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The Efficiency of Fibrolytic Enzymes to Improving the Nutritive Quality of Local Raw Materials for Aquafeed

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Abstract: A study was undertaken to evaluate the effects of supplementation fibrolytic enzymes on digestibility to improving the nutritional quality of local raw materials (soybean meal, rice bran, cassava pulp, cassava skin) for aquafeed. The objectives of the study were to improve the quality of cassava pulp and cassava skin from industrial cassava starch production by fibrolytic enzymes to obtain more sugar that can be the energy source in aquatic animals feed. The factorial experimental design (4×3) was conducted to study 4 types of raw materials (soybean meal, rice bran, cassava pulp, cassava skin) and 3 types of enzymes (xylanase at 24,000 U/kg, cellulase at 10 U/kg and mannanase at 750 U/kg). The result found that total sugars (glucose, xylose, maltose, mannose) from *in vitro* digestion from rice bran digestion was the highest (6.477 ± 107 mg/g) and significant differences from other raw materials ($P < 0.05$). However, total sugars of cassava pulp (0.900 ± 0.031 mg/g) and cassava skin (0.543 ± 0.013 mg/g) was not significantly difference from soybean meal (1.459 ± 0.054 mg/g) ($P > 0.05$). In soybean meal, rice bran and cassava pulp all three enzymes showed no significant differences ($P > 0.05$) in digestibility efficiency interestingly, cellulase exhibited significantly better digestibility with cassava skin. Total sugar from Cellulase, Xylanase and Mannanase were 0.484 ± 0.215, 0.128 ± 0.025 and 0.287 ± 0.055 respectively. Mannanase enzyme had the ability to digest fiber and released sugar from cassava pulp better than cellulase enzyme and xylanase enzyme, respectively.

Keywords: fibrolytic enzymes, digestibility, local raw materials, aquafeed

Introduction

Aquaculture is recognized as the fastest-growing agribusiness sector and has thus become an important component of the global food supply (FAO, 2014). However, the increased production of intensively reared fish species necessitates the supply of high quantity and sustainable feed ingredients in balanced formulated diets for warm-water fish species. In recent years one prominent area of research in aquaculture nutrition has focused on the replacement of fishmeal with plant-based protein ingredients to support the globally expanding aquaculture industry and to ensure its sustainability (Gatlin *et al*., 2007; NRC, 2011). However, most plant-based feedstuffs have a wide variety of anti-nutritional factors such as phytic acid, non-starch polysaccharides (NSP) and protease inhibitors, which may impair nutrient utilization, as well as impair fish performance and health (Francis *et al*., 2001; NRC, 2011). Attempts to improve the digestibility of plant proteins have included the use of hydrothermal treatments such as extrusion, as well as fractionation of crops to reduce the content of anti-nutritional factors and increase their protein concentration. While these approaches have been shown to improve protein and energy digestibility of numerous plant protein feedstuffs, the digestibility of many ingredients remains low because of a lack of the enzymes needed for breaking down the complex cell wall structure that encapsulates other nutrients (Glencross *et al*., 2007; NRC, 2011). The application of exogenous enzymes as feed additives to improve nutrient digestibility of plant-based feedstuffs has been researched extensively in poultry and swine and is now broadly used throughout the world as a way to reduce the anti-nutritional effects of NSP and phytic acid, increasing the utilization of carbohydrates and phosphorus, respectively (Adeola *et al*., 2011). In aquaculture, the use of phytase to improve phosphorus utilization from plant feedstuffs has emerged quite rapidly (Cao *et al*., 2007; Cheng *et al*., 2004; Dalsgaard *et al*., 2009; Kumar *et al*., 2012). However, in the case of carbohydrase enzymes, their use in aquaculture feeds has not been as nearly as widespread, regardless of their positive effects on nutrient digestibility (Adeola *et al*., 2011). Carbohydrases include all enzymes that catalyze a reduction in the molecular weight of polymeric carbohydrates,

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but more than 80% of the global carbohydrase market is accounted for by two dominant proteins, xylanase and glucanase (Benjamin, 2003). In addition, other commercially available carbohydrases include α-amylase, β-mannanase, α-galactosidase and pectin-ase. Practically all feed-relevant carbohydrases are in the same group of proteins, the hydrolase/glycosidase family, hydrolyzing carbohydrate polymers to generate decreased molecular weight oligosaccharides or polysaccharides (Adeola *et al*., 2011). Because the study of carbohydrase supplementation in aquaculture feeds is recent and scarce, this review is an attempt to compile most of the research completed to date in this regard, describing the specific effects of this group of enzymes on nutrient digestibility and fish perform-ance, to encourage additional study in this subject and suggest opportunities for future research.

