THE EFFECTIVITY TEST OF SHEEP RUMEN LIQUOR ENZYME ADDED TO PALM KERNEL MEAL ON ITS DECREASE OF CRUDE FIBER AND APPARENT DIGESTIBILITY COEFFICIENT FOR CATFISH *Pangasius hypophthalmus* DIET

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(*Received 27 May 2011; Accepted 28 September 2011*)

ABSTRACT

Two experiments were conducted to evaluate the hydrolysis of fiber content in palm kernel meal (PKM) by sheep rumen liquor enzyme and to know the apparent digestibility coefficient of hydrolyzed PKM for catfish *Pangasius hypophthalmus*. The first trial examined effectivity of sheep rumen liquor enzyme to decrease crude fiber content of PKM. The added volume of sheep rumen liquor enzyme was 0, 20, 40, 60, 80, and 100 mL/kg PKM and then it was incubated for 0, 12, and 24 hours. A factorial completely randomized experimental design consisted of 2 variables and triplicates were selected. The second trial was conducted to evaluate the apparent digestibility coefficients of hydrolized PKM for catfish. Apparent digestibility coefficients were determined using chromic oxide indicator added to both reference and test diets. The feed ingredients used in the trial were hydrolyzed PKM (PKMe) and unhydrolyzed PKM (PKM). Ten fishes with weighing around 20 g were used in the trial and held in 80 l tanks. Feces were collected from three replicate groups of fish using a fecal collection column attached to fish rearing tank. PKM hydrolyzed with 100 mL/kg and incubated for 24 hour showed the lowest crude fiber content (6.99%) among the treatments ($P<0.05$). Apparent digestibility coefficient of hydrolyzed PKM was 57.57% compared with unhydrolyzed PKM 15.31%. Based on the evaluation in those parameters it was concluded that sheep rumen liquor enzyme added to PKM was effective to decrease crude fiber content of PKM and improve apparent digestibility coefficient of PKM for catfish.

KEYWORDS: ***apparent digestibility coefficient, catfish, crude fiber, palm kernel meal, sheep rumen liquor enzyme***

INTRODUCTION

Palm kernel meal (PKM) which is a by-product of the palm kernel oil industry has been widely used as a feed ingredient for livestock. Proximate analysis showed that PKM has a protein content between 13.6%-17.45% (Sundu *et al.,* 2003; Orunmuyi *et al.,* 2006; Hadadi *et al.,* 2007), crude fat ranged between 17.1%–21.55% (Sundu *et al.,* 2003; Hadadi *et al.,* 2007) and crude fiber reached 18.33%-21.3% (Sundu *et*

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al., 2003; Orunmuyi *et al.,* 2006). The high crude fiber content of PKM due to requires to be reprocessed for using as fish feed raw material use as raw material for fish feed. The limitations of fish in digesting crude fiber. Crude fiber can be used maximum of 7% for fish diets and in the range of 3% to 6% crude fiber for catfish diets (Robinson *et al.,* 2001).

One effort of processing of high-fiber feedstuffs that have been developed in the field of animal husbandry is the use of rumen liquor to decrease crude fiber content of feedstuffs. Sheep rumen liquor is a source of alternative materials that are cheap and easily used as a source of enzyme hidrolase (Moharrery & Das, 2002). Rumen liquor contains of fiber degrading enzyme including α-amylase, galactosidase, hemisellulase, cellulase, and xylanase (Williams & Withers, 1992). Development of utilization technology of rumen liquor in cattle feed inspires the aquaculturist to develop this technology in fish feed. One of the fishery commodities which are potential and have been developed by the community are catfish. Information digestibility of high fibrous feedstuffs in catfish has not been obtained yet. Base on the information above, the objective of the research was to evaluate the effectivity of sheep rumen liquor enzyme added to palm kernel meal on its decrease of crude fiber content and apparent digestibility coefficient for catfish, *Pangasius hypophthalmus* diet.

MATERIALS AND METHODS

The study was conducted in two trials that are the effectivity test of sheep rumen liquor enzyme to decrease the crude fiber content on palm kernel meal (PKM) and digestibility trials of PKM which have been hydrolyzed by rumen liquor enzymes for catfish diet.

