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Determination of True Amino Acid Digestibility and Metabolizable Energy in Fermented Palm Kernel Meal with *Aspergillus wentii* TISTR 3075 for Chickens

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ABTRACT

The determination of energy and digestibility values of new added-value products is important for feed formulation. True amino acid digestibility and metabolizable energy of feedstuffs were evaluated in adult meat type crossbred chickens. Sulfur amino acids (cysteine and methionine) and lysine were limiting amino acids in palm kernel meal (solvent extract; PKM) and fermented palm kernel meal (FPKM) with *Aspergillus wentii* TISTR 3075. The FPKM with *Aspergillus wentii* TISTR 3075 lead to an increase in true amino acids digestibility except for arginine. The apparent metabolizable energy (AME; DM basis) of corn, PKM and FPKM were 3,628.88, 2,201.83 and 2,080.26 kcal/kg, respectively. The true metabolizable energy (TME) of PKM (DM basis: 2,958.21 kcal/kg) was slightly higher than FPKM (DM basis: 2,843.01 kcal/kg) while the nitrogen-corrected apparent metabolizable energy (AME_n), nitrogen-corrected true metabolizable energy (TME_n) of PKM (DM basis: 2,411.84 and 2,603.33 kcal/kg, respectively) were significantly higher than FPKM (DM basis: 2,153.11 and 2,333.07 kcal/kg, $P = 0.03$ and $P = 0.02$ respectively). *Aspergillus wentii* may use up nitrogen-free extract (NFE) in PKM as an energy source during the fermenting process, resulting in a lower metabolizable energy of FPKM when compared to PKM (without fermentation).

Keywords: Chicken, fermented palm kernel meal, amino acid availability, metabolizable energy

INTRODUCTION

The possible anti-nutritional factors (mannan and galactomannan) discourage the poultry and swine production industry generally from using palm kernel meal (PKM). Moreover, PKM contains a number of non-starch polysaccharides (NSPs) which are mostly indigestible. Of the total NSPs in PKM 78 % is mannan, 3 % is arabinoxylans, 3 % is glucoroxylans which have been found to be water-insoluble and 12 % is cellulose [1]. These problems could be solved by using exo-mannanase or enzyme producing components {endo-β-mannanase - EC 3.2.1.78 and exo-mannanase (β-manosidase, EC 3.2.1.25)}, particularly mannanase, galactosidase and cellulase. Some potential indigenous enzyme producers include *Aspergillus niger* (lipase, protease, cellulase and xylanase) and *Aspergillus* sp. (mannanase, phytase, cellulase and xylanase) [2]. The degraded carbohydrates thus formed have been found to be readily absorbed and could supply nutrients needed by chicken and swine. The mannan-oligosaccharides produced could function as prebiotics to improve the animal's health [3-5].

The energy necessary for maintaining a bird's general metabolism and for producing meat and eggs is provided by the energy-yielding dietary compounds, primarily carbohydrates and fats, as well as protein. A nitrogen-corrected metabolizable energy (ME_n) and nitrogen-corrected true metabolizable energy (TME_n) values differ substantially for some ingredients, such as rice bran and wheat middlings. Therefore, ME_n values should not be indiscriminately interchanged with TME_n values for purposes of diet formulation [6]. Fermented palm kernel meal (FPKM) with *Aspergillus* spp. might improve the utilization of oligosaccharides, a group of carbohydrates, which are poorly digested and also amino acids by poultry.

The objective of this study was to evaluate the amino acid availability, AME, AME_n, TME and TME_n of PKM (solvent extracted) and FPKM (with *A. wentii* TISTR 3075) used to formulate diets for chickens.

MATERIALS AND METHODS

Aspergillus wentii TISTR 3075 was cultivated in solid-state fermentation with the medium (palm kernel meal - solvent extracts purchased from Chumporn palm oil industry public company limited). After incubation for 48 h at 30 °C (for producing mannanase, cellulase and xylanase), the fermented material (FPKM *A. wentii* TISTR 3075) was harvested and oven dried at 55 °C.

