CHAPTER 5
DISCUSSION

5.1 Screening of β-glucanase producing microorganisms

5.1.1 β-glucanase production

In this study 10 fungal strains (*Pennicillium* sp. KPFC 678, *Aspergillus* sp. KPFC 947, *Aspergillus* sp. KPFC 919, *Aspergillus* sp. KPFC 614, *Aspergillus niger* KPFC 16 and *Aspergillus* sp. KPFC 277) produced β-glucanase except *Nectria* sp. KPFC 770. Based on the activity at pH 3.0 and 7.0, *Aspergillus* sp. KPFC 947, *Aspergillus niger* KPFC 919, *Aspergillus niger* KPFC 16, *Aspergillus* sp. KPFC 614, *Aspergillus* sp. KPFC 277 and *Pennicillium* sp. KPFC 678 were selected for further studies.

Tang *et al*. (2004) produced β-glucanase by *Bacillus subtilis* ZJF-1A5 in liquid state fermentation and gave 251 U/ml activity at 48 h with optimized medium. It was 1.4 times higher than the original medium. Compared with Jecu (2000), wheat straw and wheat bran were suitable for β-glucanase production under solid state fermentation (SSF) with 74% moisture content at pH range of 4.5–5.5, 14.8 international units (IU) endoglucanase activity: ml were obtained in 96 h. This study used agricultural by-product for β-glucanase production which was aimed to reduced cost.

Tapingkae *et al*. (2003) found that *Aspergillus* sp. FAS 128 was the most efficient strain for enzyme production, especially pentosanase, β-glucanase and amylase. The activities of pentosanase, β-glucanase and amylase were approximately 2,500, 3,000 and 1,500 U/g enzyme, respectively, compared to our studied β-glucanase production by many *Aspergillus* sp. and *Penicillium* sp. which were approximately 1,200-1,300 U/ml at pH 3.0 and 500-1,000 U/ml at pH 7.0.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

5.1.2 Evaluation of safety

Several selected strains of fungi and bacteria are already being successfully used in biotechnological processes for producing various substances. Such strains are known to be stable and not connected to toxicological or hygienic concerns. Thus, the selected microorganisms in this project had to be tested for several kinds of toxins which are mostly found in animal feed and cause problems for animals. These included cytotoxicity, ochratoxin and aflatoxin because previous report showed that *Aspergillus* sp. and *Penicillium* sp. produced aflatoxin or ochratoxin, other toxins produced by these genera was rarely found (พันทิพา, 2544).

5.1.2.1 Cytotoxicity test

In summary, the results of the tests showed that the produced enzyme was not toxic to a baby hamster kidney cell line (BHK) and a human liver hepatocyte cell line (HepG2). Thus, 6 microorganisms (*Aspergillus niger* KPFC 16, *Aspergillus* sp. KPFC 277, *Aspergillus* sp. KPFC 614, *Pennicillium* sp. KPFC 678, *Aspergillus niger* KPFC 919 and *Aspergillus* sp. KPFC 947) were safe for animals.

Liver and kidney are important target organs of toxic effects of chemicals, since they are primarily involved in metabolism and excretion of chemicals. Therefore, hepatoxicants and nephrotoxicants were selected using the baby hamster kidney cell line (BHK) and a human liver hepatocyte cell line (HepG2) to elucidate the feasibility of *in vitro* cytotoxicity assay to predict liver and kidney target-organ toxicity (Plumb *et al*., 1989).

Acute toxicity testing in animals is typically the initial step in the evaluation of the health effects of β-glucanase enzyme preparation produced by controlled solid-state fermentation of a selected pure culture of the fungus, and its primary purpose is to provide information on potential health hazards that may result from a short-term exposure. Recent studies suggest that *in vitro* methods might be helpful in predicting acute toxicity and estimating toxic chemical concentrations *in vivo*. Some results (Zhang *et al*., 2007) have illustrated that cytotoxicity data *in vitro* may be useful in identifying an appropriate starting dose for *in vivo* studies, and thus may potentially reduce the number of necessary animals for such determinations.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Previous work by Tapingkae (2003) was tested for cytotoxicity of *Aspergillus* sp. FAS 128 by lung cell line of mice. The results showed that *Aspergillus* sp. FAS 128 had cytotoxicity effect to the lung cell line, which is not a target organ to toxic effect by eating. In the mean time, enzyme used in animal feed would be diluted with small amount of enzyme mixed up with large amount of other feed ingredients.

