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Effect of xylanase and cellulase supplementation on growth performance, volatile fatty acids and caecal bacteria of broiler chickens fed with palm kernel meal-based diet

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Abstract

In this study, the effect of xylanase and cellulase supplementation in palm kernel meal (PKM) based diet on growth performance, volatile fatty acids (VFAs) and the caecal bacterial populations of broiler chickens were investigated. Seventy five day old male Cobb broiler chicks were randomly allocated to three dietary treatment groups receiving T1 (20% PKM-based diet without enzyme), T2 (20% PKM-based diet with xylanase) and T3 (20% PKM-based diet with cellulase). Each enzyme was supplemented at an amount of 200U/kg of feed. Weekly body weight gain and feed intake were recorded. All chickens were slaughtered on day 35 and the caecum content was aseptically collected for VFAs quantification and bacterial enumeration. Supplementation of xylanase and cellulase in PKM diets had different effect on the growth performance, the number of caecal bacterial population and VFAs produced. A significant reduction in the cumulative feed intake of birds fed cellulase-supplemented PKM compared to xylanse-supplemented and unsupplemented PKM diet was observed. However, the final body weights gain and cumulative feed conversion ratio (FCR) were not significantly different between the treatment groups. Determination of VFAs production of the caecal contents demonstrated a significant difference in the production of iso-butyric and n-valeric acid among treatment groups. The number of total viable bacteria, lactic acid bacteria (LAB) and coliform in caecal samples were also enumerated. Significant difference was observed in the number of caecal bacteria population between the treatment groups. The effect of xylanase and cellulase on weight gain of broiler chickens was strongly related to the feed intake rather than due to the decrease in the number of pathogenic bacteria in the caeca. Xylanase supplementation was beneficial in enhancing cumulative feed intake, weight gain and FCR of the broiler chickens, but did not entirely reduce the number of pathogenic caecal bacteria. However, cellulase supplementation reduced all parameters observed for growth performance and the number of caecal bacteria.

Key words: Xylanase, Cellulase, Palm Kernel Meal, Bacteria, Broiler

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Introduction

The price of conventional feedstuffs for broiler chickens is constantly increasing. This major constraint has drawn attention of many farmers to use non-conventional feed ingredients such as palm kernel meal (PKM) that contains high amount of non-starch polysaccharides (NSPs) component. The inclusion of PKM in the diet of broiler chickens is still low due to limited nutrient content particularly essential amino acid (e.g. lysine and methionine), grittiness (Sundu et al., 2006) and high in fiber especially in the form of NSPs (Sekoni et al., 2008). The composition of NSPs in PKM varies among the PKM produced. According to Jaafar and Jarvis (1992), the NSPs comprises of 58% mannans, 12% cellulose and 4% xylan; however, Dusterhoft et al. (1992) reported that the NPSs of PKM comprises 78% mannan, 12% cellulose, 3% glucoronoxylans and 3% arabinoxylans, respectively.

Due to low digestive enzymic activity and their tendency to create a viscous environment in the intestinal lumen, the digestion of NSPs fractions in poultry tends to be more variable (Choct and Anisson, 1992; Józefiak et al., 2004). PK can be hydrolyzed with the help of enzymes produced by the caecal microflora or by supplementation of poultry diets with specific enzymes (Choct et al., 1999; Józefiak et al., 2004). Bedford (2002) and Luo et al. (2009) had demonstrated the existence of interaction between enzymes and the host animal, its microflora, and also dietary ingredients. For example, the supplementation of exogenous enzymes such as xylanase in commercial diet helps in degradation of arabinoxylans fraction to sugars such as xylose and xylo-oligomers. This sugar can be fermented by caecal bacteria, which can stimulate the production of VFAs and the growth of specific beneficial bacteria (Bedford, 2000; Engberg et al., 2004).

Fermentation in the foregut such as rumen and hindgut (caecum or colon) produces VFAs, which can be metabolized as important energy sources for both ruminant and monogastric animals (Guo et al., 2003). The production of VFAs varies between different substrates, possibly due to selection by specific microbial species, which were best able to ferment specific compounds (Guo et al., 2003). The VFAs provides energy to the host that would otherwise be not utilized in the absence of microbial fermentation (Donalson et al., 2008). In addition, VFAs may also inhibit the growth of some pathogenic bacteria such as Salmonella spp. (McHan and Shotts 1993; Józefiak et al., 2004).

