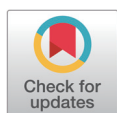


Effects of dietary palm kernel meal and β -xylanase on productive performance, fatty liver incidence, and excreta characteristics in laying hens

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Competing interests

No potential conflict of interest relevant to this article was reported.

Abstract

The objective of the present experiment was to investigate the effect of dietary palm kernel meal (PKM) and β -xylanase supplementation on productive performance, egg quality, fatty liver incidence, and excreta characteristics in laying hens. A total of 320 Hy-Line Brown laying hens (33 weeks of age) were allotted to 1 of 4 treatments with 8 replicates in a feeding trial. Each replicate consisted of 10 consecutive cages with 1 hen per cage. The corn-soybean meal-based control diet was prepared. Additional diet was prepared by including 10% of PKM in the control diet with a partial replacement of corn, soybean meal, and animal fat. In addition, 0.025% β -xylanase was supplemented at the expense of celite to those 2 diets to produce 4 treatment diets in a 2 × 2 factorial arrangement. All hens were provided the diet and water ad libitum for 8 weeks. Results indicated no significant interactions between inclusion of dietary PKM and β -xylanase for all measurements; therefore, the main effects were mainly discussed. Hens fed diets containing 10% PKM had greater ($p < 0.05$) feed intake and yolk color than those fed diets containing no PKM. However, dietary PKM did not influence fatty liver incidence and excreta characteristics. Dietary β -xylanase supplementation had no effects on all measurements, regardless of inclusion of PKM. In conclusion, PKM can be a potential feed ingredient for laying hens at the inclusion of 10% in the diet. It appears that dietary β -xylanase used in the current experiment has little effect on layer productivity, regardless of inclusion of 10% PKM in the diet.

Keywords: β -xylanase, Egg quality, Excreta characteristics, Laying hen, Palm kernel meal, Performance

INTRODUCTION

Palm kernel meal (PKM) is a by-product of palm kernel during palm oil extraction, which is mostly produced in Southeast Asia countries (e.g., Indonesia and Malaysia). Because of its nutritional values

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Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Choi WJ, Kim KE, Kil DY.

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Ethics approval and consent to participate

The protocol for the current experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University (IACUC No. 2020-00042).

and low production cost, PKM has been considered an economical ingredient for poultry diets [1]. It has been reported that inclusion of PKM up to 40% in poultry diets has no adverse effects on productive performance [2,3]. However, poultry nutritionists often hesitate to use the large amounts of PKM in diets because of its adverse properties such as dark color and poor essential amino acid profiles. In addition, PKM contains the relatively high amounts of nonstarch polysaccharides (NSP), which is well-known as an antinutritional factor due to impairment in nutrient digestion and absorption in the gastrointestinal tract of animals [4,5]. Therefore, dietary supplementation of NSP-degrading enzymes (NSPase) is widely used in the feed industry when PKM is included in the diet. In particular, dietary β -mannanase is often supplemented to diets containing PKM because β -mannan is the major NSP in PKM [6]. It has been reported that dietary supplementation of β -mannanase in diets containing PKM improved productive performance of broiler chickens and laying hens [7,8]. Dietary β -xylanase may also be considered a potential NSPase in diets containing PKM because xylan is also present at the high amount in PKM [6]. Supplementation of β -xylanase in diets containing PKM was reported to improve growth performance of broiler chickens [9]. However, there is currently little information regarding the interactive effects of dietary PKM and β -xylanase supplementation in laying hens.

Therefore, the objectives of the present experiment were to investigate the effects of dietary PKM and β -xylanase supplementation on productive performance, egg quality, fatty liver incidence, and excreta characteristics in laying hens.