5.1.2.2 Aflatoxin test

The extracted samples of microorganisms cultured in wheat bran soybean medium were tested for aflatoxin. The results showed that all of the investigated samples contained less aflatoxin than the allowable amount at 20 ppb regulated by The Food and Drug Administration (U.S. Food and Drug Administration,1994). The substantial aflatoxin certainly came from the contaminated raw material in wheat bran soybean medium. It can be said that 6 microorganisms were not aflatoxin producing microorganisms. Binder *et al*.(2007) showed that mycotoxin occurrence in Asia and the Pacific region, for all 122 soybean samples tested, aflatoxin was found in maximum of 13 μg/kg. However, there was not evidence of aflatoxin contamination in 98 wheat samples tested. Thus, a small amount of aflatoxin certainly came from soybean meal. Similarly, Tapingkae (2003) found a little amount of aflatoxin in fermented medium by *Aspergillus* sp. FAS 128 (14-15.5 ppb) which contained less aflatoxin than the allowable amount (20 ppb) regulated by FDA. From these results, it can be said that *Aspergillus* sp. FAS 128 is safe for aflatoxin.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

5.1.2.3 Ochratoxin test

Many agree that levels of ochratoxin designed for human or animal consumption should lie between 10-20 ppb. Some foreign markets have set regulation which limits ranging from 5 to 50 ppb (Boutrif and Canet 1998). The ochratoxin level in extracted sample from *Aspergillrus* sp. and *Penicillium* sp. were 10.04-24.65. Thus, the ochratoxin level of 6 microorganisms had less than the allowable (50 ppb) level. In addition, the use of fermented product in animal feed would possibly be reduced when small amount of enzyme was mixed with large amount of animal diet.

According to the results, the selected strains for next study would be *Aspergillus* sp. KPFC 277, *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 919 because these 3 microorganisms showed safety for animal and gave highest β-glucanase activities at both pH 3.0 and 7.0.

5.1.3 Resistance to pH, heat treatment and proteolytic enzyme

Enzymes, as proteins, are subjected to denaturation by heat, pH and oxidizing agents. Enzymes fed to animals must survive heat encountered during pelleting, prolonged storage, and to attack by other feed components. Moreover, once inside the gastrointestinal tract, β-glucanase is subjected to conditions that can cause inactivation, such as the acidic pH of the stomach contents in the presence of pepsin, and further proteolytic attack from pancreatic proteases in the small intestine.

5.1.3.1 Resistance to pH and proteolytic enzyme

The result showed that when exposed to pH 3.0 with pepsin, β–glucanase activity of *Aspergillus* sp. KPFC 919, *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 was still nearly 100%. Thus, at pH 3.0, which is closer to the pH found after feeding (in stomach), β-glucanase activity was relatively unaffected. Pepsin did not affect the stability of β-glucanase, as reported by Inborr and Gronlund (1993), and by Yu and Tsen (1993) with bromelain, papain and cellulase. pH was clearly the most important factor affecting β-glucanase stability

After passing through the proventriculus and gizzard, the digesta passes through the small intestine, with exposure to pancreatic enzymes and $NaHC0_3$. The pH in the duodenum and jejunum ranges from 5.3 to 7.3 (Riley and Austic, 1984; Ward *et al*., 1987). The result showed that when exposed to pH 7.0 with pancreatin β–glucanase, activity of *Aspergillus* sp. KPFC 919, *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 was still nearly 100%. The results indicated that β–glucanase activity of 3 microorganisms was resistant to pH 7.0 and pancreatin. This suggested that enzyme should survive after passing the small intestine of the animal.