A few studies had been conducted to estimate the effect of PKM inclusion with enzyme supplementation on broiler chickens growth performance (Sundu et al., 2005) and digestibility (Sekoni et al., 2008). The supplementation of exogenous enzymes particularly NSPases in PKM-based diets is to reduce the detrimental effect of NSPs component on the digestibility of feeds and growth performance of birds; hence, improving the feed digestibility efficiency by broilers (Sundu et al., 2006). However, no study has reported the effect of enzyme supplementation in PKM-based diets on the caecal bacteria of broiler chickens. This study was therefore conducted to determine the effect of xylanase and cellulase supplementation on the

growth performance, VFAs production and caecal bacterial population of broiler chickens fed with PKM as a basis diets.

Material and methods

A commercial xylanase (*Thermomyces lanuginosus* expressed in *Aspergillus oryzae*) and cellulase (from *Trichoderma reesi* ATCC 26291) from Sigma-Aldrich were used for this experiment. The xylanase and cellulase were added in each treatment diets to provide a guaranteed minimum of 200 units per kg of feed, at an inclusion rate of 80 g and 200 g per tonne, respectively. The activity of cellulase enzyme was defined as the amount of a unit enzyme that will deliberate 1.0µmole of glucose from cellulose in one hour at pH 5.0 at 37˚C (two hour incubation time).

In the feeding trial, a total of 75 day old male Cobb broiler chicks were obtained from a commercial hatchery. The chicks were weighed and randomly allocated to three dietary treatments in a completely randomized design. Each treatment consisted of five replicates with five birds per replicate. The dietary treatments were based on maize supplemented with 20% PKM with or without enzyme supplementation. The inclusion of PKM has been reported by several authors that the optimum inclusion of PKM in broilers diet is 20%; without having any detrimental effect on the growth performance of birds (Alimon, 2004; Adrizal et al., 2011; Saenphoom et al., 2013). The feed and water were provided *ad libitum*. The experimental period was divided into two phases: starter (1 to 21 days) and grower period (22 to 35 days). The composition of the experimental diets is shown in Table 1.

The nutrient contents of the experimental diet were formulated according to Cobb 500 nutrient requirement. Birds were vaccinated against Newcastle disease, infectious bronchitis and infectious bursal disease as per recommended at day 3 and 14. MyVac 201 was used against Newcastle disease and infectious bronchitis and MyVac IBD V877 was used against infectious bursal disease. Both vaccines were purchased from Pharmaceuticals Sdn. Bhd. Malaysia. All chicks were provided with 24 hour light for the first four days, followed by lighting at night for 21 days. The chickens were inspected daily and dead birds were removed. In the fifth week of the experiment, all chickens from each pen were slaughtered. The contents of caecum were aseptically collected and pooled for VFAs quantification and bacteria enumeration.

Feed intake and body weight gain of the experimental broiler chickens were measured on weekly basis. The VFAs were measured following the procedure outlined by Wang et al. (2005). Briefly, approximately 1g of digesta was diluted with distilled water (1:1 wt/vol) in a screw-capped tube. After homogenization and centrifugation, 1 mL of clear supernatant was transferred into ampula, 0.2 mL metaphosphoric acid solution was added and subjected to another homogenization before it was placed on ice for at least 30 min to allow

Table 1: Ingredient composition and nutrient level (%) of the experimental diet.

	Diets	
	Starter (0-21 days)	Finisher (22-35 days)
Ingredients(% use)		
Corn grain	40.73	46.35
Soybean meal	28.0	26.0
Fish meal	7.0	3.63
PKM	20.0	20.0
Crude palm oil	1.0	1.0
Dicalcium phosphate	1.39	0.38
Salt	0.35	0.35
Limestone	1.0	1.6
L-lysine	0.12	0.32
DL-methionine	0.16	0.11
Vitamin premix[1]	0.13	0.13
Mineral premix[2]	0.13	0.13
Nutrient analysis		
Dry matter (%)	86.41	88.77
Protein (%)	21.34	20.51
Energy (Kcal/kg)	3441.80	3541.1
Crude Fiber (%)	7.54	8.22
Crude fat (%)	3.30	3.37
Calcium (%)	0.96	0.9
Phosphorus (%)	0.57	0.49
Digestible amino acids (%)		
Lysine	1.47	1.41
Methionine	0.35	0.44

