



## Effects of Dietary Inclusion of Palm Kernel Cake and Palm Oil, and Enzyme Supplementation on Performance of Laying Hens

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**ABSTRACT** : A total of 392 twenty eight week-old laying hens was used to study the effects of dietary inclusion of solvent-extracted palm kernel cake (PKC) (0%, 12.5% and 25%) and enzyme (mixture of mannanase,  $\alpha$ -galactosidase and protease) supplementation (0 kg/t, 1 kg/t and 2 kg/t) on the performance of laying hens. The levels of PKC did not significantly influence nitrogen corrected true metabolizable energy (TME<sub>n</sub>) of the diets. Enzyme-supplemented PKC had significantly higher AME and TME<sub>n</sub> values than PKC diets with no enzyme supplementation. Dietary inclusion of 12.5% and 25% PKC in the diets of laying hens did not adversely affect mean egg production or daily egg mass. However, layers consumed significantly more PKC-based diets and had significantly poorer feed conversion ratios (FCR) than controls. However, the feed intake and FCR of hens provided the 12.5% PKC-based diets with enzyme supplementation at 1 kg/t did not differ from the controls. Dietary inclusion of PKC or enzyme did not affect eggshell quality, but egg yolk colour was significantly paler when layers were fed the 25% PKC diet. (**Key Words** : Palm Kernel Cake, Enzyme, Laying Hens)

### INTRODUCTION

Malaysia is the world largest producer of palm oil, a major edible oil. Palm kernel cake (PKC) is a major by-product of palm kernel oil. There is a wealth of literature on the potential of PKC as a source of protein and energy for livestock (Alimon and Hair-Bejo, 1995). Palm kernel meal generally contains 17-21% protein, 10-17% crude fibre, 4-5% ash and ether extract values of 0.7-0.9% depending on the efficiency of oil extraction from the kernel (Nwokolo et al., 1977; Onwudike, 1986). Although large quantities of PKC are available for feed, the use of PKC in the feed industry is mainly limited to the ruminant sector. Palm kernel meal is not widely used in the poultry industry because of its high fibre and low energy contents. Although the subject of using PKC in poultry diets has been studied by several researchers (Onwudike, 1986; Zulkifli et al.,

2003; Mustafa et al., 2004), the recommended levels of inclusion seem to vary from one study to another. Longe (1984) noted poorer egg production for layers fed 20% PKC, while other authors found that 20% PKC could be used in layer diets without detrimental effects on performance (Yeong and Mukherjee, 1983; Ngoupayou, 1984). Onwudike (1988) reported that levels as high as 40% for laying hens could be used without reducing performance.

Several studies have shown that enzymes with mannanase activity could break down the non-starch polysaccharides (mannans) of PKC, hence improving its nutritive quality (Dusterhoft et al., 1993a,b,c; Daud et al., 1997). Work in our laboratory suggest that an enzyme mixture of mannanase,  $\alpha$ -galactosidase and protease (PKCase) from Alltech, Inc. (Kentucky, USA) has the ability to increase the metabolisable energy of PKC (Chong, 1999). Supplementation of PKCase significantly increased the release of reducing sugars in PKC and soybean meal by 26.8%-67.4% and 20%-30%, respectively. Thus, the objectives of the study were to determine the effects of different inclusion levels of PKC and enzyme supplementation on performance of laying hens under the hot, humid tropical climate.

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Table 1. Dietary treatments<sup>1</sup>

	Levels of enzyme		
	0 kg/t	1 kg/t (1E)	2 kg/t (2E)
12.5% PKC	Diet L2	Diet L3	Diet L4
25.0% PKC	Diet L5	Diet L6	Diet L7

<sup>1</sup>Diet L1 = a basal corn-soybean meal diet without palm kernel cake or enzyme supplementation.

## MATERIALS AND METHODS

### Birds, husbandry and experimental procedures

A total of 392 Lohmann Brown laying hens (28 weeks old) were purchased from a local farm. The hens were placed in individual wire-floored cages (0.3 m wide×0.4 m length×0.4 m height) arranged in a single tier within a conventional open-sided house (with cyclic temperatures, minimum, 25°C; maximum, 34°C) in Malaysia. Eight hens were fed from a single trough and considered as one replicate. There were seven replications for each dietary group. A total of seven rows of battery cages was used and the birds received 16 h of light per day. A randomized block design was used for the study in which the seven diets were randomly assigned to each row (or block). Feed intake per replicate was determined weekly. Eggs were collected once daily (1030 h) and egg production was recorded. Eggs were classified as normal, cracked, deformed-shell, soft shell or no-shell. All eggs produced during a 2-day period (Tuesday and Wednesday) every week were individually weighed. Eggs specific gravity was measured once weekly (Wednesday) on all eggs collected on a single day. The saline solutions for egg specific gravity measurements were in increments of 0.005 and ranged from 1.060 to 1.100. Before every gravity solutions were verified with a hydrometer, and water or salt was added as required. During the last day of the experiment, four eggs from each replicate were broken and measured for yolk colour with a Roche's Yolk Colour Fan.