The feedstuffs were tested: yellow corn (as standard), palm kernel meal (solvent extract; PKM) and fermented palm kernel meal with *A. wentii* TISTR 3075 (FPKM). Thirty two chickens (10-week-old males meat type) were taken from the floor pens for each trial. Eight birds for each test feedstuffs were randomly sampled and stocked in individual cages. Eight birds were used for estimating endogenous losses of energy, nitrogen, and amino acids. All the birds were deprived of feed for 24 h to ensure that their alimentary canals were empty, i.e. without feed residues [7,8]. This was followed by another 48 h period of feed deprivation, during which the total excreta voided from each bird was collected and frozen. The amounts of each tested feedstuff were 30 g. A stainless steel funnel with 35 cm stem (external diameter: 1.3 cm, internal diameter: 1.1 cm) was used in the precision-feeding technique. Excreta were collected at 48 h after precision-feeding. The total excreta were kept in plastic trays. The ground sample and excreta samples were assayed for gross energy (GE) in an Isoperibol bomb calorimeter (Leco AC 500). Feedstuffs were then analyzed for dry matter (DM) by oven-drying, nitrogen (N) by Kjeldahl procedures, crude fiber, ether extract and ash by proximate analysis [9]. Calcium and total phosphorus were determined according to the method described by AOAC [9]. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined according to the method described by AOAC and NFTA [9-11]. The concentration of hemicellulose was calculated as the difference between NDF and ADF. Amino acid content was determined using an Amino Acid Analyzer.

True amino acids were calculated with the following formula [8,12]:

$$\text{Apparent amino acid digestibility (AAAD)} = \frac{(\text{amino acid intake} - \text{amino acid output})}{\text{amino acid intake}} \tag{1}$$

$$\text{True amino acid digestibility (TAAD)} = \text{AAAD} + \frac{(\text{endogenous amino acid output})}{\text{amino acid intake}} \tag{2}$$

Apparent metabolizable energy (AME), nitrogen-corrected apparent metabolizable energy (AME_n), true metabolizable energy (TME) and nitrogen-corrected true metabolizable energy (TME_n) were calculated with the following formulae [13]:

$$\text{AME} = \frac{(\text{EI} - \text{EO})}{\text{FI}} \tag{3}$$

$$\text{AME}_n = \text{AME} - \left(\frac{8.22 \times \text{ANR}}{\text{FI}}\right) \tag{4}$$

$$\text{TME} = \text{AME} + \left(\frac{\text{FEL}}{\text{FI}}\right) \tag{5}$$

$$\text{TME}_n = \text{TME} - \left(\frac{8.22 \times \text{ANR}}{\text{FI}}\right) - \left(\frac{8.22 \times \text{FNL}}{\text{FI}}\right) \tag{6}$$

where EI is the gross energy intake.
EO is the gross energy output.
FI is the feed intake (g).
FEL is the fasting energy loss.
FNL is the fasting nitrogen loss.
ANR is the apparent nitrogen retained.
8.22 is the corrected factor to zero N balance [14].

Statistical analyses of the data as completely randomized design were performed (One-way ANOVA followed by DMRT). F values were given with levels of significance.

RESULTS

Chemical Compositions

Chemical compositions based on DM basis of PKM and FPKM are summarized in **Table 1**.

Table 1 Analysis of the tested ingredients (% as DM basis).

Chemical compositions	Palm Kernel Meal	FPKM *A. wentii* TISTR 3075
Moisture	9.52	5.72
Crude Protein	16.99	21.36
Ether Extract	0.24	0.71
Crude Fiber	13.71	13.16
Ash	9.07	13.32
Nitrogen Free Extract	59.99	51.45
ADF	47.14	45.47
NDF	77.56	73.32
Hemicellulose	30.42	25.46
Calcium	0.30	0.33
Total Phosphorus	0.62	0.75
Gross Energy (kcal/kg)	4,106.77	3,924.81
Essential amino acids		
Methionine	0.34	0.39
Threonine	0.49	0.68
Valine	0.69	0.89
Isoleucine	0.46	0.63
Leucine	0.99	1.30
Phenylalanine	0.64	0.71
Histidine	0.62	0.65
Lysine	0.39	0.59
Arginine	1.92	1.80
Non-essential amino acids		
Cysteine	0.22	0.26
Aspartic acid	1.23	1.61
Serine	0.68	0.82
Glycine	0.70	0.87
Glutamic acid	3.10	3.12
Alanine	0.64	0.89
Tyrosine	0.37	0.48
Proline	0.52	0.71

Dry matter, crude protein, ether extract, ash, calcium and phosphorus in FPKM tended to be higher than those in PKM. In contrast, nitrogen free extract and gross energy tended to be lower. Ash content in FPKM was 46 % higher than PKM, However, calcium and phosphorus in FPKM were higher than in PKM, which were 10 and 20 %, respectively.

