Songklanakarin J. Sci. Technol.
39 (1), 101-108, Jan. - Feb. 2017

http://www.sjst.psu.ac.th

Original Article

The effect of increased levels of dried coconut meal supplemented with an enzyme cocktail® on diet utilization in growing pigs

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Received: 30 September 2015; Revised: 12 January 2016; Accepted: 26 April 2016

Abstract

The experiment was conducted to determine the effect of increased levels of dried coconut meal supplemented with an enzyme cocktail® on diet utilization in growing pigs. A 4x4 latin square design was used in this study. Four crossbred (Duroc x Landrace x Large White) barrows averaging 17.88±0.96 kg in body weight were allotted 4 diets, diet 1 (the control diet), diet 2, diet 3 and diet 4 (5, 10 and 15% dried coconut meal in each diet with an enzyme cocktail®, 1 kg of the enzyme cocktail containing the activities of phytase 1,000,000 units, amylase 5,000,000 units, xylanase 3,500,000 units, beta-glucanase 2,000,000 units, cellulase 1,500,000 units, pectinase 1,000,000 units and mannanase 800,000 units; 500 g/t of feed, at a level 0.05% in the diets, respectively). Pigs were raised in individual metabolism cages. Faeces and urine samples were collected 4 times a day for 5 days for data collection. The results showed that the nutrient digestibility percentage of dry matter, crude protein, crude fat, ash, nitrogen-free extract, blood urea nitrogen, digestible energy (kcal/kg) and metabolizable energy (kcal/kg) were not significantly different ($P>0.05$) in pigs fed with different diets. However, pigs fed with 5, 10 and 15% dried coconut meal in the diet with an enzyme cocktail® at a level 0.05% had significantly ($P<0.05$) higher digestible fiber than pigs fed with the control diet, and increased (linear and quadratic contrast, $P<0.05$). Pigs fed with the control diet (diet1) had significantly ($P<0.05$) higher digestible apparent biological value than pigs fed with 15% dried coconut meal in a diet with an enzyme cocktail® at a level 0.05% but not significantly different ($P>0.05$) from the other groups. Apparent biological values contrarily reduced (linear and quadratic, $P<0.05$) with increasing level of dried coconut meal in pig diets. In conclusion, our data indicate that pigs fed with 5% dried coconut meal in a diet with addition of an enzyme cocktail at a level of 0.05% can show obviously increasing the highest digestibility of crude fiber (79.25%) without impairing nutrient digestibility.

Keywords: dried coconut meal, enzyme cocktail®, diet utilization, pigs

1. Introduction

Coconut meal (frequently called copra meal) is produced by coconut oil extraction or the expeller method (Austin *et al.*, 2001) and is often fed to monogastrics like poultry and pigs in subtropical and tropical countries where it is readily available (Février *et al.*, 2001; Jaworski *et al.*, 2014). There are many reports about supplementing coconut meal or copra meal for animal feed because coconut meal, which is a by-product of the coconut industry, contains about 21% crude protein (Austin *et al.*, 2001) but also a low amount of some essential amino acids (Atinyao *et al.*, 2001), and coconut meal is also high in fiber, mannose polysaccharide (Khuwijitjaru *et al.*, 2012), starch, arabinose, xylose, mannose, galactose, glucose and cellulose (10, 5, 28, 12, 7, 3 and 54 g kg^{-1} DM, respectively) (Knudsen, 1997). Dried and grated coconut meal is low in protein as a result of heat damage but it is high in fiber. Dried and grated coconut meal contains levels of moisture, crude protein, crude fat, crude fiber and ash at 3.80, 2.25, 61.30, 8.80 and 2.00%, respectively (Cocjin, 1991). However, Somkuna *et al.* (2013) reported that dried

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coconut meal contains about 2.6% crude protein and 26% crude fiber which limits the use of the meal for simple-stomached animals (Mcdonald *et al.*, 1995) because the high fiber content tends to interfere with digestibility resulting in the retention of nitrogen and other nutrients (Atinyao *et al.*, 2001). The high fiber content is an important factor that limits its use in swine diets (Kim *et al.*, 2001).