The Effectivity Test of Sheep Rumen Liquor Enzyme

In the first trial, the enzyme was isolated from rumen liquor of sheep that was provided grass as a feed. The rumen liquor was centrifuged at 10,000 rpm for 20 minutes in 4^{o}C conditions, then the supernatant was reacted with 60% ammonium sulfate using magnetic stirer and stored for 24 hours at 4^{o}C. Furthermore the rumen liquor was centrifuged and the formed sediment used as a source of enzyme (Budiansyah *et al.,* 2010; Fitriliyani 2010). The obtained enzyme were tested for its activities including activities of cellulase (Ghosse, 1987), amylase and protease (Bergeyer & Grassi, 1983), and lipase (Tietz and Friedreck referenced *in* Barlongan, 1990). The effectivity of enzymes in decreasing crude fiber of PKM was tested by adding the different volume of enzyme into the PKM, namely: A (0 mL/kg), B (20 mL/kg), C (40 mL/kg), D (60 mL/kg), E (80 mL/kg), and F (100 mL/kg of PKM) with a incubation period of 0, 12, and 24 hours (Fitriliyani, 2010). A factorial completely randomized experimental design consisted of 2 variables and triplicates was selected. Crude fiber content and levels of *Neutral Detergent Fiber* (NDF), *Acid Detergent Fiber* (ADF), cellulose, hemisellulosa and lignin of PKM were observed before and after incubation (Takeuchi, 1988; Van Soest, 1991). Data were analyzed statistically using analysis of variance (ANOVA) and followed by Duncan test (Steel & Torrie, 1993).

Apparent Digestibility Test of PKM

The feed ingredients used in the first trial were hydrolyzed PKM (PKMe) and unhydrolized PKM (PKM). The second trial was set up in completely randomized design with 3 treatments (100% reference diet as control, 70% reference diet and 30% PKMe as diet A, 70% reference diet and 30% PKM as diet B) and triplicates (Watanabe, 1988). Apparent digestibility coefficients were determined using chromic oxide indicator added to the reference diet and test diets. The feed composition and proximate composition of feed are presented in Table 1 and 2.

Ten fishes with weighing around 20 g were used in the trial and held in 80 L tanks for 15 days. Water temperature and dissolved oxygen concentration were maintained at 30^{o}C and 7,0 mgL^{-1} respectively. Fish were fed at satiation and feeding frequency was adjusted three times per day (Watanabe, 1988). Feces were collected from three replicate groups of fish using a fecal collection column attached to fish rearing tank. They were dried immediately at 110^{o}C for 4-6 hours. Fecal samples were analyzed for moisture, chromic oxide, protein, calcium, phosphorus, and energy. Apparent digestibility coefficient were calculated based on the procedure of Takeuchi (1988). At the end of the feeding trials. The feed consume, total digestibility, nutrient digestibility, ingredient digestibility and survival

Table 1. Composition of reference and test diet (%)

Composition	Reference Diet (100% commercial)	Diet A (30% PKMe)	Diet B (30% PKM)
Comercial feed	96.5	66.5	66.5
PKMe	0	30	0
PKM	0	0	30
CMC	3	3	3
Cr_2O_3	0.5	0.5	0.5
Total	**100**	**100**	**100**

PKMe : Hydrolyzed PKM
PKM : Unhydrolized PKM
CMC : Carboxy Methyl Cellulosa

Table 2. Proximate composition of the reference and test diet (in dry weight)

Proximate composition	Reference diet (100% commercial feed)	Diet A (30% PKMe)	Diet B (30% PKM)
Protein	28.83	25.20	24.43
Fat	7.12	6.97	7.72
Ash	12.59	12.45	10.69
Crude Fiber	4.66	5.36	8.36
NFE	46.80	50.02	48.80
GE (ckal/100 g feed)*	423.85	414.94	412.67
C/P**	14.703	16.466	16.892

NFE : Nitrogen Free Extract
* GE : *Gross energy*
** C : Energy; P: Protein

rate were measured. Data were analyzed statistically using analysis of variance (ANOVA) and followed by Duncan test (Steel & Torrie, 1993).

RESULTS AND DISCUSSION

Activity of Sheep Rumen Liquor Enzyme

The result of measurement of cellulase, amylase, protease, and lipase activity are presented in Figure 1. The measurement result showed that activity of the cellulase enzyme was greater among others. The values of enzyme activity from the largest to lowest are cellulase (0.31 ± 0.015), amylase (0.14 ± 0.016), protease (0.11 ± 0.016) and lipase enzyme (0.03 ± 0.011) respectively.