In Thailand, the main feed ingredients used in aquaculture feed production of Thailand has been soybean meal and soybean products (41%) following by fishmeal (22%), wheat flour and wheat products (13%), rice products (7%), corn (6%), cassava products (5%), land animal protein sources (3%), plant protein sources (2%) and fish oil (1%), respectively. Meanwhile, domestic fishmeal production in 2016 was 3% of all feed ingredients, soybean and soybean meal made up of about 25%, and other carbohydrate-producing plants accounted for 60% of total production base on the information of the Thai Feed Mill Association (2017). High-quality fishmeal is a priority for aquaculture feed production especially for shrimp and marine fish and this has significantly affected feed cost. The supply of fish meals from local production has been insufficient and the rest had to be imported. Recently, local fish meal production faced a challenge from Illegal, Unreported and Unregulated (IUU) fishing. This situation has a negative impact on aquaculture businesses and may become more problematic in the future due to the possibility of the issue resulting in non-tariff trade barriers. Research on the use of alternative feed ingredients in aquaculture feeds is needed. However, feed ingredients from local raw materials and waste from agriculture and industry have been utilized by many competing interests. In Thailand, most raw materials and waste from agriculture and industry have been utilized by other sectors and competing uses such as livestock, pet foods, fertilizers, biofuels and in some cases by direct human consumption (Laddawan, 2017). There are many factors that affect the selection of alternative local feed ingredients. Non-nutritional aspects are price, palatability, market availability (domestic or imported), physical characteristics (easy to use/to transport), presence of contaminants and market acceptability (eg. IUU fishing, GMOs, labor issues and carbon footprint. Meanwhile, nutritional aspects such as proximate composition, amino acid profile, fatty acid profile, energy content, vitamin and mineral contents, anti-nutritional factors and nutrient digestibility are definitely additional factors that must be taken into account (Laddawan, 2017).

The success and sustainability of aquaculture depend on minimizing the operational cost of feed that in general comprises 50–60% of the total cost in intensive farming. The major feed ingredient, fish meal, is expensive and there is increasing competition with other livestock industries for the available static supply of fish meal. Hence, the incorporation of plant-derived materials in fish feeds is receiving increasing attention. One of the main constraints in the utilization of plant ingredients in aquaculture is the presence of indigestible carbohydrates, which consist primarily of non-starch polysaccharides (NSPs). These form a part of the cell wall structure of cereals and legumes. The presence of NSPs in the diet interferes with feed utilization and adversely affects the performance of the animal. Supplementation of NSP-degrading enzymes in feed mitigates the adverse effects of NSPs. The effects of NSPs in pigs and poultry have been widely studied; however little information exists for fish.

The objectives of the study were to improve the quality of cassava pulp and cassava skin from industrial cassava starch production by fibrolytic enzymes to obtain more sugar that can be the energy source in aquatic animals feed. Which these sugars can be a source of energy in feed to increase growth in aquatic animals and to study the efficiency of the xylanase enzyme, cellulase enzymes and mannanase enzymes in the digestion of Soybean meal, Rice bran, Cassava pulp, and Cassava skin obtain sugar that can be a source of energy in aquatic animals.

Materials and Methods

Animal feed ingredients

Soybean meal, Rice bran, Cassava pulp and Cassava skin provided from the cassava starch factory in Mukdahan province, Thailand. All ingredients have dried with sun and sieved pass through 180 µm mash size and determined chemical composition in all ingredients according to proximate analysis (AOAC., 2000) for dry matter, ash, lipids, protein, fiber to NDF (hemicellulose, cellulose-lignin), ADF (cellulose-lignin) and energy values.

Fibrolytic enzymes (Non-starch polysaccharidase; NSP ase)

Enzyme Xylanase and Enzyme Cellulases that are commercially sold from ABvista, USA. The enzyme Mannanase is commercially sold from Elanco, USA.

