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Protein quality of *Aspergillus niger*-fermented palm kernel cake

(Kualiti protein hampas isirung kelapa sawit terfermentasi dengan *Aspergillus niger*)

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Key words: fermented palm kernel cake, protein quality, chemical evaluation, rat bioassay

Abstract

This study was conducted to evaluate the nutritional improvement especially on protein quality of *Aspergillus niger*-fermented palm kernel cake (fPKC). Results of proximate and chemical analyses showed an improvement in nutritional quality of fPKC compared to untreated PKC. The crude protein of fPKC (24.7%) increased significantly compared to the value in untreated PKC (17.5%). The total essential amino acid in fPKC was significantly increased (6.3%) compared to the value in untreated PKC. The fPKC contained 15.7% of total amino acid, accounted for 63.4% of the crude protein. Rat bioassay on protein quality was conducted for 28 days to determine protein efficiency ratio (PER), in vivo apparent protein digestibility and feed conversion ratio (FCR) of fPKC. Results showed that when fPKC was fed as the only protein source during the experiment, the diet did not support rats' growth. Rats fed diets of fPKC recorded a mean body weight loss of 24.4 g PER, and FCR of fPKC also showed negative values (–1.5 and –6.5, respectively), while the apparent digestibility value of fPKC was 22.6%. This indicated that fPKC may not be fed as the sole protein source in the diet of animals.

Introduction

Palm kernel cake (PKC) is the solid residue left behind after the extraction of oil from the kernels of oil palm fruits. It is abundantly produced throughout the year in Malaysia and this guarantees its supply and availability as a major ingredient for livestock feeding. The low digestibility of PKC by poultry is affected by three main factors: high shell content, unfavourable fibre composition and low metabolisable energy (ME) value, reported to be 6.2 MJ/kg (Chin 2002). The application of enzymes for saccharification of fibrous material has received much attention. In Malaysia, this approach is being pursued in the context of improving PKC through biological processes such as fermentation since there are possibilities for the improvement of digestibility and amino acid availability in the fermented product (Kompiang 1993). Apart from this, fungal growth under solid state fermentations has also been found to be more suitable for low technology

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applications and there is hardly any waste disposal at the end of the process because the whole product may be used directly in animal feeds (Iluyemi et al. 2006).

As for determination of protein quality, biological evaluation is the best tool for judging the quality of protein since various factors determine the ultimate quality of the protein in vivo. Thus, the objective of this study was to determine the protein quality of fermented PKC using chemical analyses (amino acid profile and proximate composition) and rat bioassay. Rat was selected as the model animal in this study because a number of studies have demonstrated that fungal protein-enriched agricultural residues can be utilized by simple-stomached animals such as rats (Alexander et al. 1979; Mathot et al. 1992; Devrajan et al. 2004).

Materials and methods

Solid substrate fermentation (SSF) of PKC

Raw material "expeller pressed palm kernel cake" was bought from a FELDA factory in Serting, Negeri Sembilan, Malaysia. Fermented PKC (fPKC) was produced using the established solid substrate fermentation (SSF) technique (Abdul Rahman et al. 2005) employed by the PKC group in MARDI. The inoculum used in this study was spore suspension (1 x 107 spore/ml) of *Aspergillus niger*, strain FTCC 5003. The PKC substrate and distilled water were sterilized at 121 °C for 20 min. The sterile PKC substrate (1.3 kg) in tray system was mixed with distilled water and 0.5% of inoculum to get 50% moisture content. The culture was incubated in an incubator at 30 °C for 66 h. The fermented PKC was then dried in an oven at 60 °C. The sample was then ground, mixed and sieved to get smaller particles (less than 2 mm) and homogenized.

Proximate and amino acid analyses

Proximate analyses (crude protein, moisture content, ash and crude fat) of the samples were determined according to standard methods described by the AOAC (2000). Dry matter and nitrogen-free extract (NFE) were determined by differences. Fibre analyses were carried out according to the method described by Van Soest (1963). Neutral detergent fiber (NDF) represents total fibre in plant cell walls and consists of lignin, cellulose and hemicellulose, while acid detergent fibre (ADF) represents the less digestible components which consists of lignin and cellulose. The concentration of hemicellulose was calculated as the difference between NDF and ADF, while that of cellulose as the difference between ADF and acid detergent lignin (ADL).

The amino acids were estimated by HPLC (Waters Inc., USA), subjected to pre-column derivatization and determined using AccQ Taq method as described by the manufacturer (Waters Inc., USA). Samples were hydrolysed with 6 N hydrochloric acid (HCL) to break the peptide bonds of protein. The amino acid analyser detected 15 amino acids (acid-stable amino acids) which commonly occur in food proteins except cystine, cysteine, methionine and tryptophan. The four types of amino acids are labile under acid hydrolysis conditions and require separate method of analysis. The amino acid cystine, cysteine and methionine were therefore first oxidized with performic acid, under controlled conditions, to convert into their residues of cysteic acid and methionine sulphone. These acid-stable residues were then freed from the protein by hydrolysis with 6 N HCl (Mason et al. 1980). For tryptophan, the amino acid was released using an alkaline hydrolysis (Hugli and Moore 1972).

Diet formulation

Diets for the study were formulated based on proximate analysis results (*Table 1*) and recommendation by AOAC (2000) for determination of protein efficiency ratio (PER). Other components included in the diet were mineral mixture (USP XVII), vitamin mix AOAC (CA 40055), cellulose, sucrose, corn starch and corn oil (Mazola). The samples evaluated were casein from Animal

Table 1. Proximate analysis of raw materials (% wet weight)

Analyses	Casein	PKC	Farmented PKC
Crude protein (%)	81.93 ± 0.24	16.39 ± 0.07	20.59 ± 0.30
Moisture content (%)	10.01 ± 0.02	6.09 ± 0.05	9.17 ± 0.10
Ash (%)	1.52 ± 0.08	3.99 ± 0.10	5.40 ± 0.04
Crude fat (%)	0.18 ± 0.01	5.68 ± 0.16	3.57 ± 0.02
Nitrogen (%)	13.12 ± 0.04	2.62 ± 0.01	3.30 ± 0.05

Data are presented in the mean values, n = 3

Table 2. Rat diet composition with 10% reference/test protein (g/kg feed)

Diet composition	Casein diet	PKC diet	Farmented PKC diet
Test protein	122.05	610.22	485.58
Mazola corn oil	79.77	45.34	62.68
Ash mixture (AOAC)	48.15	25.63	23.76
Moisture	37.79	12.85	5.47
Vitamin mixture (AOAC)	10.00	10.00	10.00
Cellulose	10.00	0	0
Sucrose	346.12	147.98	206.25
Corn starch	346.12	147.98	206.25

Data are presented in the mean values, n = 3

Table 3. Proximate analyses of formulated rat diets

Analyses	Casein diet	PKC diet	Farmented PKC diet
Crude protein (%)	10.22 ± 0.11a	10.69 ± 0.31a	10.45 ± 0.13a
Moisture content (%)	8.36 ± 0.26a	6.69 ± 0.11c	7.47 ± 0.06b
Ash (%)	4.56 ± 0.03b	4.72 ± 0.13b	5.12 ± 0.06a
Crude fat (%)	7.76 ± 0.10a	7.74 ± 0.16a	7.88 ± 0.02a
Nitrogen (%)	1.64 ± 0.02a	1.72 ± 0.05a	1.68 ± 0.02a

Data are presented in the mean values ± SD (n = 3). Values with different letters within same row are significantly different (p <0.05)

Nutrition Research Council (ANRC) which was used as reference protein, untreated PKC and fPKC. The diet composition (g/kg) for each test protein is illustrated in *Table 2*. After diet preparation for each type of test protein, another proximate analysis was carried out to ensure the diet formulation (*Table 3*) was done correctly following the recommendation by AOAC (2000).

Experimental animals

The *Sprague Dawley* strain rats were bought from Animal House, Universiti Kebangsaan Malaysia, Bangi, Selangor. All rats were provided 15 g of diet (mash form) every day and water *ad libitum*. Eight male weanling rats (24 days old) per observation were placed in wire-bottomed cages and randomly assigned by treatment to individual cages. All rats were acclimatized to the new environment and fed mash form of standard rat chow diet for a week before starting the experiment. On day one of the assay period, the mean average weights of rats fed casein, PKC and fPKC diets were 69.8, 70.8 and 72.6 g respectively. During the assay, the temperature in the laboratory was maintained at 21 ± 2 °C, with a 12 h light/dark cycle.

Rat bioassay

Protein quality study of fPKC using rat bioassay was conducted over a period of 28 days to determine protein efficiency ratio (PER), in vivo apparent protein digestibility

of fPKC and feed conversion ratio (FCR). Body weight was recorded on day 0 and every two days thereafter for 28 days. For determination of feed intake, left over feed were collected daily and weighed. PER was calculated using standard recommended equations (Jood and Singh 2001).

$$\text{PER} = \frac{\text{Gain (or loss) in body weight (g)}}{\text{Protein consumed (g)}}$$

The feed conversion ratio (FCR) was obtained by dividing the amount of food consumed during the assay with the weight gain.

$$\text{FCR} = \frac{\text{Amount of food consumed (g)}}{\text{Weight gain (g)}}$$

Food intake and faecal output data were recorded daily for 9 days (days 10 through 18) of the 28-day study period to determine the in vivo apparent protein digestibility. The in vivo apparent protein digestibility was calculated as follows:

$$\text{In vivo apparent protein digestibility (\%)} = \frac{\text{N in diet (g)} - \text{N in faeces (g)} \times 100}{\text{N in diet (g)}}$$

Statistical analyses

All statistical computations were performed with the ANOVA procedure followed by Duncan New Multiple Range Test (DMRT) (SAS Inst. 1996).

Results and discussion

Proximate analyses

The proximate and chemical analyses were calculated based on dry matter basis (*Table 4*). The data indicated that the crude protein of fPKC (24.7%) was significantly higher than the value in untreated PKC (17.5%). The increase in the protein content of the fPKC could be attributed to the reduction of biomass after fermentation, which resulted from the utilization of the carbon content by the fungus. According to Mathot et al. (1992), about 20% of the initial substrate (DM) was lost during fermentation, especially starch and hemicellulose which were the main sources of carbon for the fungus. Apart from this, the increase in the amount of the microbial biomass in the form of single-cell proteins might possibly account for the increase in the protein content of the *A. niger* products (Oboh et al. 2002).

Results on fibre analyses also showed a general improvement in nutritive value of fPKC compared to untreated PKC (except for the ADL value). NDF and ADF levels in fPKC were significantly decreased from 79.0% to 50.3% and 46.8% to 35.8%, respectively. The significant reduction in hemicellulose (32.2–14.5%) and cellulose (34.4–21.5%) might be attributed to fibre degradation by fungal enzymes. On the other hand, the increase in the ADL content of the fPKC could be attributed to the reduction of biomass after fermentation. These findings were similar to the reports by Mathot et al. (1992), Kompiang (1993) and Iluyemi et al. (2006) on the utilization of *A. niger* in improving the nutritional value of barley, cassava and PKC, respectively. SSF has also been reported to improve the nutritive value of food legumes and cereals by decreasing the levels of antinutrients (Dhankher and Chauhan 1987) and increasing protein digestibility (Taylor and Taylor 2002).

The total ash content increased (41.5%) significantly in fPKC compared to the value in untreated PKC (*Table 4*). The difference in the total ash content might be due to the differences in the mineral concentration of the samples. All minerals and trace elements analysed in the fPKC were significantly higher compared to the level in untreated PKC, except for copper and phosphorus (Marini et al. 2006). The increased of ash content in fPKC also caused by the loss of organic matter during the fermentation process. Chavez et al. (1988) reported that the ash content of fermented product was higher than in the original material and the lower gross energy value of the samples was consistent with their higher ash contents.

Table 4. Chemical composition of fermented palm kernel cake (fPKC) and untreated palm kernel cake (PKC) (% dry matter basis)

Chemical composition (%)	PKC	fPKC
Acid detergent fiber (ADF)	46.8 ± 0.2a	35.8 ± 0.1b
Acid detergent lignin (ADL)	12.4 ± 0.3b	14.3 ± 1.1a
Ash	4.1 ± 0.0b	5.8 ± 0.0a
Cellulose	34.4 ± 0.3a	21.5 ± 1.1b
Crude fat	10.7 ± 0.1a	4.1 ± 0.4b
Crude fiber	16.8 ± 0.7a	14.5 ± 0.3b
Crude protein	17.5 ± 0.3b	24.7 ± 0.2a
Dry matter	94.8 ± 0.0a	91.2 ± 0.0b
Hemicellulose	32.2 ± 0.3a	14.5 ± 0.3b
Moisture content	5.2 ± 0.0b	8.8 ± 0.1a
Neutral detergent fiber (NDF)	79.0 ± 0.3a	50.3 ± 0.2b
Nitrogen free extract (NFE)	45.9 ± 0.9a	42.2 ± 0.6b

Data are presented as mean values ± SD (n = 3). Values with different letters within the same row are significantly different (p <0.05)

Amino acid profiling

Table 5 shows the amino acid profiles of untreated PKC and fPKC. Compared to untreated PKC, the increase of total amino acid and essential amino acid (EAA) in fPKC were significant (p <0.05). The total amino acid content in fPKC accounted for only 63.35% of crude protein, lower than the value in untreated PKC (85.09%). The difference between total amino acid and crude protein could be accounted for by the presence of non-protein nitrogen (NPN) such as nucleic acid and chitin in the fungal mycelia (Mathot et al. 1992). In the fermented substrates, about 60–68 % of the total N is present as α amino N, the remaining N might be associated with the cell wall chitin of fungus, chitosan as polymeric hexosamine and in the purine and pyrimidine bases of the nucleic acids and nucleotides. Therefore, fPKC contained high percentages of NPN.

The increment of total amino acid in the fPKC was 5.5% and it was significantly (p <0.05) increased compared to the values in the PKC. This happen because most of the amino acid contents in fPKC were significantly increased compared to the values in PKC, except for arginine, methionine, glutamic acid and cystine (*Table 5*). Based on Onwudike (1996), PKC protein had a poor amino acid balance, with lysine being a major limiting amino acid. However, the result showed that by fermentation with *A. niger*, lysine content was significantly increased from 0.49% in PKC to 0.64% in fPKC.

The differences in total amino acid and chemical constituents of fPKC reported by Iluyemi et al. (2006) with the results of this study might be due to the differences in the strain of *A. niger* used and fermentation conditions such as fermentation temperature, medium and duration. Modifications and improvements in fermentation process of the fPKC should be applied to reduce the non-protein nitrogen contents, such as nucleic acid, which have no known nutritional value (Mathot et al. 1992). The nutritional value of protein is considered high if the composition of its essential amino acid content is close to the essential amino acid profile required in the diet of animals.

Hydrolysed protein shows better availability since low molecular weight peptides and amino acids are released. Therefore, higher in vivo protein digestibility values should be expected in fPKC, since there is a marked increase in amino acids after fermentation. The increase in hydrophobic amino acids such as isoleucine, leucine and lysine was also

Table 5. Amino acid profiles of fermented palm kernel cake (fPKC) and untreated palm kernel cake (PKC) (% dry matter basis)

Amino acid (%)	PKC	Fermented PKC
Arginine	2.06 ± 0.11a	1.52 ± 0.03b
Threonine	0.52 ± 0.02b	0.72 ± 0.02a
Valine	0.89 ± 0.01b	1.04 ± 0.01a
Lysine	0.49 ± 0.03b	0.64 ± 0.00a
Isoleusine	0.63 ± 0.04b	0.78 ± 0.01a
Leusine	1.07 ± 0.03b	1.23 ± 0.01a
Phenylalanine	0.68 ± 0.02b	0.79 ± 0.01a
Tryptophan	0.11 ± 0.00b	0.13 ± 0.01a
Histidine	0.27 ± 0.00b	0.34 ± 0.01a
Methionine	0.26 ± 0.02a	0.22 ± 0.01b
Total essential amino acid	6.98 ± 0.24b	7.42 ± 0.09a
Aspartic acid	1.38 ± 0.05b	1.62 ± 0.02a
Serine	0.71 ± 0.00b	0.78 ± 0.02a
Glutamic acid	3.44 ± 0.05a	2.78 ± 0.04b
Glycine	0.74 ± 0.01b	0.95 ± 0.02a
Alanine	0.59 ± 0.20b	0.82 ± 0.00a
Proline	0.53 ± 0.01b	0.64 ± 0.01a
Tyrosine	0.29 ± 0.03b	0.46 ± 0.01a
Cystine	0.20 ± 0.01a	0.19 ± 0.01a
Total non-essential amino acid CP	7.87 ± 0.31a	8.25 ± 0.12a
Total amino acid	14.85 ± 0.40b	15.67 ± 0.15a
Total amino acid as % of crude protein	85.09 ± 2.69a	63.35 ± 0.41b

Data are presented as mean values ± SD (n = 3). Values with different letters within the same row are significantly different (p <0.05)

important, due to the effects that these amino acids have on the physical and functional properties of food proteins (Mahmoud 1994).

Rat bioassay

In the rat bioassay, all rats survived at the end of the observation study and gained weight except for the fPKC group (*Figure 1*). For the control (casein diet) groups, the higher growth rate recorded by rats might be due to higher feed intake (*Figure 2*) and nutritional quality of the diet (Aning et al. 1998). This would suggest that feed intake plays a very vital role in growth (Aning et al. 1998). Apart from the possible lower protein quality of the fPKC, the negative growth rate recorded by rats fed with fPKC diet could be attributed to a lowest in feed intake compared to other rats' group (*Figure 2*). The decrease in feed intake in this group might be due to variations in flavour and appearance of the feed compared to the uniformity of the standard rat feed. Other than that, the decrease in body weight might be due to the inability of the animals to fully utilize the ingested feed. Indirectly, this suggested that certain essential nutrients required for the survival of rats might be lacking in the feed or it may contain indigestible components. The high fibre content of the fPKC (14.5%) might reduce the digestibility and availability of the nutrients (Eggum 1970).

Results in *Figure 1* also showed that when fPKC was fed as the sole protein source during the experiment, the diet did

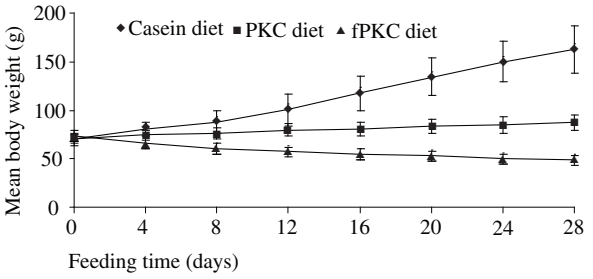

Figure 1. Mean body weights of rats fed with ANRC casein, untreated and fermented PKC diet (values are mean ± SD, n = 8)

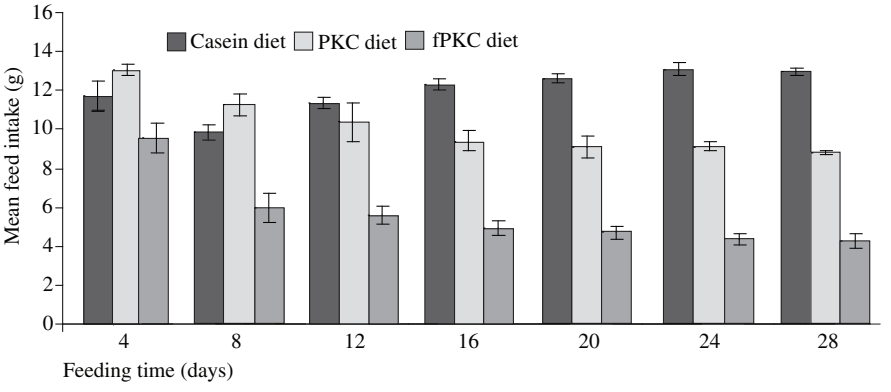

Figure 2. Mean feed intake of rats fed with ANRC casein, untreated and fermented PKC diet (values are mean ± SD, n = 8)

not support the rats' growth. This indicated that fPKC could not be fed as the sole protein source in animals' diet. Rats fed diets containing fPKC had a mean body weight loss of 24.4 g whereas those fed diets containing untreated PKC had a mean body weight gain of 16.5 g (*Table 6*).

A feeding trial on broilers however showed that fPKC based feed mixed with other sources of protein from fish meal and soybean meal was acceptable and the metabolisable energy (ME) of *A. niger*-fermented PKC increased from 6.2–9.0 MJ/kg (Daud et al. 2003). Results in this study were similar to those of Joshi et al. (2000) who successfully fed fermented apple pomace to broilers but not to rats. They attributed this phenomenon to the possibility of the rats might have had insufficient amount of enzymes required for utilization of feed high in fibres as was in the fPKC. The negative value of PER for fPKC (*Table 6*) could also be due to the high fibre content and lower feed intake. Based on Weiss and Scott (1979), the fibre depressed intake and increased bulkiness consequently caused growth depression (*Figure 1*).

Although there were no previous reports on the PER value of *A. niger*-fPKC, the lower N digestibility of fungal protein compared with casein had been previously reported by Alexander et al. (1979) and Mathot et al. (1992) who attributed it to the resistance of the rigid cell walls of fungi to enzymes digestion. Fungal cell walls contain a specific N compound called chitin. Chitin is an insoluble polymer and according to Smith et al. (1975), chitin digestibility ranged between 60% and 92% (depending on the fungal strain). Another possibility that could have caused the decreased feed intake in animals was the presence of a toxic substance from fungal secondary metabolite (Kazanas et al. 1984) in the fPKC. Other

Table 6. Protein efficiency ratio (PER) and feed conversion ratio (FCR)

Protein source	Weight gain (g)	Total diet consumed (g/rat/28 days)	Protein in diet (%)	Protein consumed (g/rat/28 days)	PER	FCR
Casein	92.7 ± 19.1a	335.9 ± 48.7a	10.3	34.6 ± 5.0	2.7 ± 0.2a	3.7 ± 0.3b
PKC	16.5 ± 5.8b	284.3 ± 26.5b	10.7	30.4 ± 2.8	0.5 ± 0.2b	19.0 ± 6.1a
Fermented PKC	–24.4 ± 2.6c	157.7 ± 12.6c	10.4	16.3 ± 1.3	–1.5 ± 0.2c	–6.5 ± 0.7c

Data are presented as mean values ± SD (n = 8). Values with different letters within the same column are significantly different (p <0.05).

Table 7. In vivo apparent digestibility of PKC, fermented PKC and casein

Protein source	Total diet consumed	Nitrogen in diet (%)	Total nitrogen consumed	Nitrogen in dried faeces (%)	Total nitrogen in dried faeces	Apparent digestibility (%)
Casein	109.0 ± 17.2	1.6 ± 0.0	1.8 ± 0.3	3.4 ± 0.0	0.16	90.8 ± 1.6a
PKC	88.7 ± 9.0	1.7 ± 0.1	1.6 ± 0.2	2.6 ± 0.0	0.87	45.7 ± 5.7b
Fermented PKC	46.4 ± 6.0	1.7 ± 0.0	0.8 ± 0.1	3.6 ± 0.0	0.59	22.6 ± 9.7c

Data are presented as mean values ± SD (n = 8). Values with different letters within the same column are significantly different (p <0.05)

than that, the fPKC diet was least acceptable probably due to the undesirable odour of the feed. Therefore, there is a need for reformulation of feed comprising fPKC in order to make a balanced and attractive feed for rats. Reports by Singh and Narang (1992) and Devrajan et al. (2004) showed that the digestibility of reconstituted feed comprising fermented and unfermented apple pomace was comparable to that of the standard rat feed.

A report by Mathot et al. (1992) similarly showed that the PER values for barley protein and soybean meal were significantly higher than that of the fermented barley protein but lower than that of casein. The fermented barley PER value was probably underestimated because of the relatively low methionine and cystine content in the experimental diet of the feed as well as the high non-protein nitrogen content (Mathot et al. 1992). These differences may, to a great extent, be ascribed to significant differences in body weight gain and in total protein intake, although they may also reflect differences in the protein quality.

In contrast, Wang et al. (1968) reported that wheat fermented with *Rhizopus oligosporus* gave PER value higher than that of the original grain. This discrepancy can be explained by the choice of substrate, fungal strain and fermentation process which were all quite different. The slow growth of rats fed with PKC diet tallied with the result of PER value of the diet (*Table 6*). The low PER value of PKC diet was to be expected because of the high fibre content of PKC (*Table 4*). Results in *Table 6* also showed that the FCR value of PKC diet was significantly higher (p <0.05) than the FCR value for casein. This could be due to the negative effect of high fibre consumption on feed intake and weight gain. A similar result was observed by Raupp et al. (2004) on rats given a partially hydrolyzed cassava solid waste as a fibre source.

The in vivo apparent digestibility of fPKC and PKC were much lower compared to casein (*Table 7*). This could be due to the fibre, odour and taste factors mentioned earlier. The insoluble fibre constituent from fPKC and PKC most likely promoted the greatest faecal bulking, faecal weight and

defecation frequency in rats as compared to that from casein. Post-mortem examinations showed that the rats fed with untreated PKC and fermented PKC were unable to utilize the ingested feed. These results are similar to those reported by Raupp et al. (2004) for partially hydrolyzed cassava waste in model rats. A similar decrease in digestibility of fermented apple pomace (Devrajan et al. 2004) and diets containing purified fibres, either pectin or cellulose (Davies et al. 1991) in rats, has also been reported.

Conclusion

Results from this study showed that fPKC may not be fed as the sole protein source in the diet of animals. Modifications and improvements in the fermentation process of the fPKC should be made to reduce the non-protein nitrogen content. The problem of feed acceptability in rat bioassay could be overcome by mixing with other more palatable ingredients in a complete diet. Therefore more studies using different animal species for an extended period of time, with different formulations based on fPKC are needed to determine the suitability of such feeds, especially the long-term effects on the animal. Further research on toxicology needs to be carried out to determine any side effects, if any, of secondary metabolites in the fPKC.

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References

Abdul Rahman, A.R., Daud, M.J., Noraini, S., Jame'ah, H., Azlian, M.N., Ahmad, A. and Zainal Abidin, A.R. (2005). Influence of inoculum size during solid substrate fermentation of palm kernel cake in tray system. *Proc. of 26th. Malaysian society of animal production annual conference*, Terengganu, p. 59. Serdang: UPM

Alexander, J.C., Kuo, C.Y. and Gregory, K.F. (1979). Biological evaluation of two thermotolerant filamentous fungi as dietary protein sources for rats. *Nutr. Rep. Int. 20:* 343–351

Aning, K.G., Ologun, A.G., Onifade, A., Alokan, J.A., Adekola, A.I. and Aletor, V.A. (1998). Effects of replacing dried brewer's grains with sorghum rootlets on growth, nutrient utilization and some blood constituents in the rat. *Animal Feed Science Technology 71:* 185–190

AOAC (2000). *Official methods of analysis.* Washington, DC: Association of Official Analytical Chemists

Chavez, E.R., Touchburn, S.P. and Moo-Young, M. (1988). Microbial biomass protein from the fungus *Chaetomium cellulolyticum.* I. Composition and nutritive evaluation. *Anim. Feed Sci. Technol. 22:* 1–11

Chin, F.Y. (2002). Utilization of palm kernel cake (PKC) as feed in Malaysia. *Asian Livestock 26(4):* 19–23

Davies, J.R., Brown, J.C. and Livesey, G. (1991). Energy values and energy balance in rats fed on supplements of guar gum or cellulose. *British Journal of Nutrition 65:* 415–433

Daud, M.J, Noraini, S. and Marini, A.M. (2003). Biotechnological improvement of palm kernel cake (PKC). *Proc. of Int. conf. on animal nutrition (ICAN 2003).* 3–5 Mar. 2003, Putrajaya, p. 1–10. Serdang: MARDI

Devrajan, A., Vinod, K.J., Kuldeep, G., Chander, S. and Brij, B.L. (2004). Evaluation of apple pomace based reconstituted feed in rats after solid state fermentation and ethanol recovery. *Brazilian Archives of Biology and Technology 47(1):* 93–106

Dhankher, N. and Chauhan, B.M. (1987). Effect of temperature and fermentation time on phytic acid and polyphenol content of rabidia fermented pearl millet food. *Journal of Food Science 52:* 828–829

Eggum, B.O. (1970). The protein quality of cassava leaves. *Brit. J. Nutr. 24(3):* 761–768

Hugli, T.E. and Moore, S. (1972). On alkaline hydrolysis of tryptophan. *J. Bio. Chem. 247(9):* 282–288

Iluyemi, F.B., Hanafi, M.M., Radziah, O. and Kamarudin, M.S. (2006). Fungal solid state culture of palm kernel cake. *Bioresource Technology 97:* 477–482

Jood, S. and Singh, M. (2001). Amino acid composition and biological evaluation of the protein quality of high lysine barley genotypes. *Plant Foods for Human Nutrition 56:* 145–155

Joshi, V.K., Gupta, K., Devrajan, A., Lal, B.B. and Arya, S.P. (2000). Production and evaluation of fermented apple pomace feed in broilers. *J. Food. Sci. Technol. 37(6):* 609–612

Kazanas, N., Ely, R.W., Fields, M. L. and Erdman, J.W. (1984). Toxic effects of fermented and unfermented sorghum meal diets naturally contaminated with mycotoxins. *Applied and Environmental Microbiology 47(5):* 1118–1125

Kompiang, P. (1993). Prospect of biotechnology on improvement of nutritional quality of feedstuffs. *IARD Journal 15(4):* 86–90

Mahmoud, M.I. (1994). Physicochemical and functional properties of protein hydrolysates in nutritional products. *Food Technology 48(10):* 89–95

Marini, A.M., Yatim, A.M., Babji, A.S., Annuar, B.O. and Noraini, S. (2006). Evaluation of nutrient contents and amino acid profiling of various types of palm kernel cake (PKC). *Journal of Science and Technology in the Tropics 2:* 135–141

