,000 rpm for 5 min, then add 200 µl of supernatant in 0.4 N NaOH 1 ml and incubate at 40 degrees Celsius for 15 min. Next, add 200 µl of Folin's reagent and incubate at room temperature for 10 min. The solutions are measured absorbance at 660 nm by spectrophotometer (Kattakdad & Jintasataporn, 2018).

3.3 Cellulase activity assay

To determine the activity of cellulase, carboxymethyl cellulose was used as a substrate which digested by cellulase and then liberated β-glucose or reducing sugar was measured by using 3,5-dinitrosalicylic acid (DNS) assays. In brief, 100 µl of the solution was added to 170 µl of solution 1% Carboxymethyl cellulose at pH 7 to 8. After incubating at room temperature for 15 min, 250 µl DNS was added to stop the reaction. Boiled the sample at 100 degrees Celsius and added 1.5 ml of distilled water. The solutions are measured for reducing sugar at an absorbance at 540 nm by spectrophotometer (Kattakdad & Jintasataporn, 2018).

3.4 Calculation of enzyme %leaching

After compared absorbance with a standard curve, the values are calculated in the formula below.

$$\%\text{leaching} = \frac{\text{value}_{\text{coaitng}}\ (\text{umol/min/g feed}) - \text{value}_{\text{coating and enzyme}}\ (\text{umol/min/g feed})}{100}$$

4. Results and Discussion

The efficiency of coating material on protease and cellulase top up on shrimp feed was studied by focusing on the enzyme holding ability between four compounds, namely, chitosan, Seal 4, Pullulan, and

CMC. The results in Figure 1 presents the different value and percentage of leaching of individual coating materials. After mixing protease enzyme on feed and coated by different materials, the enzyme leaching from feed coating compared from the feed without protease enzyme supplementation and with protease enzyme supplementation. Figure 1 clearly illustrates that the protease leaching in feed coated by chitosan is higher than a group of pullulan or Seal4 or CMC coating. The enzyme leaching from the feed with the coating material and without protease supplementation comes from a natural enzyme in the feed (raw materials). According to the result, Figure 2 shows that the feed with chitosan coating has a high differentiation between feed with coating and feed with protease and coating material. In the farm, chitosan is commonly used as the post-pelleted coating. Since chitosan is biodegradable compounds and good film and forming materials, chitosan is suitable for an external shell for the capsule by a reaction of anionic polymers as alginate (Kailasapathy & Chin, 2000). The chitosan coating has been reported that can enhance the resistance to stress the environment. As chitosan is acquired from chitin by deacetylation in an alkaline media. In fact, chitosan is a copolymer comprise of (1–4)-2-acetamido-D-glucose and (1–4)-2- amino-D-glucose units (Abdou, Nagy & Elsabee, 2007). It shows the ability of antimicrobial properties in association with its cationicity and film-forming properties (Domard A & Domard M, 2001). However, coated feed with chitosan has less ability to hold the enzyme than other materials because the stickiness of chitosan can affect the dispersion of enzyme in coating materials. As a result, the protease enzyme might not be well dispersed all over the shrimp feed, causing the enzyme leaching value becomes wild variation.

On the other hand, Seal 4 presents the second-highest number of differences between feed with the coating and feed with protease and coating. It could be from the fact that Seal 4 composed of liquid gum and modified starch, which both materials are polysaccharide-based raw materials. This material is used in many kinds of food products. There are various applications as thickening agents, for instance, films, hydrogels, microspheres, nanoparticles, and matrix tablets (Jain, Khar, Ahmed, & Diwan, 2008). Its benefits also include biocompatibility and biodegradability (Shalviri Liu, Abdekhodaie & Wu, 2010). All abilities of Seal 4 lead to the thickening property; however, it still lowers the viscosity than chitosan-coated feed, leading to a higher ability to cover and disperse over the shrimp feed than chitosan coating.

