CHAPTER 1
INTRODUCTION

1.1 Overview

The gasoline price has been increasing continuously for the last 5 years. People have been trying to substitute with gasohol and bio-diesel which come from plants (corn, cassava, broken rice, etc.) and oil seeds (palm, soybean and sunflower, etc), the energy source of animal. As biofuel industry competition for feed ingredients leads to lack of animal feeds and increases their cost, thus, the utilization of new feed ingredients and agro-industrial by-product can solve this problem. As shown in Figure 1.1, the tendency of importing wheat and barley will be increased for use as feed stuffs.

However, cell walls of conventional vegetable feedstuffs, including wheat, barley, rye and oat, have higher protein content than tropical grains but contain significant quantities of non-starch polysaccharides (NSP) with more than half composing of β-glucan. Even these cereals contain approximately 0.5-5.7% β-glucan (Demirbas, 2005), β-glucan is one of the non-starch polysaccharides in cereals, and is derived from the polymerization of β-D-glucose monomers. Analysis of the chemical linkages in the β-glucan indicated the two types of linkages, i.e., β-(1,3) and (1,4). Animals lack the digestive enzymes to decompose most these chemical structures and negative effect have been documented. The result of indigestible NSPs will depress animal performance and also cause a pollution of indigestible nutrient excretes to waste. Bedford (1995) indicated that the wet faecal production of birds fed with the enzyme-supplemented diet was also reduced by more than 25% at 3 weeks and 22% at 6 weeks of age compared with control (Figure 1.2). Moreover, Leek et al.,(2007) indicated that there are benefits in the poultry house environment due to drier droppings and less ammonia excretion and there is a smaller risk of respiratory illnesses or health effects in farm workers. Moreover, at the end of their growth, animals are more homogeneous and therefore, have a much greater value at the
moment they are slaughtered. Owing to cleaner litters, poultry suffer less from skin lesions.

Figure 1.1 Quantitative and value of barley (a) and wheat (b) imports  
(Office of Agricultural Economics, 2007)
The β-glucan endosperm cell walls of barley have been identified as a main cause of poor growth rate and low nutrient digestibility. This substance increases digesta viscosity which causes anti-nutritional effects. The detrimental effect of NSPs is associated with the viscous nature of these polysaccharides. Their effects on the digestive tract and the interaction with the microflora of the gut by increasing intestinal viscosity (Figure 1.3) gives slow-down digestion rate and bound to the glycocalyx in the intestinal brush border which result in a decrease in nutrient absorption (Mathlouthi et al., 2002).

Figure 1.2 Effect of β-glucanase supplementation on wet excreta output (g day⁻¹) (Bedford, 1995)

With poorly digested diet, undigested food nutrients enter the mid-lower small intestine where the bacteria grow vigorously, being able to produce enzyme which degrade bile acids, resulting in impaired fat digestion (Mathlouthi et al., 2002) which is the rationale supporting use of NSP-enzyme feed additives to improve digestibility and bioavailability of nutrients even in relatively-undigestible diets. The supplementation of diets with microbial enzymes which degrade β-glucan and render nutrients more digestible may help overcome this problem. Earlier studies have clearly demonstrated that supplementation of diet with β-glucanase improved animal performance (Table 1.1).
Figure 1.3 Effect of rye and enzyme concentration of gut viscosity (Bedford and Classen, 1992).

Table 1.1 Growth performance in broiler an overview of the published studies

<table>
<thead>
<tr>
<th>Diet</th>
<th>Feed consumption (g)</th>
<th>Weight gain (g)</th>
<th>FCR</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>900b</td>
<td>438a</td>
<td>2.08b</td>
<td>Ankrah et al., (1999)</td>
</tr>
<tr>
<td>Barley+ E</td>
<td>1065a</td>
<td>677a</td>
<td>1.58a</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>392</td>
<td>276a</td>
<td>1.65</td>
<td>Berg et al., (1999)</td>
</tr>
<tr>
<td>Barley+ E</td>
<td>410</td>
<td>298b</td>
<td>1.58</td>
<td></td>
</tr>
<tr>
<td>Rye</td>
<td>397b</td>
<td>178b</td>
<td>2.23b</td>
<td>Mathlouthi et al., (2002)</td>
</tr>
<tr>
<td>Rye+ E</td>
<td>524a</td>
<td>281a</td>
<td>1.86a</td>
<td></td>
</tr>
</tbody>
</table>

a,b Means in the same column, within author, with different letters are significantly different (P<0.05). E = β-glucanase addition.

From the data of animal production, commodities such as enzyme, chemical substance, antibiotics and soybean are imported and tend to increase every year, especially, supplement enzyme for animal feed is expensive but may not be efficient because of time, temperature and oxidation during storage. Therefore, this project was aimed to develop the production process of β-glucanase by using microbes found in Thailand and to produce the enzyme for commercial-scale use.
The produced enzyme is more advantageous than imported enzyme because, produced enzyme is fresher, cheaper and is specific to the target feed ingredients in the diets than imported enzyme which is produced for animal manufacture in western country. As shown by Tapingkae (2003), the supplement of NSP-degrading enzyme to piglet diets suggested that feed cost of produced enzyme supplement (27.05 Baht/kg) was lower than supplement with imported enzyme (29.36 Baht/kg). The quantity of the enzyme produced is sufficient for farmer requirement, reduces the production cost and decreases the cost of pollution management. However, the main purpose was to reduce the imported enzyme and to produce this enzyme for exporting. Besides, consumers will get safety animal products which are safe from chemical substance, antibiotics and beta-agonist.

1.2 Objective of the study
To select the most efficient β-glucanase producing microorganism for β-glucanase production process.

1.3 Scopes of the study
1. To select the effective microorganism with the highest β-glucanase production, resistant to pH and stable for the pelleting process.
2. To maximize the β-glucanase production through optimization of media and cultivation condition.
3. To select an effective formulation carrier and investigate enzyme product preservation (drying).
4. To study the shelf-life of the dry enzyme at 4°C, ambient temperature and 45°C during storage.
5. To investigate the influence of crude β-glucanase on the in vitro digestibility of β-glucan in feed ingredient and productivity performance of piglets.