MATERIALS AND METHODS

Animals, experimental design, and diets

The protocol for the current experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University (IACUC No. 2020-00042). A total of 320 Hy-Line Brown laying hens (Icheon, Korea) with 33 weeks of age were allotted to 1 of 4 dietary treatments with 8 replicates in a feeding trial. Each replicate consisted of 10 consecutive cages with 1 hen per cage (37 cm \times 30 cm \times 40 cm = width \times length \times height). The experiment was conducted in a completely randomized design with a 2 \times 2 factorial arrangement with dietary supplementation of PKM and β -xylanase. The control diet was formulated mainly with corn and soybean meal. Additional diet was prepared with the inclusion of 10% PKM in the control diet with a partial replacement of corn and soybean meal. The commercial PKM was used in this experiment and nutritional compositions of PKM is presented in Table 1. In addition, 0.025% β -xylanase (Econase[®]XT, declared activity of 4,000,000 unit/g, AB Vista, Marlborough, UK) was supplemented to 2 of 4 treatment diets by replacing the same amounts of celite in treatment diets. All nutrients and energy in the experimental diets were formulated to meet or exceed recommendations of the Hy-Line International [10] (Table 2). The diets and water were provided ad libitum for 8 weeks. The cages were placed in an environmentally-controlled laying house with 16 h of lighting and 8 h of darkness. Temperature and humidity were maintained at 23 \pm 3 $^{\circ}$ C and 64 \pm 9% throughout the entire experiment, respectively.

Data and sample collection and chemical analysis

The color of diets was measured using a colorimeter (model CR-10, Konica Minolta Optics, Tokyo, Japan) to assess the color change due to the inclusion of 10% PKM.

Hen-day egg production, egg weight (EW), and broken and shell-less egg production rate were recorded daily. Feed intake (FI) and feed conversion ratio (FCR) were recorded at 4-week intervals. Egg mass (EM) was calculated based on hen-day egg production and EW. The data for productive

Table 1. Analyzed and reported nutrient concentrations of palm kernel meal (PKM)

Item	Analyzed value	Reported value ¹⁾
Gross energy (kcal/kg)	4,379	4,378
CP (%)	13.4	15.2
Dry matter (%)	93.1	91.2
Crude ash (%)	3.8	4.3
Ether extract (%)	7.2	8.4
Neutral detergent fiber (%)	70.9	66.6
Acid detergent fiber (%)	40.9	40.9
Amino acid (%)		
Arginine	1.60	1.93
Histidine	0.25	0.32
Isoleucine	0.35	0.53
Leucine	0.78	0.94
Lysine	0.38	0.44
Methionine	0.27	0.27
Methionine + cysteine	0.45	0.46
Phenylalanine	0.69	0.59
Threonine	0.45	0.47
Valine	0.66	0.76
Alanine	0.49	0.61
Aspartic acid	1.07	1.20
Glutamic acid	2.51	2.83
Glycine	0.60	0.69
Proline	0.44	0.47
Serine	0.63	0.65
Tyrosine	0.30	0.38

¹⁾Adopted from Heuzé et al. with CC-BY-NC [17].

performance were summarized for 8 weeks of the feeding trial.

Egg quality was analyzed with 12 eggs per replicate, which were randomly collected at the end of the experiment. Eggshell color was determined by the method described by Kim et al. [11]. In short, eggshell color was determined using the eggshell color fan (Samyangsa, Wonju, Korea) with different scales from 1 to 15 (1 = light white; 15 = dark brown). The CIE color scale for L^* , a^* , and b^* were also measured using a colorimeter (model CR-10, Konica Minolta Optics). Egg yolk color, haugh unit, and eggshell strength were analyzed using digital egg tester (DET-6000, Nabel, Tokyo, Japan) as reported previously [12].

At the end of experiment, the individual body weight (BW) of all laying hens was recorded. One hen with the closest average BW per replicate was chosen and euthanized by CO₂ asphyxiation. For a measure of fatty liver incidence, images of the liver attached on the body were pictured to determine the subjective fatty liver score on a scale from 1 to 5 (1 = dark red; 5 = yellowish red) [13]. In addition, the objective CIE color scale for the L^* , a^* , and b^* were also determined using a colorimeter (model CR-10, Konica Minolta Optics). Afterward, the liver was detached, weighed, and collected for measuring total lipid concentrations [14].