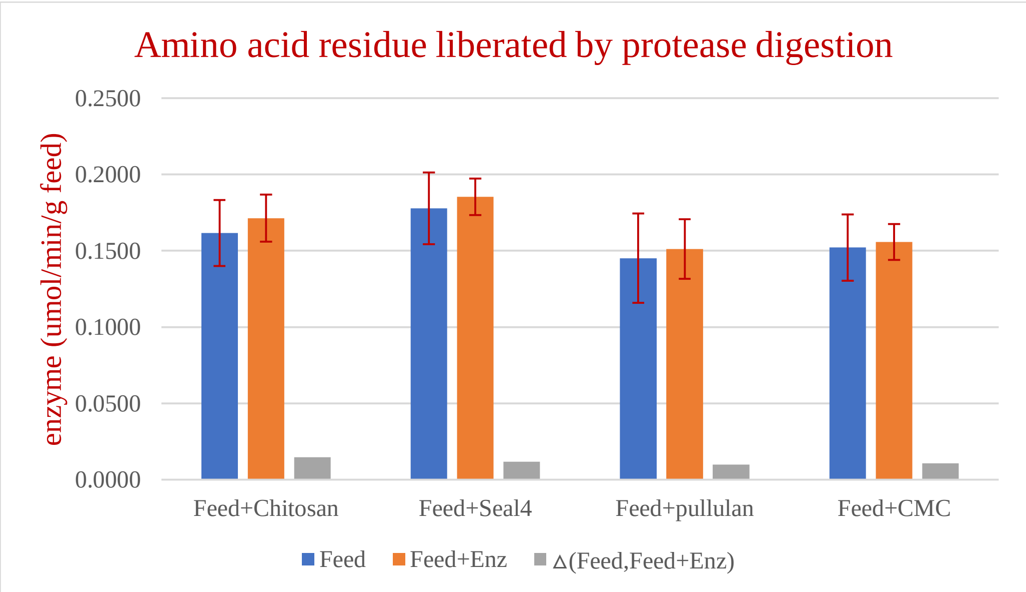

Figure 1 Concentration of protease leaching between feed and enzyme coated feed after immerse in water for 30 min

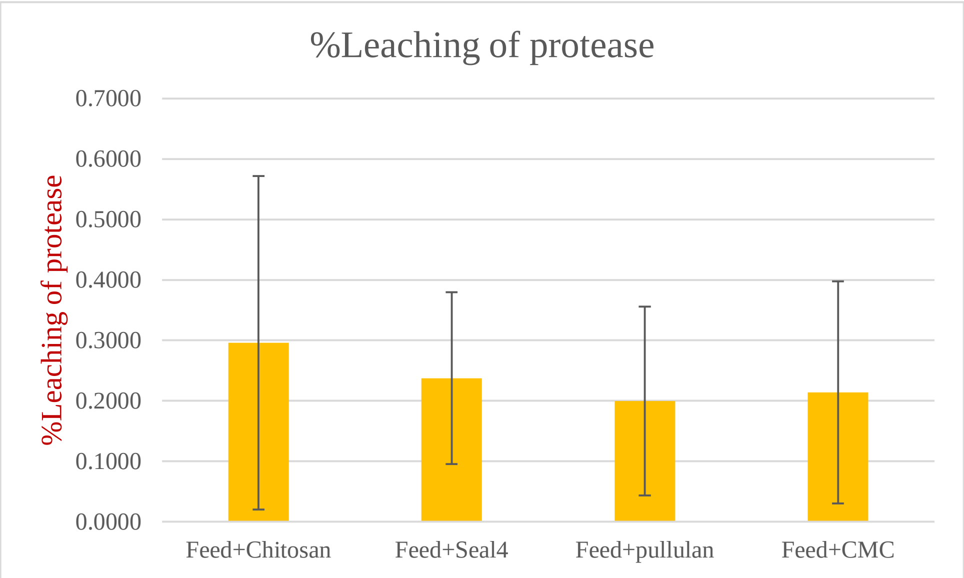

Figure 2 Percentage of protease leaching from shrimp feed coated by different materials after immersion in water for 30 min