Experiment

Experiment to two factors in the completely randomized design (Factorial 4X3 in Completed Randomized Design; Factorial in CRD):

(1) Soybean meal
(2) Rice bran
(3) Cassava pulp
(4) Cassava skin or Cassava peel

Digestibility raw materials using three types of enzymes:

(1) Xylanase enzyme at 24,000 U/kg
(2) Cellulase enzyme at 10 U/kg
(3) Mannanase enzyme at 750 U/kg

Study on digestibility

All raw materials were incubated in the pH 6.5 buffer at room temperature for 1 hour and then were boiled at 100 °C for 20 minutes. The supernatant was removed and stored at -20°C until further used. The digestibility was investigated using Bedford and Classen (1993) procedure. Reducing sugar obtained was evaluated following method of Miller (1959). Total sugar was measured using Spectrophotometer (T60 UV-Visible Spectrophotometer, PG Instruments, UK) at 550, 540, 480 and 590 nm for glucose, maltose, xylose, and mannose, respectively. The amount of reducing sugar was calculated by compared with the standard curve.

Statistical data analysis

Data collected was submitted to one-way analysis of variance (ANOVA-1). When a significant difference existed, the Duncan's test was used at 5% threshold to separate means Nwokoro *et al*. (2005) the software R program version 3.1.2 was used.

Results

Proximate analysis

Proximate analysis of soybean meal, we found that Soybean meal has 10.5% moisture, 89.5% dry matter, considering dry weight, 43.5% protein, 1.00% fat, 7.00% crude fiber, NDF (hemicellulose, cellulose-lignin) 25.56%, ADF (cellulose-lignin) 18.9%, 6% ash and total energy 2,640 cal/g, that is, the amount of hemicellulose which may be about 6.7% xylan or other hemicellulose

Our proximate analysis showed that rice bran has 10.2% moisture, 89.8% dry matter, considering dry weight (0% moisture, 100% dry matter), 12 % protein, 12% fat, 11% crude fiber, NDF (hemicellulose, cellulose-lignin) 14.73%, ADF (cellulose-lignin) 6.56%, 10.9% ash and total energy 3,120 cal/g, that is, the amount of hemicellulose which may be about 8.17% xylan or other hemicellulose.

The proximate analysis exhibited that Cassava pulp has 82.7% moisture, 17.3% dry matter, considering dry weight (0% moisture, 100% dry matter), 2.53% protein, 0.45% fat, 12.25% crude fiber, NDF (hemicellulose, cellulose-lignin) 34.08%, ADF (cellulose-lignin) 8.0%, 2.2% ash and total energy 3,826.6 cal/g, that is, the amount of hemicellulose which may be about 26.08% xylan or another hemicellulose, indicating that in cassava pulp, there are xylan or other hemicellulose is the main fiber.

Our proximate analysis showed that cassava skin has 80.0% moisture, 20.0% dry matter, considering dry weight (0% moisture, 100% dry matter), 2.02% protein, 0.51% fat, 15.05% crude fiber, NDF (hemicellulose, cellulose-lignin) 37.87%, ADF (cellulose-lignin) 26.94%, 2.4% ash and total energy 3,186.01 cal/g, that is, the amount of hemicellulose which may be about 26.08% xylan or another hemicellulose, indicating that in cassava skin, there are cellulose and lignin are the main fiber. Proximate analysis are presented in Table 1.

Digestion

The experiments of the fibrolytic enzyme by using Xylanase, Cellulase and Mannanase after the digestion process of soybean meal, rice bran, cassava pulp, cassava skin, the results of the study in Table 2 it found that fiber in soybean meal, rice bran, cassava pulp, cassava skin when digested with fibrolytic enzyme, giving the glucose, xylose, maltose, mannose means different ($P < 0.05$). Digestion of xylanase enzymes, cellulase enzymes, mannanase enzymes will give each type of sugar means statistically different ($P < 0.05$). By the three enzymes, when the digestion crude fiber in soybean meal, rice bran, cassava pulp, and cassava skin has glucose, xylose, maltose or mannose in each type of sugar from enzyme activity were not statistically different ($P > 0.05$). But the function of cellulase enzymes when

Tab. 1: Proximate composition of local raw materials for aquafeed.