Enzymes, used as feed additives, can improve the availability of plant storage polysaccharides, oil and protein, which are protected from digestive enzymes by the impermeable cell-wall structures (McDonald *et al.*, 2002). The cell walls of cereal grains are comprised of complex carbohydrates commonly referred to as non-starch polysaccharide (Choct, 1997; Thacker, 2001.) They are not degraded by pigs because pigs cannot excrete non-starch polysaccharide (NSPs) enzymes. There are several commercial enzymes which are capable of breaking down dietary fiber and they release sequestered nutrients within the fiber fraction of the consuming animal (Choct, 2006). NSP of copra meal is in the forms of mannan, galactomannan and cellulose (26, 61 and 13%, respectively). The use of mannanase alone may not be effective in digesting mannan when it is linked to galactose and cellulose. However, it was found that cellulase, galactosidase and mannanase were used with some success in the laboratory to break down these substances (Balasubramaniam, 1976; Sundu *et al.*, 2006). The effect of exogenous enzymes on growth performance and nutrient digestibility may be influenced by enzyme preparations, the physiological status of the animal and feed ingredients (Ao *et al.*, 2010; Omogbenigun *et al.*, 2004; Shim *et al.*, 2004).

The majority of research on supplementing enzymes particularly in non-ruminant diets has focused on dietary fiber. Dietary fiber is not only indigestible to digestive enzymes of mammals, but it can also reduce digestibility of nutrients and efficiency of energy utilization (Gutierrez *et al.*, 2014). Moreover, digestible energy and metabolizable energy systems may underestimate energy values for ingredients high in lipids and starch, but overestimate energy values for ingredients high in protein and fiber (Noblet *et al.*, 1994a; Noblet, 2007), because ingestion of these nutrients results in different quantities of heat increment (Black, 1995; Noblet *et al.*, 1994a). Yin *et al.* (2000) added xylanase to diets containing wheat by-products fed to 15 kg pigs and reported increasing xylanase (0-1,400 LXU/kg) yielded energy (Linear increase from 58.78 to 68.04%, P=0.003), especially in diets containing high levels of insoluble NSP. Adding an enzyme cocktail to a diet containing 20% soy hulls improved DM and energy digestibility, but not N digestibility, in 33 to 51 kg pigs (Moeser & van Kempen, 2002). Likewise, Zanotto *et al.* (2010) reported that a combination of xylanase and amylase improved the digestible energy and metabolizable energy of a corn by 2.8 and 2.9% respectively. Although improving nutrient digestion with exogenous enzyme supplementation in animal diets is not a new idea, there is limited research for dried and grated coconut meal supplemented with exogenous enzymes.

In this study, dried coconut meal is the by-product of a coconut grinding machine commonly used in fresh coconut-milk production at local markets in Nakhon Si Thammarat province in Thailand. This coconut meal is produced in large amounts every day and is cheap and available the whole year round. Usually rice by-products like rice bran are used as a traditional basal feed of pigs (Le *et al.*, 2011). Therefore, using this coconut meal from local markets as a basal replacement in the diets of pigs, supplemented with an enzyme cocktail® to improve nutrient availability in dried coconut meal, is an alternative way of improving the value of a local by-product and feed costs. The objective of the study is to determine the digestibility of pigs fed different levels of dried coconut meal supplemented with an enzyme cocktail®.

2. Materials and Methods

2.1 Dried coconut meal and an enzyme cocktail

Clean coconut meal, without husk, skin and shell produced by a coconut grinding machine at local fresh markets in Nakhon Si Thammarat province of Thailand used in this study was prepared by being exposed to the sun for 2-3 days (Somkuna *et al.*, 2013.) It was previously analyzed by the method of AOAC (2000) for formulating experimental diets as shown in Table 1; A-Zyme® F2 from Asia Star Animal Health Co., Ltd. was the enzyme cocktail® used in this study. The recommended level is 500 g per tonne of feed. 1 kilogram of the enzyme cocktail contains phytase 1,000,000 units, amylase 5,000,000 units, xylanase 3,500,000 units, beta-glucanase 2,000,000 units, cellulase 1,500,000 units, pectinase 1,000,000 units and mannanase 800,000 units.