Almirall and Estieve-Garcia (1995) studied the stability of β-glucanase produced from *Trichoderma longibruchiutum* to low pH with pepsin and the stability to high pH and pancreatin. The results showed that when exposed to pH 2 (with and

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

without pepsin) followed by restoration to pH 5, β-glucanase retained 20% of relative activity at 15 min, and was almost inactivated at 45 min. when the pH was increased to 3.2 (with and without pepsin), β-glucanase retained most activity, losing only 10% of its relative activity after 90 min. Similar to our study, β-glucanase produced from *Aspergillus* sp. lost approximately 10% of its relative activity after incubating with pepsin. For the stability to high pH with pancreatin, at pH 8.5 (without pancratin), the relative activity of β-glucanase decreased progressively, with only 30% of activity surviving at 90 min. With pancreatin added to the pH 8.5 solution, the enzyme was almost completely inactivated at 15 min. Exposure to pH 7 (without pancreatin) did not affect the activity of β-glucanase when measured at pH 5. But with pH 7, with pancreatin, reduced the activity to 50% after 15 min, and to 15% after 90 min. Contrast to our study, exposure to pH 7.0 with pancreatin did not reduce the β-glucanase activity.

5.1.3.2 Resistance to heat

The effect of pelleting temperature on the stability of crude β-glucanase produced by *Aspergillus* sp. KPFC 919, *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 were evaluated by measuring the residual activity at pH 3.0 and 7.0, 40 °C after heating in water bath at 75 °C for 2 and 5 min, comparing with the crude β-glucanase without the heat treatment.

After incubation for 2 min, the β-glucanase residual activity at pH 3.0 of *Aspergillus* sp. KPFC 919 *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 were 53.60, 76.60 and 46.53 %, respectively and decreased to 52.15, 71.10 and 34.52 %, respectively after incubating for 5 min.

After incubation for 2 min, the β-glucanase residual activity at pH 7.0 of *Aspergillus* sp. KPFC 919 *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 were 62.13, 58.79 and 49.28 % and decreased to 62.11, 56.09 and 44.62 %, respectively after incubating for 5 min.

Compared to Almirall and Estieve-Garcia (1995), the thermal stability of β-glucanase produced from *Trichoderma longibruchiutum* was incubated for various times and temperatures in a 0.1 M sodium acetate buffer pH 5. Exposure at

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

70°C for 10 min reduced the activity to 65%. At 80°C for 10 min, the activity decreased to 20%, and at 100°C for 10 min, the enzyme was almost totally inactivated. If compared with pelleting conditions, enzyme should not be inactivated by short heating from 75 to 85 °C and short time less than 10 min. It can be said that β-glucanase produced from *Trichoderma longibruchiutum* can survive.

Therefore, it was likely that β-glucanase from *Aspergillus* sp. KPFC 919 *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 could be used under pelleting condition 75-85 °C (less than 1 min) or meal diets without heat condition with little loss of β-glucanase activity. It can be concluded that the crude β-glucanase from *Aspergillus* sp. KPFC 919, *Aspergillus* sp. KPFC 947 and *Aspergillus* sp. KPFC 277 were thermally stable.

However, activities retained by *Aspergillus* sp. KPFC 277 preparation exhibited the high enzyme activity at pH 3.0 and highest enzyme activity at pH 7.0 and temperature (40 °C) as in the gastrointestinal tract of an animal, non-toxic to animal cells (a baby hamster kidney cell line and a human liver hepatocyte cell line) and contained aflatoxin (3.55 ppb) and ochratoxin (14.76 ppb) which were less than the allowable amounts. Furthermore, it was stable to gastrointestinal tract condition, stable at temperatures up to 75 °C for at least 15-30 sec in feed pelleting process. Therefore, *Aspergillus* sp. KPFC 277 was the best microorganism and subsequently chosen for the next investigations.