At the end of the feeding trial (41 weeks of age), 24 laying hens (i.e., 6 hens per treatment) were selected and randomly placed in metabolic cages with one bird per cage (35.2 cm × 45.0 cm × 55.3 cm = width × length × height) to measure excreta characteristics based on the method described by

Table 2. Ingredient composition and nutrient concentration of the experimental diets

Item	Inclusion levels of palm kernel meal (%)	
	0	10
Ingredients (%)	100.00	100.00
Corn	56.35	45.57
Soybean meal (46% CP)	20.08	17.36
Corn gluten meal (58% CP)	4.02	4.50
Palm kernel meal	0.00	10.00
DDGS	7.00	7.00
Animal fat	1.00	3.89
L-Lysine (78%)	0.16	0.24
DL-Methionine (98%)	0.12	0.14
L-Threonine (98%)	0.00	0.03
Choline chloride	0.04	0.07
Tricalcium phosphate	0.86	0.90
Limestone	9.66	9.59
Salt	0.25	0.25
Sodium bicarbonate	0.12	0.12
Vitamin premix ¹	0.12	0.12
Mineral premix ²	0.12	0.12
Phytase	0.05	0.05
Celite ³	0.05	0.05
Calculated energy and nutrient content		
AME _n (kcal/kg)	2,692	2,695
CP (%)	18.00	18.00
Amino acid (%)		
Lysine	0.82	0.82
Methionine + cysteine	0.67	0.67
Methionine	0.40	0.42
Threonine	0.57	0.57
Tryptophan	0.16	0.15
Calcium (%)	4.00	4.00
Available phosphorus (%)	0.35	0.35
Analyzed energy and nutrient content		
Gross energy (kcal/kg)	3,622	3,742
CP (%)	18.4	15.7
Dry matter (%)	90.6	91.7
Crude ash (%)	11.0	10.9
Ether extract (%)	4.1	6.9
Neutral detergent fiber (%)	11.7	16.0
Acid detergent fiber (%)	4.8	6.5
Feed color (CIE value)		
L*	63.4	54.0
a*	7.7	6.7
b*	32.7	27.3

¹Provided per kg of the complete diet: vitamin A, 10,000 IU (retinyl acetate); vitamin D₃, 4,500 IU; vitamin K₃, 3.0 mg (menadione dimethylpyrimidinol); vitamin B₁, 2.50 mg; vitamin B₂, 6.50 mg; vitamin B₆, 3.20 mg; vitamin B₁₂, 18.0 µg; biotin, 180 µg; folic acid, 1.9 mg; niacin, 60 mg.

²Provided per kg of the complete diet: cobalt, 1,200 µg (CoSO₄); copper, 19.0 mg (CuSO₄); iron, 72 mg (FeSO₄); iodine, 1.5 mg (Ca[IO₃]₂); manganese, 144.0 mg (MnO); selenium, 360 µg (Na₂SeO₃); zinc, 120 mg (ZnSO₄).

³0.025% β -xylanase (Econase[®]XT, declared activity of 4,000,000 unit/g, AB Vista, Marlborough, UK) was supplemented to treatment diets by replacing the same amounts of celite.

CP, crude protein; DDGS, dried distiller's grains with solubles; AME_n, nitrogen-corrected apparent metabolizable energy.

Ogunji et al. [15] (Table 6). The excreta moisture score was visually measured on a scale from 1 to 4 (1 = normal dry droppings and coning; 2 = slightly loose droppings, some coning but no free water; 3 = loose droppings with slight coning and some free water; 4 = extremely loose droppings with no coning and large amounts of free water). Excreta samples were also collected daily from each cage to analyze the actual moisture concentrations. Excreta moisture concentrations were determined using drying oven at 100 °C for 12 h [16].

The samples for PKM and experimental diets were dried and finely ground for analyzing the concentrations of crude protein (CP; method 990.03), dry matter (DM; method 930.15), crude ash (method 942.05), ether extract (method 2003.05), neutral detergent fiber (method 2002.04), and acid detergent fiber (method 973.18) as followed by the AOAC methods [14]. In addition, the concentrations of amino acids in PKM were analyzed by the AOAC (method 982.30) [14]. The concentrations of gross energy in the samples were analyzed using a bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA) with benzoic acid used as the calibration standard [14].