Proximate Analysis	Raw material			
	Cassava Pulp	Cassava Skin	Soybean Meal	Rice Bran
Dry matter (%)	17.30	20.00	89.50	89.80
Protein (%)	2.53	2.02	43.50	12.00
Lipid (%)	0.45	0.51	1.00	12.00
Fiber (%)	12.2	15.05	7.00	11.00
Ash (%)	2.20	2.40	6.00	10.90
NFE (Nitrogen Free Extract (%))	65.23	59.57	32.00	43.90
NDF	34.08	37.87	25.56	14.73
ADF	8.0	26.94	18.9	6.56
Total Energy (Kcal/kg)	3,826.60	3,186.01	2,640.00	3,120.20

Tab. 2: The amount of reducing sugar obtained from the digestion of soybean meal, rice bran, cassava pulp and cassava skin with fibrolytic enzyme.

Raw Matterials	Enzymes	Glucose (mg/g)	Xylose (mg/g)	Maltose (mg/g)	Mannose (mg/g)
Soybean Meal	Xylanase	0.132 (0.04)^{b}	0.101 (0.03)^{b}	0.181 (0.05)^{b}	0.048 (0.01)^{b}
	Cellulase	0.146 (0.04)^{b}	0.112 (0.03)^{b}	0.201 (0.05)^{b}	0.055 (0.02)^{b}
	Mannanase	0.139 (0.03)^{b}	0.107 (0.02)^{b}	0.192 (0.03)^{b}	0.046 (0.02)^{b}
Rice Bran	Xylanase	0.408 (0.18)^{a}	0.312 (0.14)^{a}	0.560 (0.25)^{a}	0.198 (0.07)^{a}
	Cellulase	0.732 (0.30)^{a}	0.559 (0.23)^{a}	1.004 (0.41)^{a}	0.232 (0.06)^{a}
	Mannanase	0.716 (0.18)^{a}	0.547 (0.14)^{a}	0.982 (0.25)^{a}	0.226 (0.06)^{a}
Cassava Pulp	Xylanase	0.049 (0.03)^{b}	0.037 (0.02)^{b}	0.067 (0.04)^{b}	0.011 (0.00)^{c}
	Cellulase	0.054 (0.00)^{b}	0.041 (0.00)^{b}	0.074 (0.00)^{b}	0.008 (0.01)^{c}
	Mannanase	0.059 (0.02)^{b}	0.045 (0.01)^{b}	0.080 (0.03)^{b}	0.019 (0.00)^{c}
Cassava Skin	Xylanase	0.037 (0.01)^{b}	0.028 (0.01)^{b}	0.051 (0.01)^{b}	0.012 (0.00)^{bc}
	Cellulase	0.146 (0.07)^{b}	0.111 (0.05)^{b}	0.199 (0.09)^{b}	0.028 (0.01)^{bc}
	Mannanase	0.083 (0.06)^{b}	0.063 (0.01)^{b}	0.114 (0.02)^{b}	0.027 (0.001)^{bc}
P-value	Raw Materials	0.000	0.000	0.000	0.000
	Enzymes	0.063	0.063	0.063	0.562
	Interaction	0.204	0.201	0.203	0.967

*** $^{a-b}$ Mean values in a column with disparate superscripts differ significantly (P<0.05).**

digested in the soybean meal, rice bran, cassava pulp, and cassava skin will give glucose, xylose, maltose or mannose in each type of sugar higher than the activity of xylanase and mannanase ($P < 0.05$) and the amount of glucose, xylose, maltose or mannose in rice bran each type of sugar higher than that of sugar from the crude fiber digestion in soybean meal, cassava pulp, and cassava skin ($P < 0.05$).

When considering the efficiency of fibrolytic enzymes, fiber in soybean meal the results (Fig. 1) show that the use of xylanase enzymes, cellulase enzymes, and mannanase enzymes digested soybean meal obtain glucose sugar, xylose sugar, maltose sugar or mannose sugar in each type of sugar were not statistically different ($P > 0.05$). When the soybean meal is digested, the maximum is xylose sugar ($P < 0.05$), next below is glucose sugar, maltose sugar and mannose sugar respectively.