The CMC is one of the thickening agents in food industries that can hold an enzyme with its structure. Besides, feed with CMC coated release amount of enzyme nearly a similar amount of feed with pullulan coating (Duran & Kahve, 2016). Carboxymethyl cellulose (CMC) is the common polysaccharide used for film blending based, then it has a high viscosity and is non-toxic. As CMC consists of the numerous hydroxyl and carboxylic groups, it shows high water binding and moisture sorption properties (Siracusa, 2012). Because of its polymeric structure and high molecular weight, CMC is also used in biocomposite film production to increase mechanical and barrier properties (Almasi, Ghanbarzadeh, & Entezami, 2010). Nonetheless, edible films made from CMC have weaknesses such as lower tensile strength. As a result, CMC can use to enhance the characteristics of other materials like a mixture film (Suderman Isa & Sarbon, 2016). Even though the stickiness of CMC affected on coating upon the shrimp feed, its ability of film agent is good enough to hold the enzyme within.

As pullulan are low viscosity materials, there is a higher opportunity to cover along with the pelleted feed. Hence, sampling the feed to measure enzyme activity is a higher opportunity to collect the enzyme from the coated feed. Pullulan is obtained from the fermentation of the fungus-like yeast Aureobasidium pullulans (*Pullularia pullulans*). Its structure consists of maltotriose trimer by α-(1,6)-linked and (1,4)- α-D-triglucosides (Farris, Unalan, Introzzi, Fuentes-Alventosa & Cozzolino, 2014). Pullulan is one of the coating materials nowadays due to its certain peculiar properties, for example, barrier property against oxygen and carbon dioxide. However, in the beginning, pullulan well-known as an edible coating such as a thin layer of material coated directly on the surface of the food product (Pavlath & Orts, 2009). As a result of low protease enzyme leaching from shrimp feed, pullulan is a biopolymer that has many interesting properties.

The study on shrimp feed with cellulase and coating material of chitosan, Seal4, pullulan, and CMC, after immersion in the water for 30 min, the results in Figure 3 illustrates the difference between feed with coating materials and feed with celluase and coating material. Generally, after topping-up the enzyme and coating materials, then they will immerse in water. It has some enzyme leaching from the feed, which is high or low amount depending on the quality of the coating material. Figure 4 illustrates that feed with cellulase and chitosan coating is the highest percentage of leaching so that chitosan has a lower capability to hold cellulose enzyme than pullulan, Seal 4, and CMC. According to each material structure and its property, each coating material presents different viscosity. The thicker coating seems to attach with enzyme better than fluid coating. Also, the fact that cellulase enzyme is a liquid form, the mixed ability, and coating ability might be higher than protease enzyme in powder form. The liquid enzyme has a higher opportunity with a coating technique than the powder enzyme since the liquid is better for dispersion within coating materials.

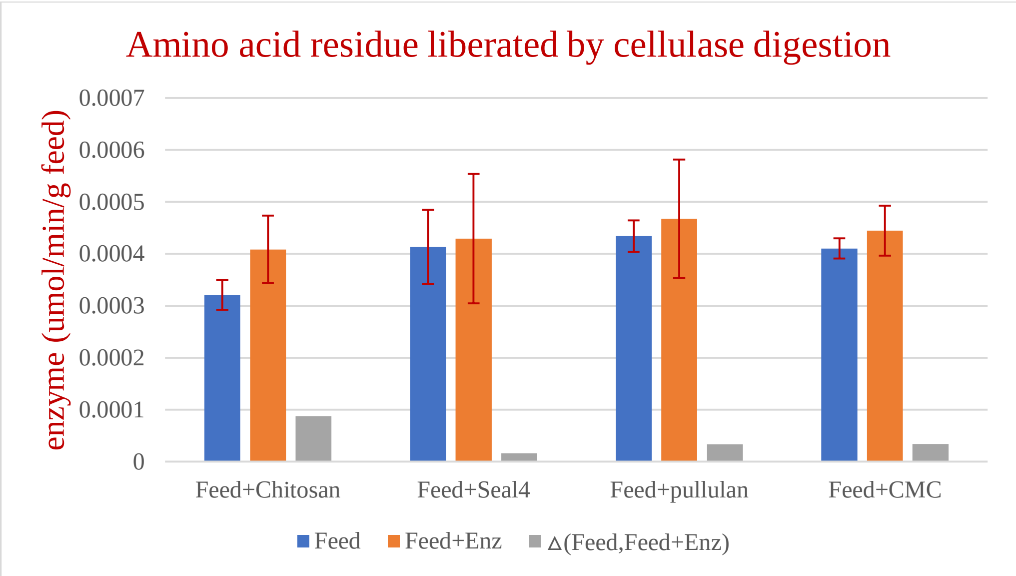

Figure 3 Concentration of cellulase leaching between feed and enzyme coated feed after immersion in water for 30 min