Table 1. Chemical composition of dried coconut meal (as-fed basis)

Item (%)	dried coconut meal
Moisture	8.23
Protein (CP)	3.02
Ether extract (EE)	20.46
Ash	2.78
Crude fiber (CF)	28.14
Nitrogen free extract (NFE)[1]	37.73
Calcium (Ca)	0.11
Phosphorus (P)	0.09
Gross energy (GE) (kcal/kg)	1,900.00
Metabolizable energy (ME) (kcal/kg)[2]	2,726.07

[1] NFE = 100-(% moisture + % Ash + % EE + % CF + % CP)
[2] Digestible energy (DE) = 4151- (122 x % Total ash) + (23 x % CP) + (38 x % EE) - (64 x % CF), R^2 =0.89 (Noblet and Perez, 1993) used to calculate ME = DE x (96 - (0.202 x % CP)) / 100) (Asplund and Harris, 1969; NRC, 1988)

2.2 Experimental design and data collection

The four healthy crossbred (Duroc x Landrace x Large White) barrows were allotted according to a 4 × 4 Latin square design. The pigs (average initial body weight of 17.88±0.96 kg) were individually housed in 50 cm × 120 cm × 75 cm (Siriwathananukul, 2011) stainless steel metabolism cages with floors made of strong slatted plastic. The height of the each cage from the ground was 75 cm. There was a stainless steel sheet placed underneath each cage for urine collection which flowed through a stainless steel funnel to a plastic container covered with a clean filter cloth for contamination protection. Four experimental diet treatments were randomly assigned, they were 0% dried coconut meal and an enzyme cocktail (diet 1), 5, 10 and 15% dried coconut meal with 0.05% an enzyme cocktail® (diet 2, diet 3 and diet 4, respectively). The nutrient composition of growing pigs was computed to meet the requirement of the NRC (1998 and 1988) recommendation as shown in Table 2. The 4 feeding periods consisted of 7 days diet acclimation (Moreira *et al.*, 2004) followed by 5

Table 2. Composition and chemical analysis of experimental diets for growing pigs (20-60 kg) (as fed basis)

Ingredients (%)		Diet 1	Diet 2	Diet 3	Diet 4
Broken rice		33.59	26.55	19.17	11.94
Corn meal		22.41	28.09	34.14	40.01
Defat rice bran		15.00	10.00	5.00	-
Dried coconut meal		-	5.00	10.00	15.00
Fish meal (55 % CP)		8.50	8.50	8.50	8.50
Soybean meal (44 % CP)		14.35	15.67	16.99	18.31
Palm oil		5.00	5.00	5.00	5.00
Dicalcuimphosphate		0.05	0.04	0.05	0.08
L-lysine		0.20	0.20	0.20	0.20
DL-methionine		0.10	0.10	0.10	0.10
Vit-Min-premix[1]		0.60	0.60	0.60	0.60
Salt		0.20	0.20	0.20	0.20
Enzymes cocktail®		-	0.05	0.05	0.05
Total		**100.00**	**100.00**	**100.00**	**100.00**
Calculated nutrients (%)	**(NRC)**				
CP	18.00[2]	18.00	18.00	18.00	18.00
EE	-	2.17	3.68	5.20	6.71
CF	-	3.79	5.04	6.29	7.53
Ca	0.74[3]	0.74	0.74	0.75	0.76
Avai-P	0.33[3]	0.33	0.33	0.33	0.33
Lys	0.95[2]	1.15	1.15	1.16	1.16
Met+Cys	0.54[2]	0.73	0.72	0.72	0.71
Trp	0.17[2]	0.21	0.21	0.21	0.21
Thr	0.61[2]	0.71	0.70	0.70	0.69
DE (kcal/kg)	3,400.00[2]	-	-	-	-
ME (kcal/kg)	3,265.00[2]	3,270.00	3,270.00	3,270.00	3,270.00
Analyzed composition (%)					
DM		89.95	87.55	86.85	83.87
CP		18.92	18.73	18.16	18.02
EE		3.98	3.71	4.71	5.03
CF		3.86	6.72	7.01	7.97
Ash		5.63	4.66	4.46	4.29
NFE		57.56	53.73	52.51	48.56
GE (kcal/kg)		4,301.11	4,290.24	4,266.76	4,189.02