5.2 Optimization of crude β-glucanase production

5.2.1 Effect of carbon source on β-glucanase production

Mostly, wheat bran is abundant in nutrient sources and commonly utilized in various enzyme productions. Unfortunately, these raw materials are rather expensive compared to raw materials such as rice bran (solvent extract), corn, broken rice and rice bran, which are cheap and generally available in the country. In order to maximize β-glucanase production and simultaneously reduce its production cost, type and proper concentrations of substrates were investigated. From the result, the highest β-glucanase production at both pH 3.0 and 7.0 were achieved by cultivation in rice bran (solvent extract). Tang *et al*. (2003) also reported that polysaccharides are better carbon sources than monosaccharides and disaccharides for the β-glucanase

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

production. Barley flour, soluble starch, wheat bran and corn flour were the better carbon sources for β-glucanase production by *Bacillus* sp. than glucose, sucrose, maltose, glycerol and lactose. However, barley flour would be the best carbon source for β-glucanase production because barley flour has high content of β-glucan which is an inducer of β-glucanase production. In our study, rice bran (solvent extract) had β-glucan only about 0.2 % lower than barley flour, thus rice bran (solvent extract) had some advantage as the best carbon source for β-glucanase production.

Membrillo *et al*. (2008) showed that the characteristic of substrate was related to microbial growth as well as enzyme production, size of sugar cane bagasse fibers strongly influences the profile of enzymatic activities in solid-state fermantation. Particle size is extremely important since it affects the surface area to volume ratio of the particle which determines the fraction of the substrate which is initially accessible to the microorganism and the packing density within the surface mass. The exposed surface area of cellulose is more important than the actual amount of cellulose present. For a constant geometry, the surface area to volume ratio increases as the particle size decreases (Mitchell *et al*., 1992). The size of the substrate determines the voidspace which is occupied by air. Since the rate of oxygen transfer into the void space affects growth, the substrate should contain particles of suitable sizes to enhance mass transfer. Smaller particles stimulate greater growth (Molony *et al*., 1984; Muniswaran and Charyulu, 1994).

Morover, in solid-state fermentation, water is present in the solid substrate whose capacity for liquid retention varies with the type of material and an effect on aeration rate depends on the porosity. Thus, in our study, rice bran (solvent extract) had smallest particles that had optimum porosity for aeration rate and capacity for liquid retention.

Furthermore, these substrates had nearly optimal pH (5.9) for *Aspergillus* sp. species. Recent studies (Shankar and Mulimani , 2007; Jecu, 2000; Chantasartrasamee *et al*., 2005) showed the optimum pH of the medium for enzyme production by *Aspergillus* sp. which range from 4.5-5.5. For chemical composition, rice bran (solvent extract) had high level of protein about 14-15%, nearly wheat bran (14-16%), that could enhance microbial growth as nitrogen source. Moreover, the amount of

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

fiber of rice bran (solvent extract) was also high (13-15%) that could stimulate β-glucanase production by *Aspergillus* sp.

5.2.2 Effect of C: N ratio

In our study, addition of soybean meal to the medium did not improve β-glucanase production by *Aspergillus* sp. KPFC 277 because rice bran (solvent extract) already had high level of protein about 14-15% that was sufficient and could enhance microbial growth as nitrogen source.

Contrast to previous study by Tang *et al*. (2003), β-glucanase produced by *B. subtilis* ZJF-1A5 was associated with nitrogen source, yeast extract was the best nitrogen source, followed by soybean flour. All inorganic nitrogen sources chosen in the experiments were not favorable for cell growth and enzyme production.

Membrillo *et al*. (2008) showed that some strains preferred addition of nitrogen sources for lignocellulytic enzyme. *Pleurotus ostreatus* Strain IE-8 fungus produced the highest levels of all enzymes: xylanases and cellulases, both without the addition of ammonium sulfate but the highest laccase activity was obtained in the presence of ammonium sulfate. However, addition of ammonium sulfate did not affect enzyme production in strain CP-50. It could be possible that selected microorganism (*Aspergillus* sp. KPFC 277) did not prefer addition of organic nitrogen source but preferred inorganic nitrogen source. Thus, the effect of inorganic sources and other nitrogen sources was investigated in the next study (5.2.3).

5.2.3 Effect of media additives

Some of nutrients might not be adequate for β-glucanase production, thus, this study evaluated the media additives which could improve enzyme production.

From the result, the addition of urea or ami solution did not improve β-glucanase production by *Aspergillus* sp. KPFC 277. Thus, inorganic acid or nitrogen source from agroindustry by-product was not suitable for this process. In a previous study by Tang *et al*. (2004), poor growth of *Bacillus subtilis* and low production of β-glucanase were observed when urea or $NaNO_3$ or $(NH_4)_2SO_4$ was used as nitrogen source, pH of broth increased sharply when $NaNO_3$ was used as nitrogen source.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

However, $(NH_4)_2HPO_4$ yielded better growth but poorer production of β-glucanase, high content of phosphate in medium was not favorable to β-glucanase production.