Result of enzyme activity analysis showed that the cellulase enzyme activity of 0.31 IU/ml/minute was higher than the activity of amylase, protease and lipase enzymes. The high activity of cellulase enzyme due to the sheep rumen liquor was taken from the sheep that was provided grass as feed. A grass has a high fiber content, so that in the rumen, the sheep requires more cellulase enzyme to digest grass. It causes cellulase enzyme activity obtained in this study is greater than the others. Moharrery & Das (2002) stated that the activity of enzymes in the rumen liquor depended on the composition or treatment of food. Agarwal *et al.* (2002) reported that lambs weighing 23.5 kg that were fed milk until 8 weeks and passed with 50% concentrate and 50% grass until the age of 24 weeks found that the enzymes pre-

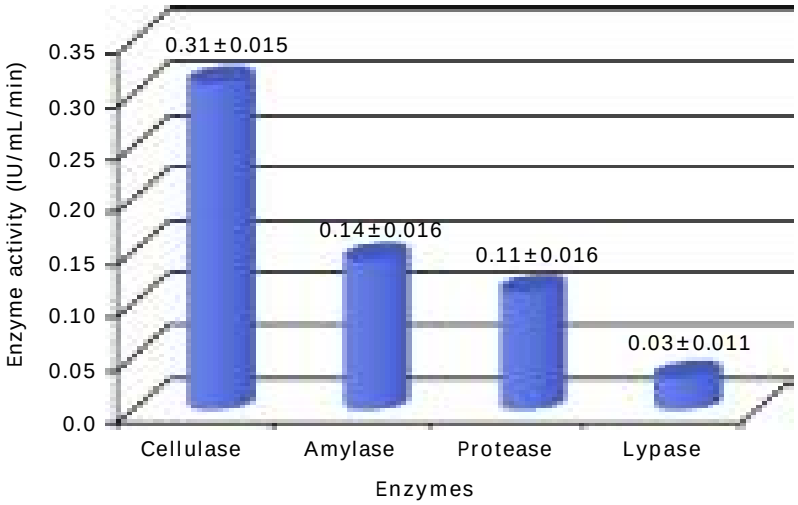

Figure 1. Activities of cellulase, amylase, protease and lypase enzymes in extracts of sheep rumen liquor

sented in rumen liquor were carboxymethyl celulase with 3.60 umol of enzyme activity of glucose per hour per mL, alpha amylase 0.33 umol of glucose per minute per mL, 0.29 umol xylanase xylosa per minute per mL, beta-glucosidase 0.20 umol p-nitrophenol per minute per mL, alpha-glucosidase 0008 mol p-nitrophenol per minute per mL, 0.05 umol urease NHS-N per minute per mL and 452.7 ug protease hydrolysis of protein per hour per mL.

Crude Fiber Content and Levels of Glucose Dissolved

The results of crude fiber content analysis and the decrease of crude fiber content in hydrolized PKM (PKMe) are presented in Table 3.

The result of analysis of dissolved glucose in PKM showed that dissolved glucose content increase by adding volume of enzyme and the incubation period (Table 4).

The result of analysis of variance showed that the combination of volume of sheep rumen liquor added to PKM and incubation period give the significantly effect on its decrease of crude fiber content. The crude fiber content of unhydrolized PKM was higher than the others on the length of incubation time at 12 and 24 h but it is not significantly different at incubation period 0 hours. Crude fiber content in all treatments showed a significant decrease in 24 hours incubation period compared with 0 and 12 hours incubation period. The lowest crude fiber value of 6.69% was achieved by adding enzyme 100 mL/kg for 24 hours incubation period. The value of 6.69% is significantly different from other treatments at 24 hour incubation period by adding enzymes 20, 40, 60, and 80 mL/kg of PKM which show