Amino Acid Availability

The amino acid availability of PKM and FPKM *A. wentii* TISTR 3075 are presented in **Table 2**.

Table 2 True amino acid digestibility coefficient and amino acid availability (% as DM basis) and comparison with broiler requirement.

Amino acid	True amino acid digestibility coefficient		True Available Amino acid		Broiler requirement	
	Palm Kernel Meal	FPKM *A. wentii* TISTR 3075	Palm Kernel Meal	FPKM *A. wentii* TISTR 3075	[6] [2]	[15] [3]
Crude protein			-	-	23.00	22.00
Cysteine	0.57	0.61	0.1253	0.1456	(Cys+Met) 0.90	0.86
Methionine[1]	0.99	1.00	0.3400	0.3700	0.50	0.46
Aspartic acid	0.98	0.94	1.1945	1.4270	-	-
Threonine[1]	0.93	0.94	0.4601	0.6014	0.80	0.74
Serine	0.96	0.95	0.6560	0.7298	(Ser+Gly) 1.25	-
Glutamic acid	0.96	0.89	2.9614	2.6251	-	-
Glycine	0.98	0.95	0.6893	0.7806	(Ser+Gly) 1.25	-
Alanine	0.97	0.93	0.6244	0.7872	-	-
Valine[1]	1.00	0.95	0.6997	0.8023	0.90	0.75
Isoleucine[1]	0.95	0.88	0.4401	0.5183	0.80	0.68
Leucine[1]	0.93	0.89	0.9205	1.1045	1.20	1.25
Tyrosine	0.83	0.81	0.3100	0.3660	(Phe+Tyr)	-
Phenylalanine[1]	0.91	0.86	0.5858	0.5785	1.34	-
Histidine[1]	0.90	0.91	0.5590	0.5518	0.35	-
Lysine[1]	0.89	0.77	0.3458	0.4329	1.10	1.22
Arginine[1]	0.95	0.91	1.8111	1.5535	1.26	1.35
Proline	0.97	0.91	0.5052	0.6125	0.60	-

[1]Essential amino acids

[2]0 - 21 days, total amino acids (as fed basis 90 %DM)

[3]0 - 16 days, digestible amino acids (as fed basis)

Due to the insufficiency of several limiting amino acids (**Table 2**) in PKM and FPKM *A. wentii* TISTR 3075, the use of sulfur amino acids (cysteine and methionine) and lysine can improve the quality of their protein. High arginine content in PKM and FPKM *A. wentii* TISTR 3075 were found. There were also low true cysteine and lysine digestibility. The true digestibility coefficient of FPKM *A. wentii* TISTR 3075 was relatively lower than PKM, especially in the case of lysine.

Metabolizable Energy

The metabolizable energy values of 3 feedstuffs are summarized in **Table 3**.

Table 3 Metabolizable energy determination (DM basis).

Item	Feedstuffs			
	Yellow Corn	Palm Kernel Meal	FPKM *A. wentii* TISTR 3075	SEM
AME, kcal/kg	3,628.88[a]	2,201.83[b]	2,080.26[b]	166.2333
AME_n, kcal/kg	-	2,411.84[a]	2,153.11[b]	143.4715
TME, kcal/kg	-	2,958.21[a]	2,843.01[a]	140.6480
TME_n, kcal/kg	-	2,603.33[a]	2,333.07[b]	136.7496
N	8	8	8	

[a,b] Means in the same rows with different superscripts are significantly different ($p < 0.05$).

The mean fasting energy was estimated to be 2,559.7 kcal/kg and the mean fasting nitrogen loss was estimated to be 1.85 g. The mean AME levels in yellow corn were significantly higher than in PKM and FPKM (P = 0.0001). The mean AME and TME levels in PKM tended to be higher than those in FPKM *A. wentii* TISTR 3075, but a significant difference was not found (P = 0.32 and P = 0.269, respectively). However, the AME_n and TME_n values for PKM were significantly higher than FPKM (P = 0.03, and P = 0.02, respectively).