Except carbon and nitrogen, other macro-nutrients such as P,S,K, Mg, Ca, Na and Fe were essential nutrients for microbial growth. Thus, the addition of mixed minerals to the medium might improve enzyme production. But, this study showed that the addition of minerals (K_2HPO_4 2 %, $MgSO_4.7\ H_2O$ 0.4 %, $CaCl_2.2H_2O$) had no effect, the macrominerals in rice bran (solvent extract) were sufficient for microbial growth and enzyme production. Contrast to Tang *et al*. 2004, the composition of fermentation medium optimized KH_2PO_4, $MgSO_4$ -$7H_2O$ and $CaCl_2$ were mixed to the medium and improved β-glucanase production by *Bacillus subtilis*.

5.2.4 Effects of initial moisture content on β-glucanase production

In this study, the substrate-distilled water ratio of 1:1.5 (w/v) was found to be optimum for enzyme synthesis by *Aspergillus* sp. KPFC 277.

In solid-state fermentation, water is used in limited quantity. The results indicated that lower or higher amounts than the optimum significantly affects the process productivity. Higher moisture level decreases porosity, changes substrate particle structure, promotes development of stickiness, reduces gas volume and exchange and decreases diffusion, which results in lowered oxygen transfer and enhanced formation of aerial mycelium (Lonsane *et al*., 1985). Several workers reported that the microbial growth is restricted by the liquid film adsorbed to the solid surface (Ferreira *et al*., 1999; Tunga *et al*., 1998). This is probably because oxygen uptake in SSF is directly from the gas phase and, to a lesser extent, from the liquid associated with the solids. The confinement of gas transfer to the liquid film on the substrate surface is also stressed.

Two factors may therefore, be operative here. In the lower range of moisture content, availability of water acts as the limiting condition, this condition reduces the solubility of nutrients provided to the organism by solid substrate, a lower degree of swelling and higher water tension (Zadrazil *et al*., 1995), while in the upper range of moisture content, oxygen availability and swelling of the substrate increase, thereby, facilitate better utilization of the substrate by microorganisms. Reductions in enzyme yields with high moisture content (Gessesse and Mamo, 1999; Tunga *et al*., 1998;

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Archana and Satyanarayana, 1997) and with low moisture content (Ferreira *et al*.,1999; Tunga *et al*.,1998; Nigam, 1990) have also been reported.

5.2.5 Effect of inoculum size and time course profiles on β-glucanase production

From the result, it can be seen that ~ 1×10^4 of the spore suspension was best for β-glucanase production, giving the maximum enzyme yield of 297,880 and 108,790 U/g medium at pH 3.0 and 7.0, respectively.

Higher concentrations of inoculum were inhibitory for enzyme production and minimum enzyme activity was obtained with the highest inoculum concentration ~ 1×10^6. Higher concentration of inoculum results in increased competition for carbon source and nutrients (could lead to fungal biomass production), which leads to exhaustion of nutrients and this imbalance results in reduced enzyme production. Ramachandran *et al*. (2005) and Ghanem *et al*., (2000) reported similar findings with *Rhizopus* sp.

Importance of inoculum size on microbial fermentation processes is widely accepted. It is clear that the β-glucanase production steadily increased with the increasing size of inoculum until it reached the magnitude when enzyme productivity became maximum. It has some optimum value depending on the microbial species and fermentation system. Larger inoculum size is detrimental to growth and production apart from an increase in the fermentation cost (Muniswaran and Charyulu, 1994).