When considering the efficiency of fibrolytic enzymes, fiber in rice bran the results (Fig. 2) show that the use of xylanase enzymes, cellulase enzymes, and mannanase enzymes digested rice bran obtain glucose sugar, xylose sugar, maltose sugar or mannose sugar in each type of sugar were not

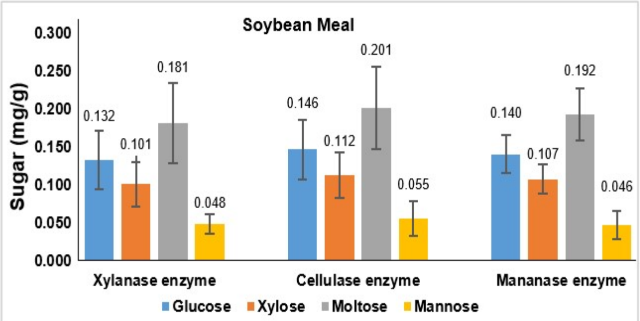

Fig. 1: The amount sugar from the soybean meal digestion with xylanase enzyme, cellulase enzyme and mannanase enzyme, the amount of each type of sugar obtained from the activity of each enzyme in the digestion of soybean meal.

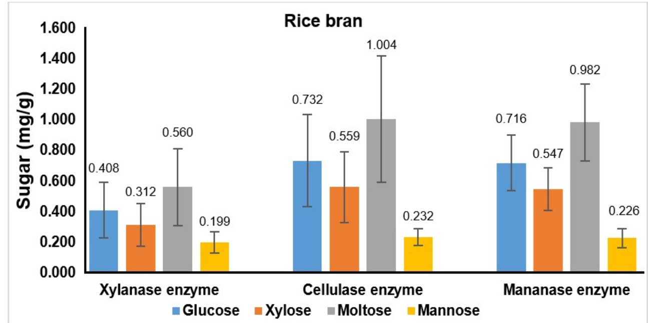

Fig. 2: The amount of sugar from the rice bran digestion with xylanase enzyme, cellulase enzyme and mannanase enzyme, the amount of each type of sugar obtained from the activity of each enzyme in the digestion of rice bran.

statistically different (P > 0.05). When the rice bran is digested, the maximum is xylose sugar (p> 0.05), next below is glucose sugar, maltose sugar and mannose sugar respectively.

Study of the efficiency of fibrolytic enzymes, fiber in cassava pulp the results (Fig. 3) show that the use of xylanase enzymes digesting cassava pulp was the most xylose sugar

(P < 0.05) while glucose sugar, maltose sugar and mannose sugar from the activity of xylanase in each type of sugar were not statistically different (P > 0.05). Digestibility fiber in cassava pulp and obtained glucose sugar, xylose sugar, maltose sugar or mannose sugar, the amount of sugar from each enzyme activity was not statistically different (P > 0.05).

Study of the efficiency of fibrolytic enzymes, fiber in cassava skin the results (Fig. 4) show that the use of cellulase enzymes digesting cassava skin was the most xylose sugar (P > 0.05) while glucose sugar, maltose sugar and mannose sugar from the activity of cellulase enzymes in each type of sugar were not statistically different (P > 0.05). Digestibility fiber in cassava skin and obtained glucose sugar, xylose sugar, maltose sugar or mannose sugar, the amount of sugar from each enzyme activity was not statistically different (P > 0.05).

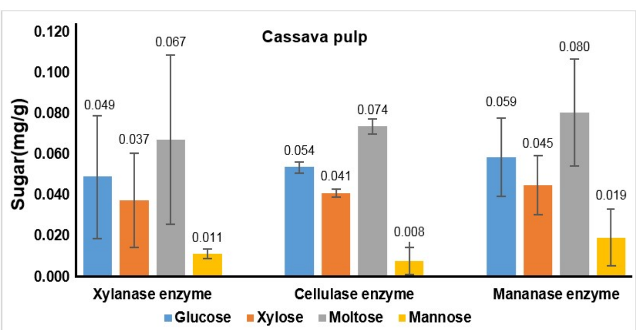

Fig. 3: The amount of sugar from the cassava pulp digestion with xylanase enzyme, cellulase enzyme and mannanase enzyme, the amount of each type of sugar obtained from the activity of each enzyme in the digestion of cassava pulp.