DISCUSSION

The chemical compositions of PKM (solvent extract) were comparable with the values reported by Chaiysongkham [16]. However, crude protein, ether extract and crude fiber were lower than the values that were recommended by Feedstuffs [15]. The nutritional components of FPKM *A. wentii* TISTR 3075 were comparable with those found by Dairo and Fasuyi [17] and Iluyemi and co-workers [18]. It was observed that treatment with a microbial inoculant might have more potential than extracts of enzymes in decreasing NDF and hemicellulose due to increased microbial respiration [19]. The increase in the ash content evidently supported the microbial enzymes, by making the minerals available for metabolic functions [17]. The main component of either mannan or galactomannan of PKM was insoluble. PKM galactomannan is composed of mannose and low galactose in which the ratio between mannose and galactose in PKM was quite high (16.3) [20]. The ratio of mannose to galactose could affect the solubility of the feedstuffs, in which pure mannan was totally insoluble, and the more galactose the more soluble it becomes. A galactose chain possibly prevents insolubility by extending the mannan macromolecule and allowing water into the space. This is dependent on the viscosity of gut contents, the speed of enzymes to reach their substrate and the rate of nutrients to reach the gut wall. Thus decreasing the rate of this process may result in decrease in the absorption of nutrients.

The amino acid profile of PKM was similar to that of previous reports [17,18] but lysine was found to be lower. FPKM *A. wentii* TISTR 3075 showed an improved quantity of amino acids (except arginine and glutamic acid). In addition, a greater than 50 % increase in the individual amino acid content of cultured barley using *A. niger* except for the sulfur-containing acids, which showed a comparatively low increase [21]. Several limiting amino acids, the use of sulfur amino acids (cysteine and methionine) by using synthetic DL-methionine and L-lysine could improved the quality of the protein. An excess of available arginine in PKM and FPKM *A. wentii* TISTR 3075 were over the broiler requirements. The high arginine content of both feedstuffs suggests that the addition of L-lysine (synthetic amino acid) becomes necessary for not only meeting the lysine requirements of the bird but also to maintain the correct ratio between arginine and lysine. An excess arginine could increase the chick's methionine requirement, thereby causing growth and feed intake depression [21]. Failure to correct the ratio could impair the performance of poultry because lysine and arginine have an antagonistic effect, an excess of one causing a deficiency in the other [23]. The optimum Arg:Lys ratio should fall between 0.8 and 1.7 depending upon dietary levels of electrolytes such as sodium, potassium, and chloride [24]. At low concentrations (15 g/kg), the arabinoxylans caused an increase in endogenous amino acid losses whereas at higher concentration (35 g/kg) a direct inhibition of protein breakdown and amino acid absorption occurred [25]. The indigestible oligosaccharides had no effect on the digestibility of most amino acids. Feeding a higher level of oligosaccharides (8 g/kg), however, may depress amino acids digestibility leading to decreased availability [26].

Metabolizable energy of PKM in this study is slightly better than reported by Feedstuffs [15], (AME: 1,772.62 kcal/kg, Gross energy: 3,815.60 kcal/kg). The AME, AME_n, TME and TME_n values in this study were also higher than the values reported [27], suggesting differences in processing and nutrient compositions. The values of GE, AME, AME_n, TME and TME_n of FPKM *A. wentii* TISTR 3075 were lower than PKM. The might possibly be due to the *Aspergillus wentii* which may use up the energy source, especially nitrogen free extract (NFE) of the substrate (PKM) during fermentation, as NFE in FPKM *A. wentii* TISTR 3075 was lower when compared to PKM. They oligosaccharides (4 to 8 g/kg of inulin, oligofructose, mannanoligosaccharide-MOS) generally had no effect on TME_n [4]. The high SEM observed in this experiment might be supported by D'Alfono and co-workers [28] who reported many representative samples of the feeds to determine gross energy content and more homogeneous of feed, even if 10 replicates were conducted, over 48 % of the variance on the TME_n estimate was attributable to uncertainty of feed energy content. This variance can be reduced dramatically by increasing the number of feed sample replicates. The nitrogen content of PKM and FPKM *A. wentii* TISTR 3075, the second largest source of variation would be expected to increase the total variance of the TME_n.