The results obtained suggested that β-glucanase production increased progressively along with increase in incubation time until 72 h in the case of *Aspergillus ficuum* (Gautam *et al*.,2002), when maximal enzyme production was recorded. In case of *Aspergillus* sp. KPFC 277, β-glucanase production increased until 96 h when maximal enzyme production was recorded. The enzyme yields declined during further incubation in both cases. The reason for the decrease in enzyme synthesis after 72 and 96 h could be due to the reduced nutrient level of medium, affecting the enzyme synthesis. Decreased enzyme yield after further incubation could also be due to poisoning and denaturation of the enzyme (Gautam *et al*., 2002).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

5.3 Preservation of crude β-glucanase as dry powder

Tapioca flour was suitable for drying process which gave the percentage of 79.78 and 99.18 % at pH 3.0 and 7.0, respectively. When correlated to previous works (Yarchai, 2003), corn flour retained the highest protease activity after drying, followed by rice flour ($P<0.05$). Compared to rice bran, soybean meal and soy protein isolate were found to be ineffective in preventing protease from heat inactivation. Tapingkae (2003) found that the best carrier was rice flour which gave 92% of pentosanase activity, the ratio between cultured diet and rice flour of 1:1, drying at 40 °C for 6 h, was suitable for drying process. Previous results showed that starch was effective in preventing enzyme from heat inactivation. Moreover, starch presents some advantages, such as it is cheap, available in large quantities, fully-biodegradable food grade and can be easily modified.

5.4 Shelf-life of crude β-glucanase powder

β-Glucanase activity of both unopened and opened decreased with increasing storage temperature and time. The activity of unopened crude β-glucanase powder stored at 4°C remained stable during experimental period (12 weeks), which the activity retained about 80 and 85 % at pH 3.0 and 7.0, respectively. Storage at room temperature resulted in decreased activity which the activity retained about 71% and 74% at pH 3.0 and 7.0, respectively and 67 and 75 % at pH 3.0 and 7.0 by storage at 45 °C. Similar change in β-glucanase activity was obtained in left-over product which was opened at the beginning. The results suggested that crude β-glucanase powder was stable with no effect of air exposure after opening.

The finding was similar to Yarchai (2003), protease activity decreased with the increasing storage temperature and time with no effect of air exposure. Morover, the results agree with Mathewson (1998), as temperature and pH can cause irreversible damage to the enzyme, keeping the water activity low can be used to control the enzyme activity during the course of processing and storage. From these results, it can be concluded that the crude β-glucanase KPFC 277 product was stable during the storage time of 0-12 weeks with no effect of air exprosure after opening.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

5.5 Other enzyme activity from Aspergillus sp. KPFC 277

The results showed that *Aspergillus* sp. KPFC 277 are capable of producing not only β-glucanase but also amylase, cellulase, xylanase and protease. The same results as Tapingkae (2003), *Aspergillus* sp FAS 128. can produce xylanase and other enzymes such as amylase, β-glucanase, cellulase, phytase and protease.

Crude β-glucanase powder of *Aspergillus* sp. KPFC 277 showed higher β-glucanase activity at pH 3.0, but at pH 7.0 imported enzymes showed slightly higher β-glucanase activity than KPFC 277. This result showed that KFFC 277 was more active at pH 3.0 than pH 7.0, contrasted to Tapingkae (2003) who found that β-glucanase produced from *Aspergillus* sp. FAS 128 was more active at pH 6.8 than 3.0. Activity assay at pH 3.0 and 7.0 suggested that other carbohydrate-hydrolyzing enzymes of KPFC 277 and protease contained much higher activity than imported enzyme, except only amylase activity which was assayed at pH 7.0.

5.6 Testing of enzyme quality by *in vitro* digestibility

The effect of the enzyme preparation on β-glucan digestibility was different among cereals. Wheat bran showed highest β-glucan digestibility, without enzyme the β-glucan digestibility is as high as 94.66%. For barley and oat, without enzyme addition, β-glucan digestibility were 54.48 and 65.98%, respectively. Contrast to Castanon *et al*. (1997), the amount of insoluble or soluble NSPs in barley and rye was not modified by the *in vitro* digestion with the pepsin and pancreatin solutions (without exogenous enzyme), which suggests that NSP are not significantly digested by nonruminant animals.