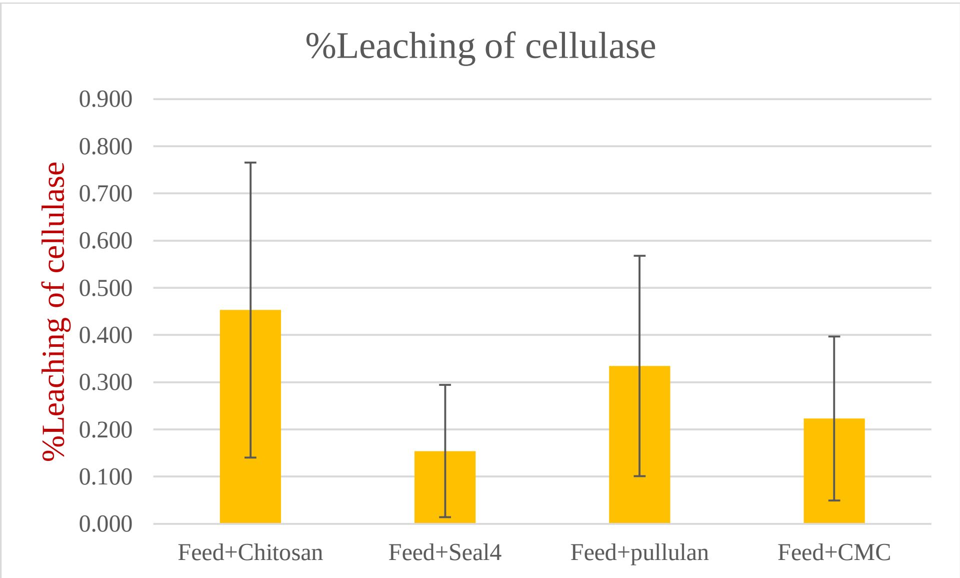

Figure 4 Percentage of cellulase leaching from shrimp feed coated by different materials after immersion in water for 30 min

Moreover, the result shows that coating with chitosan releases more amount of enzyme than the other materials. Besides, cellulase with Seal 4 is the lowest percentage of enzyme leaching. In terms of structure, all materials like Seal 4 and CMC, including pullulan and chitosan, are cellulose-based; thereby, cellulase can break down the cellulose bond and break through the coating to the environment. Seal4 based is specific gum that may not cleave by cellulase or fiber enzyme, leading to the best performance as cellulase coating materials. Hence, the plant coating materials are not suitable for cellulase coating because the coating materials will be degradable. The cellulase feed with chitosan might be degraded by cellulase in the diet that left in cellulase enzyme to water. Due to cellulase enzyme derived from the natural process of bacteria, there also has the other fiber digestion enzyme included xylanase, mannanase, glucanase, and pectinase, but cellulase is majority enzyme (Ray, 2011). The coating material used in this trial is carbohydrate. Therefore, these materials seem to be not suitable for coating on shrimp feed pellets after topping up with the cellulase enzyme.

5. Conclusion

The pullulan has the highest capability to hold the protease than the other materials due to its less stickiness that is simpler to blend with the enzyme in powder form. As the thickness of chitosan and CMC, the ability to spread on enzyme blending on feed is lower than the fluid coating. Hence, pullulan shows the least percentage of leaching in protease coating. For cellulase top up on the feed, which is liquid cellulase, the more stickiness of coating material like Seal4 and CMC shows the better performance of less leaching. Nevertheless, the ability of a cellulase enzyme can destruct most of the coating materials because their structure consists of cellulose-based composition. However, Seal4, which is gum-based, shows the potential to be the cellulase coating materials. Further study could focus on the concentration in different coating materials and observe the leaching of different enzymes, for instance, phytase and xylanase enzyme.

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