Note: [1] Each kg contains vit. A 800,000 IU, vit.D 80,000 IU , vit.E 3,000 IU, vit.K 700 mg, vit.B1100 mg, vit.B2 1,000 mg, pantothenic acid 5,000 mg, niacin 7,500 mg, choline chloride 27,000 mg,vit.B6100 mg, vit.B125 mg, biotin 16 mg, folic acid 33 mg, Fe 80 g, Zn 110 g, Cu 11 g, Mn 22 g, I 0.22 g, Se 0.18 g and santoquin 0.5 g. [2] NRC (1998) [3] NRC (1988)

days collection of faeces and urine (Rodjan, 2011). The daily feed allowance was 80-90% of feed intake in the preliminary period (Schneider & Flatt, 1975; Cai *et al.*, 1994). The water allowance was 2.5 times that of feed intake (Le *et al.*, 2007). Chromic oxide (1.0%) was added as an inert indicator to allow for digestibility determination (Favero *et al.*, 2014). The pigs were weighed individually at the beginning of each period and the data recorded. Faeces from the metabolism cages were collected and weighed separately every day of the sample collection period, in the morning (8.00 am) and in the evening (4.00 pm) (Cera *et al.*, 1989; Urynek & Buraczewska, 2003) and then the faeces were kept in plastic bags containing 10 ml of 10% formalin and stored at -20°C. Urine was collected into urine collection buckets that were placed under the metabolism cages and contained 25 ml of 25% sulphuric acid (Urynek & Buraczewska, 2003) to prevent nitrogen losses through ammonia evaporation. The collected urine was weighed and 10% of the daily urine volume was stored at -20°C (Figueroa *et al.*, 2002). The faeces and urine were stored in a deep freezer until required for analysis. At the end of a 5-day collection period, faeces from each replicate were mixed, ground and representative samples taken for proximate composition determination. The urine from each replicate was also mixed together and representative samples taken for nitrogen determination.

Blood samples were individually obtained from the jugular veins after 3 hours of feeding in each data collection period (Eggum, 1970). 3 to 5 ml blood was obtained for the blood urea nitrogen (BUN) assays. The blood samples were centrifuged at 2,000 x g for 20 min and the harvested plasma was frozen at -20°C until analysis (Whang and Easter, 2000). The procedures utilized for the determination of Dry matter (DM) and Nutrients were determined in accordance with the methods described by Banerjee (1978). Apparent biological values (ABV) were conducted according to the methods described by Pellet and Young (1980). The digestible energy (DE) and metabolizable energy (ME) were determined using the equations by Matterson *et al.* (1965); Silveira *et al.* (2015).

2.3 Chemical analysis

Samples of the experimental diets and faeces were analyzed according to the methods of AOAC (2000). Gross energy (GE) of the experimental diets, faeces and urine samples, was measured by an isoperibol bomb calorimeter (Leco AC-500). Blood samples were analyzed for BUN contents at Thung-Yai Veterinary Diagnostic center, Faculty of Veterinary Science, Nakhon Si Thammarat province, by an automatic clinical chemistry analyzer (ZY-220).

2.4 Statistical analysis

Statistical analysis of data from the experiment was analyzed as a 4 × 4 Latin square design. Duncan's multiple range test was used to compare the means of the treatments (Duncan, 1955). Variability in the data was expressed as the pooled standard error (SE) and a $p<0.05$ was considered to be statistically significant. Polynomial contrast (Linear and quadratic) was applied to determine the effects of varying inclusion levels (0, 5, 10 and 15 %) of dried coconut meal (Steel & Torrie, 1980). A probability of $p<0.05$ was considered to be statistically significant and all data were computed using a computer program.