Statistical analysis

All data were analyzed by 2-way ANOVA (analysis of variance) in a completely randomized design with the GLM procedure of SAS (SAS Institute, Cary, NC, USA). The replicate was used as an experimental unit. Outliers were checked using the UNIVARIATE procedure of SAS. The model included the effects of dietary PKM, β -xylanase supplementation, and their interaction as fixed variables. However, there were no significant interactions for all measurements. The MEANS procedure was used to calculate treatment means. Significance for statistical test was set at $p < 0.05$.

RESULTS AND DISCUSSION

The concentrations of CP in PKM (13.4%) were slightly less than previously reported values (15.2%) [17], which may be the reason why analyzed CP concentrations in diets were decreased with 10% inclusion levels of PKM (Table 2), although treatment diets were formulated to maintain equivalent CP concentrations among treatment diets. However, the concentrations of other nutrients in PKM were similar to those values reported previously [17]. Therefore, the PKM used in the current experiment can be considered the typical and representative PKM used in the commercial animal diet. Moreover, the color of diets containing 10% PKM was darker (i.e., 14.8% less lightness) than the color of diets containing no PKM, which is likely due to the dark color of PKM.

No significant interactions for all measurements were observed between dietary PKM and β -xylanase supplementation. This result was unexpected because inclusion of PKM in diets increases the amounts of viscous NSP, which is known to be detrimental on layer production; therefore, dietary β -xylanase supplementation would alleviate the negative effects. The possible reason may be that the amount of xylan in PKM is relatively low enough to exert significant effect of dietary β -xylanase [6]. Aderibigbe et al. [18] also reported no positive effects of dietary supplementation of β -xylanase in diets containing PKM on growth performance of broiler chickens. In addition, the current inclusion levels of PKM (i.e., 10%) is insufficient to induce a significant antinutritional effect.

Inclusion of 10% PKM in layer diets did not affect productive performance, except that hens fed diets containing 10% PKM had greater ($p < 0.05$) FI than those fed diets containing no PKM (Table 3). Likewise, dietary supplementation of 0.025% β -xylanase had no effects on productive performance of laying hens. This result agreed with previous experiments reporting no significant effects of inclusion of 10% PKM in diets on productive performance of laying hens [1,2]. However,

Table 3. Effect of dietary palm kernel meal (PKM) and β -xylanase supplementation on productive performance of laying hens

Item		Productive performance							
		Final BW (g)	Hen-day egg production (%)	EW (g)	EM (g)	FI (g/hen/d)	FCR (g/g)	Broken and shell-less egg (%)	
Xylanase (%)	PKM (%)								
	0	0	1,988	95.7	61	58.6	115	1.97	0.61
		10	2,032	97.3	62	60.1	117	1.94	0.39
0.025	0	0	2,018	97.0	61	59.2	115	1.94	0.93
	10	10	2,036	95.8	62	59.2	117	1.98	0.54
SEM			32.1	0.76	0.4	0.49	0.8	0.021	0.222
Main effect									
Xylanase (%)									
			2,010	96.5	62	59.4	116	1.95	0.50
			2,027	96.4	61	59.2	116	1.96	0.73
			22.7	0.54	0.3	0.34	0.6	0.015	0.157
PKM (%)									
			2,003	96.3	61	58.9	115	1.95	0.77
			2,034	96.6	62	59.7	117	1.96	0.46
			22.7	0.54	0.3	0.34	0.6	0.015	0.157
Effect (p -value)									
			0.592	0.881	0.808	0.739	0.854	0.691	0.301
			0.345	0.746	0.090	0.127	0.039	0.799	0.180
			0.692	0.081	0.758	0.143	0.791	0.177	0.680

BW, body weight; EW, egg weight; EM, egg mass; FI, feed intake; FCR, feed conversion ratio.