### Dietary treatments

A total of seven layer diets was used in the eight-week study. Diet L1 was considered as a control diet (without PKC and PKCase) and fed to layers in the control group. Two levels of PKC (12.5% and 25%) and three levels of enzyme (PKCase) were arranged in a factorial manner to yield another six diets (Table 1). PKCase is an enzyme mixture that contains 107,000 U/g of  $\alpha$ -galactosidase activity, 2,300 HUT/g of protease activity (HUT = hemoglobin unit on the tyrosine base) and 12,081 U/g of mannanase activity. Diets L2-L7 were fed to layers in the experimental groups. The composition of the diets are shown in Table 2. The nutrient levels recommended by the Lohmann Brown breeder company was used in the formulation of the diets.

### Digestibility of diets

True dry matter digestibility (TDMD) and nitrogen corrected true metabolizable energy (TME<sub>n</sub>) were determined using Sibbald's procedure (Sibbald, 1986). Six 30-week old Lohmann Brown cockerels were assigned to each diet. All the birds used in the study were fasted for 48 h to remove all digesta in the gastro-intestinal tract. Thirty grams of tested feedstuff was given to each bird through crop intubation. A similar number of birds for each type of diet were fasted throughout the experimental period to measure endogenous excretion. A plastic tray was placed under each cage and excreta were collected quantitatively for 48 h. Birds had free access to water.

For the TME<sub>n</sub> study, the excreta samples were dried at 60°C, reweighed (dry weight) and ground to pass through a 1 mm mesh screen. Gross energy was measured with an adiabatic oxygen bomb calorimeter. Total nitrogen content in the dry excreta was analysed. A correction was made for nitrogen retention, which was either positive or negative. It was done to bring the TME<sub>n</sub> data to a basis of nitrogen equilibrium. A correction factor of 8.73 kcal/kg of nitrogen was used (Titus et al., 1959).

### Statistical analyses

The study was a factorial arrangement in a completely randomized block design. All data were subjected to analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of SAS software (SAS Institute, 1996). If treatments were found to be significantly different, Tukey's multiple range test was used to determine the statistical significance among treatment least-square means.

The results obtained for hens fed the control diets were compared to those fed the experimental diets using a one-way ANOVA using the GLM procedure of SAS software (SAS Institute, 1996). If treatments were found to be significantly different, the Bonferroni (Dunn) T test (SAS Institute, 1996) was used to determine the statistical significance between treatment least-square means. The Bonferroni (Dunn) T test was used because it will avoid detecting random differences when comparing a large group of treatments.

## RESULTS

There were no significant interactions between blocks and diets. Therefore, the data were analysed as a completely randomized design experiment. When comparing data from hens fed diets L1-L7, no significant dietary effects were observed on mean egg production, hen-day egg production or daily egg mass (Table 3). Birds fed L7 diet produced significantly bigger eggs than those provided L1, L3, L4, L5 and L6 diets. On the other hand, birds fed the 25% PKC-based diet (L5) produced significantly smaller eggs than the