[1]Vitamin premix provided per tones of diets: vitamin A 50.00 MIU; vitamin D3 10.00 MIU; vitamin E 75.00 gm; vitamin K3 20.00 gm; vitamin B1 10.00 gm; vitamin B2 20.00 gm; vitamin B6 20.00 gm; vitamin 12 0.10 gm; Calcium D-Pantothenate 60.00 gm; Nicotinic acid 5.00 gm; Folic acid 5.00 gm; Biotin 235.00 g.
[2]Mineral premix provided per tones of diets: Selenium 0.20 gm; iron 80.00 gm; Zinc 80.00 gm; Copper 15.00 gm; Potassium Chloride 4.00 gm; Magnesium oxide 0.60 gm; Sodium Bicarbonate 1.50 gm; Iodine 1.00 gm and Cobalt 1.25 g.

the protein to settle. The sample was centrifuged (10,844 *g*) for 10 min and the supernatant was analyzed using gas chromatography (Agilent 69890N Series Gas Chromatography System from Agilent Technologies, USA) equipped with a flame ionization detector.

For bacterial quantification, on each sampling day, the caecum was aseptically removed and squeezed into a sterile container, placed immediately on ice and immediately transferred to laboratory. Approximately, 5 g of fresh caecal content was homogenized with 45 mL sterile peptone water and shaken vigorously for about 5 minutes before being subjected to10-fold serial dilution. Approximately 100 µL of dilutions were evenly spread onto media agar plates in triplicates. Total viable bacteria, LAB and coliform bacteria were assessed on the nutrient agar, de Man, Rogosa and Sharp (MRS) and MacConkey agar, incubated anaerobically at 37 ˚C for 48 hours. Numbers of colony-forming units were expressed as log 10 CFU/g of caecal digesta.

Proximate analysis was conducted on the feed sample to determine dry matter, ash, ether extract, crude protein, and crude fibre based on the methods describe by association of Official Analytical Chemists (AOAC, 1997). Amino acids were determined using HPLC (HP Agilent 1100 series, USA); crude protein was determined by Kjeldahl apparatus (Kjeltech Analyzer, Foss Detector, Switzerland) and gross energy was determined using an oxygen bomb calorimeter (Parr Oxygen Bomb 1108, USA).

Proximate data and effect of enzyme supplementation on growth performance, caecal bacteria counts and VFAs production were subjected to General Linear Model (GLM) procedures of SAS® 9.0 (SAS Institute). Differences were considered significant at $P < 0.05$.

Results and discussion

In this experiment mean mortality rates was recorded at 7% and was not related to the dietary treatment. The effect of dietary supplementation of 20% PKM with or without enzyme on average daily gain (ADG), average daily feed intake (ADFI), average daily feed conversion ratio (ADFCR) and average cumulative feed intake (CFI), cumulative weight gain (CWG) and cumulative FCR (CFCR) over 35 days are presented in Table 2. During the starter phase, the ADFI of birds in xylanase and cellulase supplemented bird (T2 and T3) was found not to be significantly different from unsupplemented bird. Even though, numerically the ADFI of enzyme-supplemented bird was slightly higher that unsupplemented bird, the ADG and FCR of both groups (T2 and T3) were not improved compared to birds that received no enzyme supplemented PKM-based diet. However, in the finisher phase, supplementation of xylanase had improved the ADG and AFCR of birds, whilst cellulase supplementation had poorest ADG compared to T1 and T2 group. Cumulatively, no significant differences were observed in term of performance of birds between the treatment groups.

The addition of xylanase and cellulase in PKM-based diet in the current study demonstrated a different effect on the growth performance of broiler chickens. The ADFI, ADG, and ADFCR of birds supplemented with xylanase were not significantly different from this receiving only PKM-based diet. The present data is in line with the data presented by Luo et al. (2009). It has been proposed that the effects of enzyme is depending on the age of the bird as young bird has lower capacity to stand the negative effect of NSPs in the gut. Therefore, the addition of enzyme will not have much effect on the NSPs (Choct et al., 1996). Overall, the final body weight gain of broiler chickens fed with diet supplemented with xylanase was higher compared to unsupplemented and xylanase supplemented PKM-based diet.