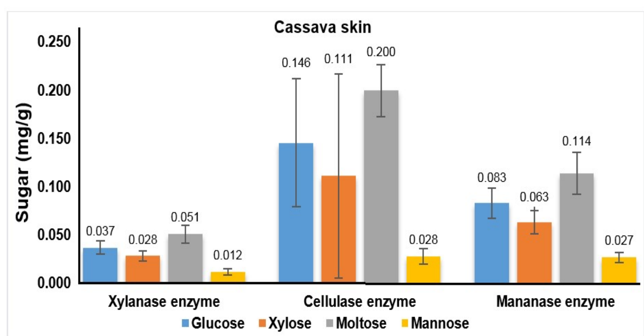

Fig. 4: The amount of sugar from the cassava skin digestion with xylanase enzyme, cellulase enzyme and mannanase enzyme, the amount of each type of sugar obtained from the activity of each enzyme in the digestion of cassava skin

Study on the efficiency of fibrolytic enzymes, fiber in soybean meal, rice bran, cassava pulp and cassava skin the results (Fig. 5) show that the digestion of soybean meal, rice bran, cassava pulp and cassava skin with xylanase enzyme, cellulase enzyme and mannanase enzyme obtain the total of sugar (glucose, xylose, maltose and mannose) were statistically different (P < 0.05) (Tab. 3). Digestibility of rice bran with cellulase enzyme obtain total sugar (glucose, xylose, maltose and mannoses) was maximum (P < 0.05). Digestion with mannanase enzyme and the digestion of soybean meal, cassava skin and cassava pulp with xylanase enzymes obtain the lowest total sugar (P < 0.05) respectively. Xylanase enzyme was more effective in digesting rice bran than soybean meal, cassava pulp and cassava skin respectively.

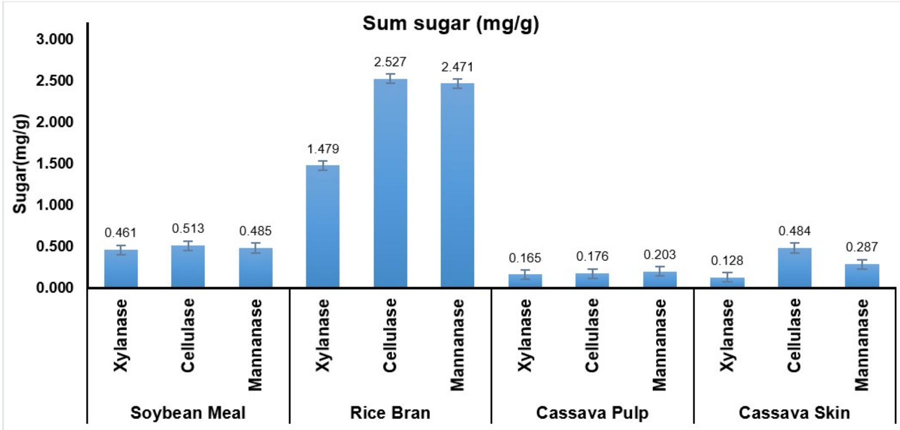

Fig. 5: Sum sugar obtained from digestion soybean meal, rice bran, cassava pulp and cassava skin with xylanase enzyme, cellulase enzyme and mannanase enzymes.

Total sugar from rice bran (glucose, xylose, maltose and mannose) were 6.477 ± 107 mg/g. Total sugars from soybean meal, cassava skin and cassava pulp digestion were 1.459 ± 0.054 mg/g, 0.543 ± 0.013 mg/g and 0.900 ± 0.031 mg/g, respectively.

Tab. 3: The amount of sum reducing sugar obtained from the digestion of soybean meal, rice bran, cassava pulp and cassava skin with fibrolytic enzyme.

Raw materials	Enzymes	Sum sugar (mg/g)
Soybean Meal	Xylanase	0.461 (0.134)[b]
	Cellulase	0.513 (0.146)[b]
	Mannanase	0.484 (0.097)[b]
Rice Bran	Xylanase	1.479 (0.580)[a]
	Cellulase	2.527 (1.004)[a]
	Mannanase	2.471 (0.587) [a]
Cassava Pulp	Xylanase	0.164 (0.096)[b]
	Cellulase	0.176 (0.014)[b]
	Mannanase	0.203 (0.063)[b]
Cassava Skin	Xylanase	0.128 (0.025)[b]
	Cellulase	0.484 (0.215)[b]
	Mannanase	0.287 (0.055)[b]
P-value	Raw Material	0.000
	Enzyme	0.065
	Interaction	0.217

*** [a – b] Mean values in a column with disparate superscripts differ significantly (P<0.05).**

Discussion

Fibrous carbohydrates in the form of cellulose are essentially indigestible by some fish species and do not make a positive contribution to their nutrition. Thus the level of crude fiber in fish feeds is typically restricted to less than 7% of the diet to limit the amount of undigested material that enters the culture system. Carbohydrates are classified according to digestibility as digestible, partially digestible and indigestible. Sugars, starches, dextrin and glycogen are digestible carbohydrates whereas, cellulose, dietary fibers and hemicellulose are indigestible carbohydrates. Galactomannans, glucomannan, pectin, inulin and pentosans are considered partially digestible carbohydrates (Oil World, 2015).