**Table 2.** Nutrient composition of layer diets

Ingredients	Diet L1	Diet L2	Diet L3	Diet L4	Diet L5	Diet L6	Diet L7
Corn	58.46	41.18	41.08	40.98	25.79	25.69	25.69
Soybean meal	28.24	28.68	28.68	28.68	27.42	27.42	27.42
Fishmeal	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Palm kernel cake <sup>2</sup>	0.00	12.50	12.50	12.50	25.00	25.00	25.00
Palm oil	1.15	5.64	5.64	5.64	9.83	9.83	9.83
Limestone	9.10	8.95	8.95	8.95	8.90	8.90	8.90
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50
PKCase <sup>3</sup>	0.00	0.00	+	++	0.00	+	++
Antioxidant <sup>4</sup>	0.01	0.02	0.02	0.02	0.02	0.02	0.02
Salt (NaCl)	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Vit-min premix <sup>5</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.12	0.11	0.11	0.11	0.11	0.11	0.11
Choline chloride (60%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calculated composition (% air-dry unless otherwise indicated)							
Nutrients							
AME (kcal/kg)	2,691	2,691	2,691	2,691	2,690	2,690	2,690
Crude protein	18.20	18.08	18.08	18.08	18.13	18.13	18.13
Arginine	1.32	1.37	1.37	1.37	1.54	1.54	1.54
Lysine	1.04	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Methionine+cystine	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Tryptophan	0.19	0.26	0.26	0.26	0.27	0.27	0.27
Threonine	0.73	0.71	0.71	0.71	0.71	0.71	0.71
Crude fat	3.68	7.83	7.83	7.83	11.80	11.80	11.80
Crude fibre	3.05	4.93	4.93	4.93	6.45	6.45	6.45
Calcium	3.96	3.96	3.96	3.98	3.98	3.98	3.98
Available phosphorus	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Determined composition (% air-dry unless otherwise indicated)							
Nutrients							
Dry matter	89.89	91.58	90.58	91.24	91.58	91.54	91.56
AME (kcal/kg)	2,899	2,742	3,020	2,983	2,756	2,973	3,101
Crude protein	18.42	18.33	18.93	18.70	18.53	18.47	18.55
Crude fat	4.01	7.66	7.47	7.60	10.85	10.73	11.51
Crude fibre	2.86	4.81	4.64	4.58	6.11	6.06	6.16
Ash	10.89	12.84	11.40	14.27	13.57	13.76	13.41
Calcium	4.00	3.89	3.91	3.85	4.10	4.02	3.99
Total phosphorus	0.66	0.70	0.70	0.70	0.73	0.73	0.73

<sup>1</sup> Levels of enzyme. + = 1 kg enzyme per tonne of feed; ++ = 2 kg enzyme per tonne/feed.

<sup>2</sup> Solvent-extracted palm kernel cake.

<sup>3</sup> Enzyme mixture, Alltech Inc. USA: It contains mannanase,  $\alpha$ -galactosidase and protease activity.

<sup>4</sup> FRA<sup>®</sup> OX Dry, Franklin Products International BV.

<sup>5</sup> Supplied per kg diet: Fe 100 mg; Mn 110 mg; Cu 20 mg; Zn 100 mg; Se 0.2 mg; Co 0.6 mg; Santoquin 0.6 mg; vitamin A 6,667 IU; vitamin D 1,000 IU; vitamin E23 IU; vitamin K3 1.33 mg; Cobalamin 0.03 mg; thiamin 0.83 mg; riboflavin 2 mg; folic acid 0.33 mg; biotin 0.03 mg; pantothenic acid 3.75 mg; pyridoxine 1.33 mg.

other groups except those fed an enzyme supplemented (2 kg/t) 12.5% PKC-based diet (L4). Birds fed the control (L1) and enzyme-supplemented (1 kg/t) 12.5% PKC-based (L3) diets consumed significantly less feed than those fed the 25% PKC diets (L5, L6 and L7). The best FCR (feed intake/egg mass) was obtained from those fed diets L1 and L3, whereas significantly poorer FCR were found in birds fed diets L2, L4, L5, L6 and L7 (Table 3). Diet had no significant effect on eggshell quality (Table 4). However, egg yolk colour was significantly paler for eggs produced by birds fed the 25% PKC-based diets (L5, L6 and L7).

There were no significant differences in true dry matter retention between birds fed the control layer diet (L1) and enzyme-supplemented PKC diets (L3, L4, L6 or L7) (Table 5). Birds fed a 25% PKC-based layer diet (L5) had a significantly lower true dry matter retention than those fed the control layer diet (L1) or enzyme-supplemented PKC diets (L3, L4, L6 or L7). The control diet (L1), 12.5% PKC diet (L2) and 25% PKC diet (L5) had lower TME<sub>n</sub> values when compared to 25% PKC diet supplemented with 2 kg/t enzyme. Enzyme-supplemented PKC diets (L3, L4, L6 and L7) had significantly higher TME<sub>n</sub> values than PKC diets

**Table 3.** The effects of dietary inclusion of solvent-extracted palm kernel cake (PKC) and enzyme supplementation on the mean performance of laying hens over 8 weeks<sup>1</sup>

Layer diet	Hen-day egg production (%)	Egg weight (g)	Daily egg mass (g)	Feed intake (g/bird/day)	FCR
Control (L1)	91.5	55.1 <sup>bc</sup>	50.4	98.7 <sup>c</sup>	1.96 <sup>d</sup>
12.5% PKC (L2)	91.7	55.6 <sup>ab</sup>	51.0	105.3 <sup>b</sup>	2.07 <sup>bc</sup>
12.5% PKC+1E <sup>2</sup> (L3)	92.0	54.9 <sup>c</sup>	50.5	101.5 <sup>c</sup>	2.01 <sup>cd</sup>
12.5% PKC+2E (L4)	93.1	54.7 <sup>cd</sup>	50.9	104.7 <sup>b</sup>	2.06 <sup>bc</sup>
25% PKC (L5)	92.1	54.3 <sup>d</sup>	50.0	111.0 <sup>a</sup>	2.22 <sup>a</sup>
25% PKC+1E (L6)	92.3	55.1 <sup>bc</sup>	50.8	108.6 <sup>a</sup>	2.14 <sup>ab</sup>
25% PKC+2E (L7)	92.6	55.7 <sup>a</sup>	51.6	108.5 <sup>a</sup>	2.10 <sup>bc</sup>
Overall mean	92.2	55.1	50.7	105.5	2.08
Pooled SEM <sup>3</sup>	0.2	0.1	0.2	0.3	0.01

<sup>1</sup> Layers were 28 weeks old at the start of the experiment. FCR = Feed conversion ratio.

<sup>2</sup> Enzyme mixture, Alltech Inc. USA: It contains mannanase,  $\alpha$ -galactosidase and protease activity; 1 E = 1 kg/t and 2 E = 2 kg/t.

<sup>3</sup> Pooled standard error of the mean; data represent mean of seven replications of eight layers.

<sup>a, b, c, d</sup> Treatment means with different superscripts within a column are significantly different at  $p < 0.05$ .

with no enzyme supplementation (L2 and L5).

## DISCUSSION

The present findings suggest that laying hens are capable of maintaining production performance when 12.5% or 25% PKC was included in their diets. However, the layers consumed more PKC based diets in order to offset the poorer digestibility of PKC diets. This resulted in poorer FCR in hens fed PKC based diets. The direct relationship between feed intake and the levels of PKC was observed in the present study. It is well established that birds attempt, as a priority, to consume a certain quantity of feed necessary to meet their energy requirements (Larbeer and Leclercq, 1994). However, both the AME and TME<sub>n</sub> contents of the PKC based diets (L2-L7) were similar to those of the control corn-soy bean meal diet (L1). Thus, it is

unlikely that the hens consumed more PKC based to meet their energy requirements. There is a possibility that the hens eat to meet their protein-amino acid needs rather than their energy requirements. Parsons et al. (1984) and Edmonds et al. (1985) reported that broilers increased their voluntary feed intake when dietary protein level in a corn-soybean meal diet was reduced from 24% to 16% by replacing soybean meal with corn. The higher feed consumption of the PKC-based diets may also be attributed to the improved palatability as a result of high inclusion of palm oil. It is well documented that addition of fat stimulates feed and ME energy consumption in poultry (Zulkifli et al., 2003).

There are conflicting results in the literature on the effects of dietary PKC on egg production in chickens. Longe (1984) found that 24-week old laying hens fed 20% PKC diets produced fewer eggs than those fed a control corn-soybean meal diet although the calculated daily energy consumption of both groups of hens was similar. On the

**Table 4.** The effects of dietary inclusion of solvent-extracted palm kernel cake (PKC) and enzyme supplementation on the mean egg quality over 8 weeks from 28 weeks of age

Layer diet	Egg shell specific gravity	Egg yolk colour
Control (L1)	1.091	3.9 <sup>a</sup>
12.5% PKC (L2)	1.091	3.9 <sup>a</sup>
12.5% PKC+1E <sup>2</sup> (L3)	1.092	3.7 <sup>a</sup>
12.5% PKC+2E (L4)	1.091	3.8 <sup>a</sup>
25% PKC (L5)	1.091	2.3 <sup>b</sup>
25% PKC+1E (L6)	1.092	2.4 <sup>b</sup>
25% PKC+2E (L7)	1.092	2.4 <sup>b</sup>
Overall mean	1.091	3.2
Pooled SEM <sup>3</sup>	0.0001	0.1

<sup>1</sup> Layers were 28 weeks old at the start of the experiment, FCR = Feed conversion ratio.

<sup>2</sup> Enzyme mixture, Alltech Inc. USA: It contains mannanase,  $\alpha$ -galactosidase and protease activity; 1E = 1 kg/t and 2E = 2 kg/t.