3. Results

3.1 Nutrient digestibility

Nutrient digestibility (%) of DM, CP, EE, Ash, NFE was not significantly different ($P>0.05$) in pigs fed with different diets. Digestibility of DM was similar for all diets, averaging 89.95, 87.55, 86.85 and 83.87% (control diet1, diet2, diet3 and diet4, respectively). Digestibility of CP was similar for all diets, averaging 83.85, 85.41, 85.08 and 83.31% (control diet1, diet2 diet3 and diet4, respectively). Digestibility of EE was similar for all diets, averaging 71.34, 67.86, 60.08 and 56.37% (control diet1, diet2 diet3 and diet4, respectively). Digestibility of ash was similar for all diets, averaging 53.01, 59.95, 60.92 and 54.31% (control diet1, diet2 diet3 and diet4, respectively). Digestibility of NFE was similar for all diets, averaging 92.73, 93.78, 92.18 and 90.63% (control diet1, diet2 diet3 and diet4, respectively). However, the digestibility percentage of CF in pigs fed with 5, 10 and 15% dried coconut meal in a diet with an enzyme cocktail at a level of 0.05% (diet2, diet3 and diet4, respectively) showed significantly ($P<0.05$) higher digestible fiber than that in control (diet1) (79.25, 75.94, 76.59 and 55.12%, respectively) increasing digestibility of fiber (linear increase from 55.12 to 75.94%, $P=0.018$) (Table 3).

3.2 Energy digestibility

DE was similar for all diets, averaging 3,690.10, 3,860.52, 3,769.17 and 3,638.47 kcal/kg (control diet1, diet2, diet3 and diet4, respectively). ME was similar for all diets, averaging 3,247.11, 3,370.29, 3,357.54 and 3,121.39 kcal/kg (control diet1, diet2, diet3 and diet4, respectively) (Table 3).

3.3 Protein quality evaluation

ABV of pigs fed with 0 and 5% dried coconut meal in a diet with an enzyme cocktail at a level 0.05% in the diets had significantly ($P<0.05$) higher ABV than pigs fed with diet 4 (76.60, 76.21 and 65.10%, respectively). However, ABV reduced (linear and quadratic, $P<0.05$) with increasing level of dried coconut meal in pig diets. The blood concentration of BUN was not affected by any of the diets, averaging 9.80, 8.85, 11.03 and 13.18 mg/100 ml (control diet1, diet2, diet3 and diet4, respectively) (Table 3).

Table 3. Effects of dried coconut meal supplemented with enzyme cocktail on apparent nutrient, energy digestibility and protein quality evaluation in growing pigs (20-60 kg)

Items (%)	Experimental diets				Mean	SEM	P-value	Contrast P-value	
	Diet 1	Diet 2	Diet 3	Diet 4				Linear	Quadratic
DM	85.83	87.97	86.08	83.86	85.94	1.10	0.178	0.334	0.295
CP	83.85	85.41	85.08	83.31	84.41	1.04	0.487	0.811	0.643
EE	71.34	67.86	60.08	56.37	63.91	5.06	0.239	0.068	0.201
CF	55.12[b]	79.25[a]	75.94[a]	76.59[a]	71.73	2.41	0.001	0.018	0.003
Ash	53.01	59.95	60.92	54.31	57.05	3.49	0.362	0.828	0.376
NFE	92.73	93.78	92.18	90.63	92.33	0.62	0.057	0.096	0.112
DE (kcal/kg DM)	3,690.10	3,860.52	3,769.17	3,638.47	3,739.56	60.51	0.151	0.502	0.120
ME (kcal/kg DM)	3,247.11	3,370.29	3,357.54	3,121.39	3,274.08	83.25	0.225	0.470	0.237
ABV	76.60[a]	76.21[a]	71.11[ab]	65.10[b]	72.26	2.24	0.034	0.004	0.010
BUN (mg/100 ml)	9.80	8.85	11.03	13.18	10.72	0.96	0.078	0.074	0.121

[a, b] Means in the same row with different superscripts differ ($p<0.05$)

4. Discussion

There are evidently variations reported in the digestibility of copra or coconut meal among pigs and poultry. Nutrient digestibility of copra meal substituted in pig diets revealed that digestibility of DM and CP were not significantly different among the dietary treatments ($P>0.05$) (Kim *et al.*, 2001). Digestibility in pigs using coconut meal with DM, CP, NFE, CF, EE and Ash, GE and DE were 89.90, 20.90, 46.20, 10.50, 5.80 and 6.5%, 4.20 and 3.60 kcal/g, respectively (Creswell & Brooks, 1971). Moorthy and Viswanathan (2010) conducted digestibility and feeding values of coconut meal for white leghorn layers found that the ileal digestibility of DM, CP, EE, CF and NFE was 67.58, 71.61, 62.67, 35.99 and 74.81%, respectively.