The structure of fiber in feedstuffs is a non-starchy structure. It is a molecule of sugar in a long molecule line, therefore, called non-starch polysaccharide (NSP) which contains cellulase, hemicellulase and pectin. When these fibers are digested will make glucose, which is a monosaccharide or disaccharide called maltose and polysaccharides, as well as mannose and xylose. Soybean meal is the most important protein source used to feed farm animals. It represents two-thirds of the total world output of protein feedstuffs, including all other major oil meals and fish meal (Oseni, 2002). Its feeding value is unsurpassed by any other plant protein source and it is the standard to which other protein sources are compared (Cromwell, 1999). While it has been an accepted part of livestock and poultry diets in the USA since the mid-1930s (Lewis, 2001) soybean feed production took off in the mid-1970s and then accelerated in the early 1990s due to growing demand from developing countries. The expansion of aquaculture and prohibitions on the feed use of slaughterhouse by-products have also fueled the demand for this high-quality source of protein (Steel *et al*., 1980). Soybean meal is the by-product of the extraction of soybean oil. Several processes exist, resulting in different products. Soybean meal is usually classified for marketing by its crude protein content. There are two main categories of soybean meal, the "high-protein" soybean meal with 47-49% protein and 3% crude fiber, obtained from dehulled seeds, and the "conventional" soybean meal, with 43-44% protein, that contain the hulls. In solvent-extracted soybean meals, the oil content is typically lower than 2% while it exceeds 3% in mechanically-extracted meals (Cromwell, 2012).

Rice is a major agricultural product of Thailand with annual productivity of about 29 million tons due to suitable topography and climate nature. Commonly, consumers prefer white rice therefore brown rice is subjected to the milling process. Rice bran is an important by-product of the milling process which is enriched with fiber, proteins, oil, and important antioxidants such as vitamin E and rice oil processing: deoiled bran and soapstock. Deoiled bran is rich in proteins and amino acids and is obtained after oil extraction. Soapstock is obtained after deacidification by alkali treatment of crude rice bran oil during the refining process. It has a significant amount of y-oryzanol, which is an important component that shows many health benefits such as reduction of cholesterol in the blood and anti-aging effect etc. At present, the majority of both deoiled bran and soapstock is used as animal feed.

From the study of the proximate analysis of cassava pulp, it was found that the cassava pulp in this study had 17.3% dry matter compared with the report of Khang *et al*. (2000) that was found that 11.2% dry matter and in dry matter with 2.53% protein which has a similar value to Oseni *et al*. (2002) reported and the crude fiber in Oseni *et al*. (2002) reported at 5.0% is lower than the fiber in the cassava pulp of this study with a high fiber 12.25% which fiber as hemicellulose which may be xylan, mannan or other hemicellulose about 26.08% and contain 8.0% cellulose and lignin, indicating that in cassava pulp, there are xylan, mannan or other hemicellulose Is the main fiber. Which the nutrient value in cassava pulp may depend on the process of starch extraction (Jarernsak, 1976).

The proximate analysis of cassava skin, it was found that the cassava skin contained 80% moisture, 20.0% dry matter, compared with the report of Adegbola (1980), that was found that 20.5% of the cassava skin was dry and from the report of Nwokoro *et al*. (2005) was found that the cassava skin in dry matter with 4.3% protein and 1.0% ash, which is higher than the cassava skin in this study. It was found that the cassava skin in the dry matter with 2.02% protein and 2.4% ash, while the crude fiber in the cassava skin was reported by Nwokoro *et al*. (2005) and Adegbola (1980). In the cassava skin of this study, it was found that there were 15.05% crude fiber with cassava skin containing crude fiber as hemicellulose. Which may be xylan, mannan or another hemicellulose about 10.93% and 26.94% cellulose and lignin, indicating that in cassava skin there are cellulose and lignin as the main fiber.