For barley and wheatbran, the addition of 0.5 time of KPFC 277 resulted in increase in β-glucan digestibility, but this enzyme level did not improve β-glucan digestibility for oat. When the enzyme level was increased to 1.0 time (the same level as imported enzyme), the β-glucan digestibility of barley, oat and wheat bran was increased and did not significantly differ from imported enzyme. However, an increase in enzyme level of 1.5 times did not significantly increase β-glucan digestibility. The same results as Castanon *et al*. (1997), the lowest level of enzyme used was able to hydrolyze the solubilized NSP of rye, and increased levels of enzyme did not significantly reduce the recovery of insoluble NSP. However, the

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

effect of the enzyme preparation was different on barley than rye, this difference was probably due to differences in the structure and properties of the cell wall of NSP, which vary considerably among cereals and even among cultivars.

5.7 β-glucan analysis

The average β-glucan contents of 14 cereal grains and 2 pig diets were analysed by McCleary and Codd (1991) method. As shown in Table 4.1, barley grains have the highest β-glucan (3.78%), followed by oat (3.2%) and wheat bran (2.01%), respectively.

In a previous study, Demirbas (2005) determined mixed-linked (1-3), (1-4)-β-D-glucan contents of 14 selected cereal grains grown in Turkey. The results showed that barley and oat grains have β-glucan contents higher than the other cereals. Oat grains had the highest β-glucan ranges from 3.9% to 5.7%, barley (3.2% to 4.6%), corn (0.5-1.3%), wheat (0.5-1.0%), rice (0.4-0.9%) and flaxseeds had the lowest β-glucan ranges from 0.3% to 0.7%.

Thus**,** all cereals contain β-glucan but some cereal grains had different β-glucan contents. Variations in the β-glucan contents of cereal grains grown under different environmental conditions have been observed (McCleary and Glennie-Holmes, 1985).

5.8 Testing effect of enzyme on production performance in pigs

Average daily feed intake (ADFI) and average daily gain (ADG) of the piglets were not significantly different among the treatments ($p > 0.05$). However, at the same level of β-glucanase activity added, the piglets fed with diets containing KPFC 277 or imported enzyme tended to have a better production performance in terms of feed conversion ratio (FCR) ($P < 0.05$) than the piglets fed with without-enzyme diets. Therefore, the piglets fed with KPFC 277 or imported diet tended to have the lower feed cost per gain (31.82 and 31.81 Baht/kg, respectively) than without-enzyme diet (34.48 Baht/kg).

The result was similar to the previous study by Ankrah (1999), in some experiment, enzyme supplementation increased body weight gain but feed intake was not different. However, in some experiment, feed intake of animal was increased and

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

resulted in improvement of body weight and FCR. Almirall and Esteve-Garcia, 1995; Ankrah, 1999; Bergh, 1999; Yu, 1993 showed that crude β-glucanase for use as feed supplement in poultry barley-based diets significantly ($P<0.05$) improved feed consumption, weight gain and feed: gain ratio.

Previous study showed that animal performance could be improved by the β-glucanase supplementation, this suggested that viscosity of the small intestinal content was reduced significantly ($P< 0.05$), thereby increasing the mixing of digesta, digestive enzymes, and other necessary components required for digestion and absorption (Vahouny and Cassidy, 1985; Wang *et al*., 1992), resulting in more complete digestion of starch and protein in the small intestine of broiler chicks (Hesselman and Aman, 1986) and improved the ileal digestibilities of gross energy (GE), crude protein (CP), β-glucans, and the majority of the amino acids and the fecal digestibilities of GE, CP, and all amino acids (Li *et al*., 1996(b) and Jensen *et al*., 1998).

Other work with poultry indicated positive responses in performance when the enzyme was added to diets which high β-glucan content as shown in Table 2.7. The β-glucan content ranged from 2.6-7.6 %, depending on cultivar and environment of cultivation. In this study, the β-glucan was approximately only 0.3% which was lower than other previous studies. Thus, satisfactory results could not be expected. Thus, β-glucan was not the main cause affected animal performance but it was overall non-starch polysaccharides in the diets. There are factors influencing the success of enzyme supplementation such as the type of ingredient which have enzyme target, the conditions in the gastro-intestinal tract, the degree of hydration and body temperature of the animal. Thus, when selecting enzyme for feed additive, chemical composition of feed should be taken into account in order to get positive response.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved