Palm Kernel Cake Fermented with *Candida utilis* for Mannose-Enriched Local Feed Supply

Sundari[1] dan Sonita Rosningsih[2]

Abstract— Nutritional value evaluation on palm kernel cake (PKC) was conducted using *Candida utilis.* Experiment was assigned to Completely Randomized Design with two treatments, with fermentation and non-fermentation. Fermentation was carried on at 36°C for two days. Result showed that fermentation increased crude protein level of palm kernel cake from 22.18% to 26.07%, while NFE level diminished from 15.82% to 6.36%. Crude fiber increased not significantly in PKC and Fermented PKC namely 37.43% and 37.,84%, respectively. Crude fat decreased insignificantly, in that crude fiber of PKC and fermented PKC was 9.13% and 8.89%, respectively. Ash was 9.13% and 8.89%, respectively, and mannose increased insignificantly as much as 2.19% and 3.56%. Fiber volume fraction undergoing significant increase was hemicellulose, from 21.12% to 22.93%, while cellulose insignificantly increased from 38.9% to 41.13%, lignin insignificantly decreased from 21.12% to 19.18%. It was concluded that fermented Palm Kernel Cake product provided essential nutritional values for poultry (hemicellulose, mannane and mannose) that potentially improved poultry health.

Index Terms—*Candida utilis,* Mannose, Palm Kernel Cake.

1 Introduction

Oil palm is a promising prospect in Indonesia. Expansions on oil palm plantation are under constant improvement, particularly those recently developed in Kalimantan and Irian. This area expansion supports the prospective Palm Kernel Cake (PKC) despite the intake constraints namely high fiber (43%), low palatability, low protein (4%)/essential amino acid, and anti-nutrient such as mannan, galactomannan, xylan, and Arabinoxylan. If Indonesia produced 16.9 million tons of CPO [1], the potential byproducts were 2 million tons of palm kernel cake, 2 tons dry palm oil sludge and 4 tons dry solid heavy phase [2]. Low palatability of palm kernel cake on non ruminants made it necessary to supply other palatable feed. Nutritional content of PKC is varied, depending on the assigned oil extraction, storage and shredded palm kernel shell [3]; [4]. Crude fiber of PKC was 21,97% and the crude protein was 13,53%[5]. PKC contained 14,49% [6] crude fiber, while) reported 24% [7]. One alternative to improve feed quality was solid substrate fermentation using mold that enabled degradability of indigestible material to be more available and eventually increased nutritional value. The quality of fermented product depended on the type of microbes and solid media used. Most microbes including bacteria, fungi and yeast could produce various enzyme. Products of yeast metabolism were ethanol, citric acid, acetone, butanol, glutamate acid, lysine, nucleotides, polysaccharide and vitamins [8].

Protein component of yeast's cell wall partly consisted of enzymes like invertase, melibiose, phosphatase, glucanase, ariel-beta glucosidase, phospholipase and protease [9]. PKC fermentation using *Candida utilis* could improve nutritional value by increasing crude protein and nitrogen free extract, and decreasing fiber [5]. This fermentation caused crude fat decrease, lowered gross energy on PKC (4733,5) and FPKC (4245,5 kcal/kg) also metabolic energy of PKC (2672,54) and FPKC (1807,76 kcal/kg). Utilizing *Aspergillus niger*-fermented PKC at 15% level, 6% hydrolyzed chicken feather meal and supplementing 120 ppm Zn in ration could lower ration consumption and body weight gain, improve feed conversion ratio, increased carcass weight percentage and nutrition absorption, and lessen intestines length [10]. Palm kernel cake supplemented with cellulase enzyme could be given 15% in broiler ration [11]. Fermentation of palm oil sludge was the most effective using *Aspergillus niger* at 38^{o}C for 3 days, following 2-day enzymatic process [12]; [13]. PKC cell wall components consisted of 56.4% mannose, 11.6% cellulose, 3.7% xylose and 91.4% galactose [14]. Mannose sugar in PKC cell wall reached 45-50% [15]. It was explainable that almost 40% component in palm kernel cake was beta-mannane. Although enzymatically beta-mannane was indigestible by poultry because of the absence of mannanase enzyme, physical digestion occurred through beta-mannane degradation into minor form namely *mannan oligosaccharide* (MOS) or even manose. These substances were in charge of improving poultry body immune. As prebiotic, MOS can bind with *Salmonella sp* bacteria to reduce the population of pathogenic bacteria and increase commensal bacteria like *Lactobacillus sp*. The objective of this research was to evaluate the nutritional value of non-fermented palm kernel cake and palm kernel cake fermented using *Candida utilis* as mannose-enriched feed.

- [1]*Faculty of Agroindustry, Mercu Buana Yogyakarta University. Sedayu-Bantul 55753, Indonesia. Corresponding author: sundari_umby@yahoo.com*
- [2]*Faculty of Agroindustry, Mercu Buana Yogyakarta University. Sedayu-Bantul 55753, Indonesia. sonitarosningsih@yahoo.com*

2 Materials and Method

Research was conducted from May to June 2014 in Chemical Laboratory and Microbiology Laboratory Mercu Buana University Yogyakarta. Dry palm kernel cake (PKC) was obtained from by-product of oil palm processing in Bangka Belitung. Mold used was *Candida utilis* FNCC from PAU UGM. Research apparatus included laminar, autoclave, *Memmert*

oven , desiccator, Ohause scale, vochdoss, klem, Kjedahl bulb, a set of soxhlet extractor, silica disc, burette, Erlenmeyer glass, separating funnel, *Nasional* blender, *Retsch Mixer* vortex, magnetic stirrer, *Katterman* shaker, furnace and Cabinet dryer, *Rinnai* gas stove and gas cylinder, plastic tray, plastic bag and stirrer.

Research steps: a) making medium culture consisted of 0,3 g *bacto beef* agar; 1,5 g *bacto agar*; 0,5 g NaCl; 2,1 g glukosa; 100 ml distilled water, in which sterilization was done using autoclave at 121°C for 15 minutes to separate glucose from other components. b) Making yeast multiplication medium: All chemicals namely 1,3 g $KH_2PO_4.12H_2O$; 1,3 g $MgSO_4.7H_2O$ 1,0$KH_2PO_4.12H_2O$; 1,0 g $MgSO_4.7H_2O$; 0,01 g $FeSO_4.7H_2O$; 0,01 g $CaCl_2.2H_2O$; 0,01 g MnSO4.$4H_2O$ and 5 g NH_4NO_3; 0,01 g $FeSO_4.7H_2O$; 0,01 g $CaCl_2.2H_2O$; 0,01 g MnSO4.$4H_2O$ and 5 g NH_4NO_3 were mixed with culture medium, added with aquadest to reach 1 liter volume, then added with 50g drops. Medium acidity was made pH=4. From 1000ml compound, 250ml was taken, added two tubes to lean it, then put to aerobe shaker for 24-48 hours. From 250ml compound, 10% (75ml) was taken then put into 750 liquid culture medium, and left for 24-48 hours. c) Palm kernel cake fermentation: sterilized palm kernel cake was divided into six parts, three for non-fermented PKC (control) and each from the other three was added with medium culture to obtain 60% moistness (the added liquid was figured out after obtaining PKC water content).

Mixing steps was conducted using laminar. PKC for fermentation was placed on plastic tray, covered with aluminum foil and aerated (by punching holes), then incubated in fermenter at 36 - 37 ^{O}C for 48 hours.

The measured variables were nutrient composition (crude protein, crude fiber, crude fat, ash, NFE, mannose) and fiber fraction (cellulose, hemicellulose and lignin). Proximate analysis with AOAC method was conducted to obtain nutrient content data, while fiber fraction analysis was subject to Chesoon method[16]. Mannose analysis was conducted in Biochemicals and Nutrition Laboratory PAU IPB. The obtained data were subject to t test [17].

3 Result And Discussion

3.1. Nutrient Composition

Chemical analysis result of palm kernel cake and the fermentation product showed that fermentation could increase nutritional value of palm kernel cake (Table 1). Crude protein value of fermented palm kernel cake was higher than that of non-fermented. Despite the high crude fiber, hemicellulose had increased, showing that half of crude fiber was hydrolysable into simpler compound named mannose.

Average dry matter of PKC was higher than that of FPKC, demonstrating that during fermentation, organic compound was degraded into simpler compound in which water was released. Microbial activity used carbohydrate as carbon source. Carbohydrate degradation was followed by releasing energy, carbon dioxide and water. The released heat caused increasing substrate temperature. All organisms need energy source for living, obtained from food metabolism within the organism ecosystem [18]. Accordingly, the energy source here was carbohydrate contained in palm kernel cake and nitrogen source was the supplemented urea.

TABLE 1
NUTRIENT COMPOSITION OF PALM KERNEL CAKE (PKC) AND FERMENTED PALM KERNEL CAKE (FPKC) (% DRY MATTER)

Parameter	Treatments		T-test
	PKC	FPKC	
Dry Matter	89.43	83.90	*
Crude Protein	22.18	26.07	*
Crude Fiber	37.43	37.84	ns
Crude Fat	9.13	8.89	ns
Ash	4.74	4.94	ns
NFE	15.82	6.36	*
Mannose	2.19	3.56	ns

Note: *Value bearing different superscript on the same line showed significant difference (P<0,05).
ns = not significan (P>0.05)

Fermentation on PKC triggered change in feed nutrition content. Crude protein FPKC (26,07%) seemed higher than that of PKC (22,18%). The increasing protein content in PKC was assumed to result from supplementation of inorganic N source (urea) and mineral into substrate and microbial activity that caused proper substrate degradation. During fermentation, protein hydrolysis occurred (although in minuscule around 4%) whose product was accumulated in peptide form which was eventually hydrolyzed into amino acids. Furthermore, protein was added up inside the microbial cell. Products during growth process, besides enzyme, were extracellular enzyme protein and protein from microbial metabolism that induced the increasing crude protein [19].

Crude fiber in fermentation product also increased, as assumed to result from microbial growth that required some food substance, among which was crude fiber as substrate. In line with Satiawiharja [20] on fermentation product, medium functioned as the source of carbon, nitrogen and energy. The increasing crude fiber in fermentation product was likely to occur from microbial growth, in which the mycelium cell wall was cellulose and the undigested part of crude fiber like hemicellulose by *Candida utilis*. Fermentation process caused degradation in certain enzymes against the indigestible materials, cellulose and hemicellulose for instance, into simple glucose. In this research, the process did not occur, and might require longer period [8].

PKC crude fat lowered from 9,13% into 8,92%, but statistical measurement showed that the decrease was not significant (P<0,05). During fermentation, lipolysis occurred due to the fat consumed by the leaven for its growth some catalysis reactions induced by lipase enzyme was hydrolysis, ester-synthesis and alcoholysis [21]. By lipase enzyme activity, fat content in fermentation product decreased. This occurrence was absence in this research, as assumed due to lack of incubation period. It was different from observation by [5] that fat content decreased in palm kernel cake substrate fermented using *Candida utilis*. The decreasing substrate with high fat content such as palm kernel cake showed that *Candida utilis*

could produce lipase enzyme. The difference was also likely to occur due to material type and condition, and PKC extraction process.

The increasing ash content in FPKC was mostly due to mineral supplementation on substrate medium. Fermentation process on required a minuscule mineral to support yeast enzyme activity. Ash was composed of Ca, Mg, P and micro substance. Living organism needs very little mineral for metabolism [22] and not all of them were made into new compounds, even mostly served as co-factor in enzyme activity which woud return as original mineral after enzyme reaction. Accordingly, mineral content before and after fermentation would be detected in form of ash with the same amount.

TABLE 2
LEVEL OF CELLULOSE, HEMICELLULOSA AND LIGNIN IN PKC AND FPKC (% DRY MATTER)

Parameter	Treatments		T-test
	PKC	FPKC	
Cellulose	38.91	41.13	ns
Hemicellulose	21.12	22.93	*
Lignin	21.12	19.18	ns

*Note: *Value bearing different superscript on the same line showed significant difference (P<0,05).*
ns = not significan (P>0.05).

N-free Extract (NFE) in fermented palm kernel cake was significantly decreasing. NFE was carbohydrate building blocks. [23] stated that plant carbohydrate consisted of NFE and crude fiber. The decreasing NFE showed the assigned carbohydrate as carbon compound in cell building synthesis. From the carbohydrate composition of palm kernel cake, enzymes in fermentation product were mannanase, alpha-galactosidase and cellulase. Those enzymes hydrolyzed mannane, galactomannane and cellulose to produce simple but more carbohydrate. Carbohydrate was degraded by microbe into energy and CO_2 for the cell life to improve *Candida utilis* and eventually produced higher cell protein.

Mannose value in FPKC product increased insignificantly)>0,05) although hemicellulose value significantly increased (P<0,05) (Tabel 2). It might due to the lack incubation period. Mannose was one of mannane hydrolysis products. Mannane physical form was molecular ribbons but more flexible and less strong compared to cellulose, straight and expandable [24]. Mannane from oil palm generally posses strong hardness, high crystalline and is water-insoluble. Mannanase enzyme excreted by *Candida utilis* hydrolyzed mannane into mannose. Mannane was composed of main component of D-glucose and D-mannose. D-glucose was synthesized from glucose-1-phosphate catalyzed by *GDP-G-pyrophosphorilase* into GDP-glucose by releasing pyrophosphate and guanosine 5'- triphosphat. From GDP-D-glucose by *GDP mannose 2-epimerase* enzyme would be catalyzed into GDP-D-mannose or vice versa. If both components was catalyzed by transferase inside golgi, glucomannane would form. Around 3-5% glucomannane as matrix material of cell wall in form of hemicellulose fraction was 3-12% [24].

3.2. Fiber Volume Fraction

Cellulose, hemicellulose and lignin content did not decrease (Table 2) because of the degradation of cellulose with limited hydrogen bridge and unsystematic space between microfibrils; furthermore, crystalline cellulose was hydrolyzed and it degraded covalent bond of crystalline cellulose. Glucose was then metabolized by microbe to induce cell growth and secondary product synthesis. Accordingly, cellulose content in this research was the remaining cellulose in substrate and the one formed by the microbe as one of cell components. therefore, although substrate had been fermented, statistical value of cellulose did not show any changes.

Lignin statistical value in fermented PKC was not significantly different. Lignin was a phenylpropane unit and 5-15% methoxy cluster [25]. Lignin is resistant to chemical degradation including enzymatic. Lignin contained 61-65% C, 5-6% H and 30% O. Lignin in the wood was 17-32% of the dry matter. Coumaryl alcohol and synapil alcoholwere served as the precursor. Lignin had a strong bond with polysaccharide and cell wall protein of plant, therefore the compound was indigestible during digestion.

FPKC hemicellulose (Tabel 2) was significantly higher (P<0.05) than that of PKC because of the loosening lignocellulose bound resulted from lignocellulose activity, that helped cellulase and hemicellulose enzymes to penetrate the substrate. Hemicellulose molecule had shorter chain than cellulose and was soluble in hot acidic solution [26]. This compound bound with cellulose and lignin through Hydrogen Bridge. Hemicellulose is hydrolysable non-crystal like. Hemicellulose hydrolysis produced pentose and hexose [5].

4 CONCLUSION

Two-day fermentation of palm kernel cake using *Candida utilis* provided the essential nutrition for poultry by increasing crude protein, hemicellulose, and mannose.

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