Cassava pulp digestion with xylanase enzyme, cellulase enzyme and mannanase enzyme obtain the total amount of sugar (glucose, xylose, maltose or mannose and sum sugar), not statistically different ($P > 0.05$). Digestion with mannanase enzyme and the digestion of cassava skin with xylanase obtain the lowest total sugar content ($P < 0.05$), it was found that the main components of crude fiber in cassava skin were cellulose and hemicellulose. While cassava pulp compositions were xylan and mannan mostly.

When considering the digestion of cassava pulp and cassava skin with commercial enzymes, which is composed of fibrolytic enzymes, the main fiber found in large quantities, is the type that is primarily used such as xylanase enzymes, cellulase enzyme, and mannanase enzyme. Which from the digestion of cassava pulp and cassava skin was found that the fiber in cassava pulp and cassava skin when being digested with fibrolytic enzymes obtain glucose sugar, xylose sugar, maltose sugar and mannose sugar content is different ($P < 0.05$). The structure of fiber in cassava pulp is hemicellulose, xylan, mannan group or another hemicellulose, is the main fiber. When digested with xylanase enzyme, cellulase enzyme and mannanase enzyme will obtain the amount of glucose sugar, xylose sugar, maltose sugar and mannose sugar from the activity of enzymes that not different ($P > 0.05$). However, the structure of the fiber in the cassava skin is cellulose and lignin. When digested with cellulase enzyme obtain glucose sugar, xylose sugar, maltose sugar and mannose sugar more than the digested with xylanase enzyme and mannanaes enzyme ($P < 0.05$). Nevertheless, considering the type of sugar obtained from the operation of each enzyme was found that the xylose sugar is the main sugar obtained from the work of the xylanase enzyme, cellulase enzyme and mannanase enzyme, regardless of the structure of the main membrane, it is hemicellulose or cellulose. This is because commercial enzymes are often produced by microorganisms such as bacteria, fungi or yeasts. Which will produce many types of enzymes for living, in the production process, interested groups of enzymes that need to be the main enzyme. Such as xylanase enzyme, cellulase enzyme, mannanase enzyme and then separated for trade, but may have other enzymes mixed in, especially xylanase enzyme, amyloglucosidase enzyme, amylase enzyme maltase enzyme. That is commonly found in the production of digestive enzymes of microorganisms. Which is generally mixed in a few amounts. Which producer will not make pure enzymes because they have a high cost, moreover the xylanase enzyme is useful in digesting hemicellulose. Makes the efficiency of digestion crude fiber and carbohydrates well overall. Therefore resulting in the activity of the main enzyme that studies the digestion of crude fiber in cassava pulp and cassava skin was found that high xylose sugar. Both in the work of cellulase enzymes, xylanase enzyme whether the main fiber is cellulose or hemicellulose. Moreover, glucose sugar was more than maltose sugar and mannose sugar. Which shows the ability to digest fiber into the monosaccharide. Which is a joint effect of the activity of fibrolytic enzyme digestion into polysaccharide whether cellulase enzymes, xylanase enzyme. Then there digested are the polyseccharide and the remaining starch in cassava pulp and cassava skin as glucose. From the work of the amylase enzyme, amyloglucosidase enzyme, maltase enzymes that are mixed in the main enzyme making the fiber digestion more efficient, aquatic animals can use cassava pulp and cassava skin combined with enzyme supplementation to effectively digest feed ingredients and use sugar as more energy. The enzyme may help to overcome the depression in fiber digestion that occurs when using high concentrate diets.

Conclusion

The results in the present study suggested that digestion of crude fiber in raw materials; soybean meal, rice bran, cassava pulp and cassava skin with xylanase enzyme, cellulase enzyme, and mannanase enzyme. Soybean meal has 7.00% crude fiber, Rice bran has 11.00% crude fiber, Cassava pulp has 12.25% crude fiber and cassava skin has 15.05% crude fiber. When digested with fibrolytic enzymes, the fiber in the rice bran will obtain all types of sugar more than soybean meal, cassava pulp and cassava skin. Moreover, effective by using cellulase enzyme in digesting rice bran obtain glucose sugar, xylose sugar, maltose sugar and mannose sugar more than using xylanase enzyme and mannanase enzyme. Cassava pulp can be used for any type of enzyme that is studied. In addition, digestion of soybean meal, rice bran, cassava pulp and cassava skin will obtain xylose sugar and glucose sugar as the main sugar group. Therefore can be used as an energy source for aquatic animals. The enzyme was helping fiber digestion that occurs when using high fiber concentrate in diets.

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