increased FI was observed by feeding diets containing 10% PKM to laying hens in this experiment. The reason for this observation may be related to high amounts of fiber in PKM. Poultry has a tendency to consume diets until the energy and nutrient requirements are satisfied [19]. Dietary fiber is an antinutritional factor that is associated with decreased available energy and nutrients in diets, and therefore, increases in dietary fiber are often related to increased FI, which is required to compensate for the decreased intake of available energy and nutrients [4,20]. No differences in other productive performance such as hen-day egg production, EW, EM, and FCR also supported that hens fed diets containing 10% PKM had available energy and nutrient that are similar to those fed diets containing no PKM. Dietary β -xylanase supplementation has been reported to ameliorate antinutritional effects of NSP such as xylan in PKM [4]. However, all measurements for productive performance were not influenced by dietary β -xylanase supplementation in the current experiment. This observation may be explained by the fact that antinutritional effects of xylan are not noticeable when 10% PKM is included in diets. Perez et al. [2] and Abdollahi [7] also reported that 10% PKM in the diet had no antinutritional effects on productive performance of laying hens.

There were no differences in all egg quality measurements, except for egg yolk color, by feeding diets containing 10% PKM to laying hens (Table 4). For egg yolk color, hens fed diets containing 10% PKM had greater ($p < 0.05$) yolk color than those fed diets containing no PKM. This result agreed with previous experiments reporting that feeding diets containing PKM or fermented PKM to laying hens increased egg yolk color score [21,22]. The reason is likely that PKM contains high amounts of carotenoids, which are the main chemical compounds responsible for the yellow coloration of egg yolk [22,23]. However, egg quality was not affected by dietary β -xylanase supplementation, regardless of inclusion of 10% PKM in diets [1].

Table 4. Effect of dietary palm kernel meal (PKM) and β -xylanase supplementation on egg quality of laying hens

Item		Egg quality							
		Eggshell color ¹⁾				Egg yolk color	Haugh unit	Eggshell strength (kg/cm ²)	Eggshell thickness (μ m)
		Color fan	L*	a*	b*				
Xylanase (%)	PKM (%)								
0	0	12.7	54.4	19.8	30.4	8.4	91.4	4.4	407.0
	10	12.8	53.6	20.7	30.3	8.7	91.9	4.5	410.1
0.025	0	12.5	54.1	19.9	30.1	8.5	91.9	4.4	411.5
	10	12.5	54.1	20.3	29.7	8.7	91.7	4.5	412.0
SEM		0.27	0.50	0.36	0.48	0.06	0.79	0.10	3.16
Main effect									
Xylanase (%)									
0		12.7	54.0	20.3	30.4	8.6	91.7	4.4	408.5
0.025		12.5	54.1	20.1	29.9	8.6	91.8	4.5	411.7
SEM		0.19	0.35	0.26	0.34	0.04	0.56	0.07	2.24
PKM (%)									
0		12.6	54.2	19.8	30.2	8.4	91.6	4.4	409.2
10		12.7	53.9	20.5	30.0	8.7	91.8	4.5	411.0
SEM		0.19	0.35	0.26	0.34	0.04	0.56	0.07	2.24
Effect (p -value)									
Xylanase		0.419	0.865	0.599	0.355	0.675	0.888	0.790	0.322
PKM		0.757	0.496	0.080	0.645	<0.001	0.854	0.313	0.567
Xylanase \times PKM		0.938	0.407	0.467	0.784	0.410	0.640	0.901	0.682

¹⁾Eggshell color was measured by the color fan scale and CIE [11].

Neither inclusion of 10% PKM or 0.025% β -xylanase supplementation in diets influenced visual inspection scores of fatty liver, CIE colors, and lipid concentrations in the liver (Table 5). Fatty liver syndrome (FLS) or fatty liver hemorrhagic syndrome (FLHS) frequently occurs in laying hens, which is often associated with considerable economical losses in layer production [24]. In particular, FLS or FLHS prevails for laying hens that are raised in confined cages. The primary etiology includes dietary nutrients, environment, hormones, and genetic factors [13,25]. It has been reported that feeding high amounts of dietary fiber to laying hens may decrease the progress of fatty liver development by regulating lipid transport from or to the liver [26]. In addition, Akiba and Matsumoto [27] reported that dietary fiber increases lipoprotein lipase activity and reduces hormone-sensitive lipase activity, which was suggested as the reason for decreased liver lipid deposition in chicks. This is the reason why we hypothesized that inclusion of 10% PKM in diets may decrease the characteristics of fatty liver development; however, no such beneficial effect was observed in this experiment. This lack of response may be due to the fact that animals (e.g., age of hens) and environment (e.g., dietary compositions and stocking density) in the current experiments may not be enough to increase susceptibility to fatty liver development in laying hens.