CONCLUSIONS

The results showed that fermentation of palm kernel meal with *Aspergillus wentii* TISTR 3075, DL-methionine and L-lysine could improve the quality of their protein. High arginine content in feedstuffs suggests that the addition of L-lysine (synthetic amino acid) becomes necessary for not only meeting the lysine requirements of the bird but also to maintain the correct ratio between arginine and lysine. The AME, AME_n, TME and TME_n slightly reduced in palm kernel meal fermented with *A. wentii* TISTR 3075 compared to palm kernel meal without fermentation. This indicates that fermentation by *A. wentii* TISTR 3075 reduces the energy source in palm kernel meal. Additional fat or oil in FPKM is suggested, to meet requirements in feed formulation for birds.

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บทคัดย่อ

นิวัต เมืองแก้ว และ จารุรัตน์ ชินาจริยวงศ์

การหาค่าการย่อยได้ที่แท้จริงของกรดอะมิโนและพลังงานใช้ประโยชน์ได้ของกากเนื้อเมล็ดในปาล์มน้ำมันที่ผ่านกระบวนการหมักด้วยเชื้อรา *Aspergillus wentii* TISTR 3075 ในไก่

การหาค่าพลังงานและค่าการย่อยได้ของวัตถุดิบอาหารสัตว์ชนิดใหม่มีความจำเป็นต่อการนำไปใช้ในการประกอบสูตรอาหารสัตว์ จากการศึกษาการย่อยได้ของกรดอะมิโนในกากเนื้อเมล็ดในปาล์มน้ำมันและกากเนื้อเมล็ดในปาล์มน้ำมันที่ผ่านหมักด้วยเชื้อรา *Aspergillus wentii* TISTR 3075 ในไก่เนื้อ พบว่าขาดความสมดุลของกรดอะมิโน โดยเฉพาะกรดอะมิโนซีสตีน เมทไธโอนีนและไลซีน ยังพบว่าปริมาณกรดอะมิโนอาร์จินีนมีค่าสูงกว่าปกติ เพื่อปรับสัดส่วนให้กรดอะมิโนสมดุลขึ้นมีความจำเป็นต้องเสริมกรดอะมิโนไลซีน กากเนื้อเมล็ดในปาล์มน้ำมันหมักด้วยเชื้อรามีค่าการย่อยได้ที่แท้จริงของกรดอะมิโนที่จำเป็นต้องเติมในอาหารเพิ่มขึ้นยกเว้นอาร์จินีน สำหรับการหาค่าพลังงานใช้ประโยชน์ได้ของข้าวโพด กากเนื้อเมล็ดในปาล์มน้ำมัน (ชนิดสกัดด้วยสารเคมี) และกากเนื้อเมล็ดในปาล์มน้ำมันหมักด้วยเชื้อรา พบว่าพลังงานใช้ประโยชน์ได้แบบปรากฏมีค่า 3,628.88 2,201.83 และ 2,080.26 กิโลแคลอรี่ต่อกิโลกรัมวัตถุแห้ง ตามลำดับ โดยพลังงานใช้ประโยชน์ได้ที่แท้จริงของกากเนื้อเมล็ดในปาล์มน้ำมัน (2,958.21 กิโลแคลอรี่ต่อกิโลกรัมของวัตถุแห้ง) มีค่าค่อนข้างสูงกว่าค่าพลังงานที่วิเคราะห์ได้จากกากเนื้อเมล็ดในปาล์มน้ำมันที่ผ่านการหมักด้วยเชื้อรา (2,843.01 กิโลแคลอรี่ต่อกิโลกรัมของวัตถุแห้ง) ในขณะที่ค่าพลังงานใช้ประโยชน์ได้ปรับค่าไนโตรเจน และพลังงานใช้ประโยชน์ได้ที่แท้จริงปรับค่าไนโตรเจนของกากเนื้อเมล็ดในปาล์มน้ำมัน (2,411.84 และ 2,603.33 กิโลแคลอรี่ต่อกิโลกรัมของวัตถุแห้ง ตามลำดับ) มีค่าสูงกว่าในกากเนื้อเมล็ดในปาล์มน้ำมันหมักด้วยเชื้อราอย่างมีนัยสำคัญทางสถิติ (2,153.11 และ 2,333.07 กิโลแคลอรี่ต่อกิโลกรัมของวัตถุแห้ง $P = 0.03$ และ $P = 0.02$ ตามลำดับ) ทั้งนี้เป็นเพราะเชื้อราใช้แหล่งพลังงาน (โดยเฉพาะคาร์โบไฮเดรตที่ละลายได้ง่าย) ของกากเนื้อเมล็ดในปาล์มน้ำมันไปในระหว่างการหมัก

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