Table 3. Result of analysis of crude fiber content of hydrolized PKM

Incubation period (hours)	Volume of sheep rumen liquor enzyme/kgPKM (mL)					
	0	20	40	60	80	100
0	17.54±0.18^{a}	17.63±0.20^{a}	17.22±0.20^{a}	16.71±0.21^{b}	17.06±0.16^{b}	16.06±0.16^{c}
12	16.38±0.27^{d}	11.74±0.06^{e}	10.07±0.019^{f}	10.94±0.05^{g}	10.48±0.20^{h}	9.15±0.84^{i}
24	15.55±0.27^{j}	10.92±0.45^{g}	9.40±0.23^{k}	8.86±0.45^{i}	8.38±0.36^{l}	6.69±0.30^{m}

Different notation on the same column shows the significantly difference (P<0.05)

Table 4. Dissolved glucose content of hydrolized PKM (%)

Volume of sheep Rumen liquor/kg PKM (mL)	Incubation period (hours)	
	12	24
0	0.040±0.001	0.041±0.001
20	0.080±0.001	0.169±0.003
40	0.122±0.003	0.246±0.006
60	0.168±0.035	0.333±0.064
80	0.218±0.008	0.424±0.014
100	0.242±0.027	0.469±0.049

crude fiber content values respectively 10.92%, 9.40%, 8.86%, and 8.38% (Table 3).

The decrease of fiber content in hydrolyzed PKM is caused by cellulase enzyme activity that hydrolysis cellulose in PKM into simpler forms. The obtained result in the current study showed that the lowest crude fiber content of PKM (6,69%) was achieved by adding 100 mL/kg rumen liquor to PKM and incubation for 24 hours. While the highest increase of dissolved glucose content was 0.469%. James *et al*. (2005) reported that the increase of enzyme concentration gave effect for substrate more than the increase of temperature during the hydrolysis process. Whereas Vijaya *et al*. (2002) explained that there was indication of increasing hydrolysis proses by increasing time of incubation.

The sheep rumen liquor enzyme was used to hydrolyze *lamtoro leaves meal* and the result obtained in the study showed that 100 mL/kg rumen enzyme added on *lamtoro meal* and incubated for 24 hours decreased crude fiber content of 53.64% (Fitriliyani, 2010). The rumen liquor enzyme added on *lamtoro meal* broke down the difficult components to easily digested and can be utilized by the animal. She also reported that the sugar total content measured at 2 hours incubation produced a linear response curve while at 24 hour incubation showed quadratic response curve. Fitriliyani (2010) revealed that difference of this response caused by relationship between availability of the subtrate and incubation time. At 2 hours incubation enzyme will be hydrolyzing substrate whereas at 24 hours incubation enzyme has maximum to break down the available substrate. Result obtained in Alemawor research (2009) showed that there was a decrease of better nutrition quality by using multienzyme on feed raw material including increase of sugar total value and decrease of crude fiber, *Neutral Detergent Fiber* (NDF), *Acid Detergent Fiber* (ADF), cellulose and lignin.

The content of *Neutral Detergent Fiber* (NDF), *Acid Detergent Fiber* (ADF), cellulose, hemicellulose and lignin

Result of NDF, ADF, cellulose, hemicellulose, and lignin analysis on hydrolized PKM by sheep rumen liquor enzyme of 100 mL/kg at 24 hours incubation period and unhydrolized PKM is presented in Table 5.

Result of fiber fraction analysis such as NDF, ADF, cellulose, hemicellulose, and lignin on PKM showed that the content of NDF, ADF, cellulose and lignin decreased on hydrolized PKM, whereas hemicellulose increased. The decrease of fiber fraction content on hydrolyzed PKM is 5.17% NDF, 40.61% ADF, 36.28% cellulose, dan 43.61% lignin, while the increase of hemicellulose is 104.92%. Alemawor (2009) reported that using of multienzyme added to feed raw materials increased nutritional quality such as increase glucose dissolved, decrease crude fiber, NDF, ADF, cellulose and lignin.