The interactions between feed and enzymes were found in the DM, CP, lipid digestibility and AME of the diet (Sundu *et al.*, 2006). Kong *et al.* (2015) found that the enzyme complex addition increased the in vitro ileal digestibility of DM in copra meal ($p=0.047$). In another experiment, the addition of the mannan-degrading enzyme may have been the reason why the digestibility of all nutrients, particularly CP, increased, but the low digestibility of lipid may be due to the increased levels dietary fiber blocking the access of enzymes to cell contents (Knudsen, 1997).

Atinyao *et al.* (2001) reported that the crude fiber content of diets increased with the increase in the level of copra meal in the diets and there was also a significant interaction ($P<0.05$) between enzyme supplementation and levels of copra meal. The similar results were found in the experiment at diets in this study, their crude fiber in a diet was high when levels of using dried coconut meal in diets increased. Digestibility of crude fiber was significantly ($P<0.05$) higher in pigs fed with every level of dried coconut meal in a diet with an enzyme cocktail® when compared with the control group. However, this study observed that by increasing level of dried coconut meal with an enzyme cocktail at a level 0.05%, there was high digestibility of fiber. This high digestibility of fiber might be because enzyme can degrade insoluble NSP into more soluble NSP (Choct *et al.*, 2004). Likewise, Dekker and Richards (1976); Mcleary *et al.* (1976); Mcleary and Matheson (1986); Atinyao *et al.* (2011) reported that the specific action of the enzyme is to breakdown the β 1-4 linkage between mannose units of mannans and galactomannans and the mode of action of enzyme on enhancing nutrient digestibility may involve the degradation of the cell wall NPSs (Passos *et al.*, 2015).

Although digestibility of fiber of pigs fed with 5, 10 and 15% dried coconut meal with an enzyme cocktail at a level 0.05% in the diets was improved ($P<0.05$), digestible energy (DE) of pigs fed with 5, 10 and 15% dried coconut meal with an enzyme cocktail at this level in the diets was not significantly different ($P>0.05$) compared with the control diet. This study observed that there was a tendency the pigs fed with 5 and 10% dried coconut meal with an enzyme cocktail at a level 0.05% in the diets to have improved the digestible energy along with metabolizable energy. However, Silveira *et al.* (2015) reported that high-energy digestibility can be observed in the amount of digestible dry matter. Noblet and Perez (1993) found that a high fiber content is responsible for adverse effects on the digestible energy content of feeds for pigs. A very high apparent digestible energy content may indicate that there was increasing digestibility of some other energy source such as protein (Creswell & Brooks, 1971).

The high biological values of the diets supports the general view of maximal utilization of good quality proteins (Ekpo, 2011). Biological value reflects both availability and digestibility of maintenance needs of the animal (Elemo *et al.*, 2011). Dried coconut meal is high in fiber but its amino acids imbalanced (Somkuna *et al.*, 2013). In this study, the apparent biological value of pigs fed with diet 4 was significantly ($P<0.05$) lower than the control diet and diet 2 and was observed

that by increasing level of dried coconut meal, there was a linear decrease in apparent biological value. This might be because diet 4 had higher fiber than other groups. Kim *et al.* (2001) found that decreasing amino acids digestibility in the copra meal diet can be explained partly by the higher content of crude fiber in the copra meal. Concentration of blood urea nitrogen was highly correlated with urinary nitrogen excretion rate (Kohn *et al.*, 2005). Blood urea nitrogen from this study was not significantly different among the dietary treatments ($P>0.05$), similar is Wang *et al.* (2009) who reported that BUN was not affected by the addition of the enzyme cocktail (α-1,6-β-galactosidse, β-1,4-mannanase, and β-1,4-mannosidase) to low-nutrient corn-SBM containing DDGS diets.

5. Conclusions

The results of this study suggest that feeding pigs with 5% dried coconut meal in a diet with an enzyme cocktail at a level of 0.05% can be recommended. It does not impair nutrient digestibility.

Acknowledgements

The authors would like to thank Mr. Kanit Khoploy-klang, Assistant Professor, Faculty of Agriculture, The Rajamangala University of Technology Srivijaya, for extending all necessary help to carry out the animal trial.

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