<sup>3</sup> Pooled standard error of the mean; data represent mean of seven replications of eight layers.

<sup>a, b, c, d</sup> Treatment means with different superscripts within a column are significantly different at  $p < 0.05$ .

**Table 5.** Nitrogen corrected true metabolizable energy<sup>1</sup> (TME<sub>n</sub>) and true dry matter retention of layer diets

Layer diet	True dry matter retention (%)	TME <sub>n</sub> (kcal/kg)
Control (L1)	71.4 <sup>a</sup>	3,382 <sup>bc</sup>
12.5% PKC (L2)	62.9 <sup>bc</sup>	3,226 <sup>c</sup>
12.5% PKC+1E <sup>2</sup> (L3)	69.6 <sup>a</sup>	3,509 <sup>ab</sup>
12.5% PKC+2E (L4)	68.1 <sup>ab</sup>	3,469 <sup>ab</sup>
25% PKC (L5)	56.7 <sup>c</sup>	3,240 <sup>c</sup>
25% PKC+1E (L6)	65.2 <sup>ab</sup>	3,458 <sup>ab</sup>
25% PKC+2E (L7)	66.2 <sup>ab</sup>	3,586 <sup>a</sup>
Overall mean	65.7	3,410
Pooled SEM <sup>3</sup>	0.9	24

<sup>1</sup> Dry matter basis.

<sup>2</sup> Enzyme mixture, Alltech Inc. USA: It contains mannanase,  $\alpha$ -galactosidase and protease activity; 1E = 1 kg/t and 2E = 2 kg/t.

<sup>3</sup> Pooled standard error of the mean; data represent mean of six cockerels.

<sup>a, b, c, d</sup> Treatment means with different superscripts within a column are significantly different at  $p < 0.05$ .

other hand, Onwudike (1988) reported that laying hens could tolerate up to 40% PKC in their diets without adverse effects on egg production. However, the layers (31-week old hens) fed either control or 40% PKC diet only attained a hen-day production of 62% and a daily egg mass of 36.5 g. The present findings indicated that feeding of 25% PKC diets had no adverse effect on egg production and the hens were able to maintain a high level of egg production (92.2% egg production and 50.7 g egg mass).

The beneficial effects of enzyme supplementation on true dry matter retention (improved by 12.5%) and  $TME_n$  (improved by 8.4%) were also observed in the present study. Enzyme supplementation significantly reduced feed consumption and improved FCR in the PKC-fed groups. The similar FCR between those provided the 12.5% PKC diet with 1 kg/t enzyme supplementation and control diet suggests that the latter can be fed in laying hens without any detrimental effect on performance. Dietary inclusion of PKC or enzyme supplementation did not influence eggshell quality. Egg yolk colour, however, was significantly paler as the dietary level of PKC reached 25% and this observation is in agreement with those of Panigrahi and Waite (1998). The negligible effect of enzyme supplementation on egg shell quality has also been reported in quails (Sarıççek et al., 2005).

Zulkifli et al. (2003; 2007) and Nwe Nwe Htin et al. (2007) reported that providing diets containing high levels of palm oil enhanced growth performance and survivability of heat-stressed broiler chickens. Refined palm oil contains larger amount of vitamin E (350-450 ppm; Sambanthamurthi et al., 2000) as compared to soybean oil and coconut oil (Goh et al., 1985). Stressful conditions, particularly those related to high ambient temperatures, are well known to increase vitamin E requirements in poultry (Cheville, 1979). In the present study, however, the high addition of palm oil had negligible effect on the performance of laying hens under the hot, humid tropical conditions.

In conclusion, the present findings suggest that laying hens were able to tolerate 12.5% to 25% PKC in their diets without any adverse effect on egg production and egg mass. However, laying hens fed 12.5% and 25% PKC diets consumed significantly more feed and had a poorer FCR than those fed the control diets. The diminishing yolk colour associated with a high inclusion rate of PKC in layer diets could be a problem in Malaysia where customers prefer a darker yolk colour. Enzyme supplementation of PKC-based diets was beneficial in improving true dry matter retention,  $TME_n$  and FCR in the PKC-fed groups. Hence, enzyme supplementation remains promising and future studies should be conducted to evaluate whether a higher level of enzyme-supplemented PKC could be used in poultry diets. Earlier studies (Oyofe et al., 1989; Hinton et

al., 1990; Allen et al., 1997) have shown that mannose (from the digestion of PKC) greatly reduced the colonization of salmonella in the gastro-intestinal tract of poultry. Further study on this aspect is also warrant.

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