Apparent Digestibility Test of PKM Hydrolyzed

The observation of feed consumption, digestibility value (total, protein, Ca, P, energy, materials) and survival rate is presented in Table 6. Analysis of variance showed that feed consumption and survival rate were not significantly different among the treatments ($P>0.05$). This data indicate that the feed have a good

Table 5. Composition of NDF, ADF, cellulose, lignin and hemisellulosa on PKM and PKMe

Fiber fraction (%)	PKM	PKMe*
NDF	75.10±0.015	71.22±0.012
ADF	56.81±0.061	33.74±0.038
Cellulosa	32.22±0.061	20.53±0.012
Hemicellulosa	18.29±0.026	37.48±0.214
Lignin	23.30±0.098	13.14±0.083

* hydrolyzed PKM by sheep rumen liquor enzyme 100 mL/kg at 24-hour incubation period

Table 6. Feed consumption, digestibility value (total, protein, energy, materials), DE and survival rate

Parameters	Diet		
	Reference (100% commercial feed)	A (30% PKMe)	B (30% PKM)
Feed consumption (g)	134.14 ± 2.30^{ns}	137.63 ± 0.03^{ns}	133.74 ± 2.36^{ns}
Digestibility (%)			
Total	75.23 ± 0.49^{a}	69.93 ± 0.33^{b}	57.26 ± 0.35^{c}
Protein	91.63 ± 0.40^{a}	87.86 ± 0.57^{b}	83.66 ± 0.84^{c}
Energy	83.39 ± 0.17^{a}	72.72 ± 0.14^{b}	60.59 ± 0.02^{c}
Materials	-	57.57±0.489^{a}	15.31±0.217^{b}
DE (kcal/100gr)	353.4 ± 0.73^{a}	300.5 ± 0.16^{b}	263.9 ± 0.36^{c}
SR (%)	100.00 ± 0.00^{ns}	100.00 ± 0.00^{ns}	100.00 ± 0.00^{ns}

*) Different notation on the same row shows the significantly difference (P<0.05)

pallatability. Nutritional content, palatability, temperature, age, body weight and stomach capacity are factors that affect feed intake. The palatability of feed is determined by taste, smell and color of physical and chemical factors feed (Parakkasi, 1990).

Analysis of variance of digestibility coefficient showed significantly different among the treatments, where diet A with 30% PKMe has digestibility coefficient higher than diet B that used 30% PKM. This difference also occured on digestible energy (DE) value, where the treatments was significantly affect on DE value.

Digestibility value describes fish ability to digest feed and feed quality. Digestibility showed fraction of nutrien or feed energy that was digested and was not excreted into feces form (NRC, 1993). Result obtained in this study showed that digestibility value of feed with hydrolyzed PKM was higher than unhydrolyzed PKM. Mokoginta (1999) reported that the difference of feed and nutrient composition affected on protein and total digestibility. Base on the result of proximate analysis it was known that crude fiber content diet B (8.36%) was higher than diet A (4.66%). Halver (1989) reported that fish are less able to digest crude fiber because there are no fish gut microbes that can produce the enzyme cellulase. High content of crude fiber in fish feed will affect the digestibility and absorption of nutrients in the digestive system of fish. Protein digestibility value for all treatments in this current study showed more than 80%. According to Ranjhan (1980), crude protein digestibility depends on protein content in feed. Low protein feed has low digestibility and vice versa. Protein digestibility depend on amount of protein in digestive tract (Tilman *et al.,* 1991). The high protein content

in feed will increase protein consume and further it will affect on feed digestibility value (Wahju J., 1997). Hertrampf & Pascual (2000) reported that chicken was fed with PKM has energy digestibility, protein digestibility and crude fiber digestibility were 78.9%, 59.8%, and 24.4 % respectively. Hertrampf & Pascual (2000) recommended that using of PKM in feed ranged 5% to 10% for herbivorous and omnivorous, and 3% to 8% for carnivorous.

Result of material digestibility measurement on hydrolyzed and unhydrolyzed PKM showed that material digestibility value of hydrolyzed PKM (57.7%) was higher than unhydrolyzed PKM (15.31%). In described decreasing of hydrolyzed PKM digestibility for *Pangsius hypopthalmus* diet of 73.4% compared with unhydrolyzed PKM. The difference of feed digestibility value in this study also caused by the difference fiber fraction value of feed including NDF, ADF, cellulose and lignin. Van Soest *et al.* (1985) reported that NDF content strongly influence the ability of cattle to consume feed. Furthermore, it is said that the NDF content more than 56% reduced the level consumption of dry matter.

CONCLUSION

Based on the evaluation in those parameters it was concluded that sheep rumen liquor enzyme added to PKM was effective to decrease crude fiber content of PKM and improve apparent digestibility coefficient of PKM for catfish *Pangasius hypopthalmus*.

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