Visual excreta scores and moisture concentrations were not affected by inclusion of 10% PKM in diets (Table 6). In addition, dietary β -xylanase supplementation did not influence on the excreta scores and moisture concentrations, regardless of PKM in diets. Watery excreta is highly associated with increasing production of dirty eggs, which are easily contaminated by microbes, jeopardizing the egg safety [28]. Jimenez-Moreno et al. [29] reported that excreta moisture concentrations were positively correlated with fiber concentrations in poultry diets. However, although inclusion of 10% PKM increased fiber concentrations in diets, excreta score and moisture concentrations

Table 5. Effect of dietary palm kernel meal (PKM) and β -xylanase supplementation on fatty liver incidence of laying hens

Item		Fatty liver				
		Score	Liver color ¹⁾			Lipid concentration (%)
			L*	a*	b*	
Xylanase (%)	PKM (%)					
0	0	2.1	32.5	22.1	12.4	34.7
	10	1.9	33.3	18.9	13.2	33.0
0.025	0	2.9	34.0	20.1	15.2	32.9
	10	2.4	32.4	21.1	12.0	33.9
SEM		0.36	1.73	1.01	1.93	1.73
Main effect						
Xylanase (%)						
0		2.0	32.9	20.5	12.8	33.8
0.025		2.7	33.2	20.6	13.6	33.5
SEM		0.25	1.23	0.71	1.36	1.22
PKM (%)						
0		2.5	33.3	21.1	13.8	33.8
10		2.2	32.8	20.0	12.6	33.5
SEM		0.25	1.23	0.71	1.36	1.22
Effect (p-value)						
Xylanase		0.077	0.872	0.951	0.693	0.807
PKM		0.345	0.800	0.273	0.550	0.854
Xylanase × PKM		0.604	0.488	0.051	0.320	0.451

¹⁾Liver score was measured by the scale and CIE [13,23].

Table 6. Effect of dietary palm kernel meal (PKM) and β -xylanase supplementation on excreta characteristics of laying hens

Item		Excreta characteristics	
		Score ¹⁾	Moisture (%)
Xylanase (%)	PKM (%)		
0	0	1.9	70.5
	10	2.0	70.2
0.025	0	2.0	73.0
	10	2.2	71.8
SEM		0.10	1.22
Main effect			
Xylanase (%)			
0		1.9	70.3
0.025		2.1	72.4
SEM		0.07	0.86
PKM (%)			
0		1.9	71.7
10		2.1	71.0
SEM		0.07	0.86
Effect (p-value)			
Xylanase		0.115	0.107
PKM		0.231	0.552
Xylanase × PKM		0.685	0.697

¹⁾Excreta moisture score was measured by the scale [15].

were not influenced by inclusion of 10.0% PKM in diets. It has been reported that viscous excreta induced by soluble NSP may also increase excreta moisture concentrations [30]. Thus, we expected that dietary β -xylanase supplementation may ameliorate excreta moisture concentrations when PKM is included in diets, but we failed to find the interactive effect. No change in excreta score and moisture concentrations by feeding diets containing 10% PKM appears to explain why dietary β -xylanase supplementation had no effects on excreta score and moisture concentrations.

In conclusion, PKM can be a potential feed ingredient for laying hens at the inclusion of 10% in the diet. It appears that dietary β -xylanase used in the current experiment has little effect on layer productivity, regardless of inclusion of 10% PKM in the diet. Inclusion of 10% PKM in diets is effective in improving egg yolk color of laying hens. In addition, inclusion of 10% PKM in layer diets does not increase the dirty egg production by increasing watery excreta production.

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