Mason, V.C., Bech-Andersen, S. and Rudemo, M. (1980). Hydrolysate preparation for amino acid determination in feed constituents. *Proc. 3rd. EAAP Symp. protein metabolism nutrition 1,* p. 351–355

Mathot, P., Debevere, C., Walhain, P., Baudart, E., Thewis, A. and Brakel, J. (1992). Composition and nutritive value for rats of *Aspergillus niger* solid fermented barley. *Animal Feed Science Technology 39:* 227–237

Oboh, G., Akindahunsi, A.A. and Oshodi, A.A. (2002). Nutrient and anti-nutrient contents of *Aspergillus niger*-fermented cassava products (flour and gari). *Journal of Food Composition and Analysis 15:* 617–622

Onwudike, O.C. (1996). Oil palm (*Elaeis guineensis* Jacq.). In: *Legumes and oilseeds in nutrition* (Nwokolo, E. and Smartt, J., eds), p. 318–333. England: Chapman and Hall Publishers

Raupp, D.S., Rosa, D.A., Marques, S.H.P. and Banzatto, A.B. (2004). Digestive and functional properties of a partially hydrolyzed cassava solid waste with high insoluble fiber concentration. *Sci. Agric. 61(3):* 286–291

Singh, B. and Narang, M.P. (1992). Studies on the rumen degradation kinetics and utilization of apple pomace. *Bioresource Technol. 39(3):* 233–240

Smith, R.H., Plamer, R. and Reade, A.E. (1975). A chemical and biological assessment of *Aspergillus oryzae* and other filamentous fungi as protein sources for simple stomached animals. *J. Sci. Food Agric.:* 785–795

SAS Inst. (1996). *SAS/STAT User's Guide*: 6th edn. Cary, North Carolina: SAS Institute Inc.

Taylor, J. and Taylor, J.R.N. (2002). Alleviation of the adverse effect of cooking on sorghum protein digestibility through fermentation in traditional African porridges. *International Journal of Food Science and Technology 37:* 129–137

Van Soest, P.J. (1963). Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fibre and lignin. *Assoc. Off. Agric. Chem. J. 46:* 829–835

Wang, H.L., Ruttle, D.I. and Hesseltine, C.W. (1968). Protein quality of wheat and soybeans after *Rhizopus oligosporus* fermentation. *J. Nutr. 96:* 109–114

Weiss, H.E.C. and Scott, M.L. (1979). Effects of dietary fibre, fat and total energy upon plasma cholesterol and some other parameters in chicken. *J. Nutr. 109:* 693–701

Abstrak

Peningkatan mutu pemakanan, terutamanya kualiti protein hampas isirung kelapa sawit terfermentasi (HIKSt) dengan *Aspergillus niger* telah dinilai. Hasil analisis kimia dan proksimat menunjukkan terdapat peningkatan dalam kualiti pemakanan HIKSt berbanding dengan HIKS yang tidak dirawat. Peningkatan nilai protein kasar HIKSt adalah ketara (24.7%) berbanding dengan nilai protein kasar HIKS yang tidak difermentasi (17.5%). Jumlah asid amino perlu meningkat dengan signifikan (6.3%) dalam HIKSt berbanding dalam HIKS yang tidak difermentasi. HIKSt mengandungi 15.7% jumlah asid amino yang hanya merangkumi 63.4% sahaja daripada nilai protein kasar. Kajian bioasai tikus terhadap kualiti protein telah dijalankan selama 28 hari untuk menentukan nilai nisbah kecekapan protein (PER), nilai kebolehcernaan ketara protein secara in vivo dan kadar penukaran makanan ternakan (FCR) bagi HIKSt. Apabila HIKSt digunakan sebagai sumber protein tunggal dalam kajian pemakanan, diet berkenaan tidak dapat membantu dalam pembesaran tikus. Tikus yang diberi makan dengan HIKSt menunjukkan purata penurunan berat badan sebanyak 24.4 g. Nilai PER dan FCR bagi HIKSt juga menunjukkan nilai negatif (–1.5 dan –6.5), sementara nilai kebolehcernaan ketara bagi HIKSt ialah 22.6%. Hasil kajian dengan bioasai tikus menunjukkan HIKSt tidak boleh digunakan sebagai sumber protein tunggal dalam diet haiwan ternakan.

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