This study produced results which corroborate the findings of Nian et al. (2011) where similar effect was observed in broiler chickens supplemented with xylanase. Supplementation of xylanase into the basal diet can help in breaking down plant cell wall, releasing the nutrients by reducing the cell integrity that were previously encapsulated to improve digestive function and to promote animal performance (Bedford, 2000). As expected, the current study demonstrated the positive effect of xylanase supplementation in PKM-based diets for broiler chickens. The FCR was improved by addition of xylanase, which is congruent with earlier reports (Goa et al., 2008; Nian et al., 2011). The improvement of body weight gained and FCR of broiler chickens in the current study was somewhat similar to that reported by Friesen et al. (1992). They reported that broiler chick fed wheat-based diets supplemented with xylanase had the body weight improve by 2.9% and feed conversion by 9.2%, respectively, which in our case it has improved by 6.6% and 2.85%.

Table 2: Growth performance (means ± SE) of broiler fed 20% PKM diets supplemented with or without enzymes.

Parameters	Dietary treatment			
	T1 None	**T2** Xylanase	**T2** Cellulase	*P*
Starter (1-21 days)				
Daily feed intake (g)	58.63±0.17	61.18±2.23	61.03±1.56	NS
ADG (g)	29.31±0.51[a]	28.53±0.54[a]	26.57±0.75[b]	**
FCR	2.0±0.03[b]	2.1±0.08[a]	2.3±0.07[a]	**
Finisher (22-35 days)				
Daily feed intake (g)	158.33±4.68[a]	149.21±8.88[ab]	134.56±5.81[b]	NS
ADG (g)	61.98±1.49[ab]	66.78±1.57[a]	58.48±2.78[b]	*
FCR	2.5±0.05[a]	2.2±0.06[b]	2.3±0.06[b]	**
Cumulative				
Daily feed intake (g)	87.35±1.96	85.45±4.07	79.31±2.80	NS
ADG (g)	39.95±0.79[ab]	41.58±0.92[a]	37.94±1.51[b]	NS
FCR	2.2±0.04	2.1±0.05	2.1±0.09	NS
Initial BW (g)	38. 84±0.64	40.10±0.59	40.08±0.59	NS
Final BW (g)	1436.84±27.94[ab]	1495.23±32.5[a]	1367.81±55.48[b]	*

*Significantly different at 5% level ($P < 0.05$), **significantly different at 1% level ($P < 0.01$), ***significantly different at 0.1% level ($P < 0.001$)
NS: Not significant
[a-c]Means with different letter within a row differed significantly.

The improvement in FCR observed in this study is likely to be as a result of increase in the hemicellulose digestibility. The ability of xylanase to break down arabinoxylan polymers in PKM into smaller polymers has been shown to reduce gut viscosity and nutrient entrapment that lead to better digestion in broiler chickens (Bedford, 2000; Khattak et al., 2006; Shakouri et al., 2009).

The supplementation of cellulase enzyme in this study did not improve the performance of birds fed PKM-based diet, yet it had the poorest performance compared with other two treatment diets (T1 and T2). The result observed is in agreement with the previous study by Pontel et al. (2004). There is the possibility that the cellulase enzyme was degraded or inhibited during the passage throughout the intestinal tract (Pontel et al., 2004).

The effect of dietary supplementation of 20% PKM-based diet with or without enzyme on total viable bacterial, LAB and coliform population expressed as log 10 CFU/g of caecal digesta are presented in Table 3. In general, the number of total viable bacteria, LAB and coliform of caecal digesta were significantly different ($P < 0.01$) among the treatment groups. Xylanase supplementation increased the number of total viable bacteria and coliform bacteria in the caecal content (Table 5). The LAB was reduced significantly ($P < 0.01$) when 20% PKM-based diet was either supplemented with xylanase or cellulase.

In the current study, the supplementation of xylanase significantly increased ($P < 0.01$) the number of total viable bacteria, and coliform bacteria in the caecal contents. However, xylanase supplementation significantly reduced ($P < 0.01$) the numbers of LAB. On the other hand, cellulase supplementation had significantly reduced ($P < 0.01$) the number of ceacal bacterial population. The reduction of the LAB and increased of coliform bacterial population in the enzyme-supplemented group was in an agreement with a study conducted by Shakouri et al. (2009). High number of coliform bacteria observed in the xylanase-supplemented group may be manifested by the availability of xylobiose that derived from the utilization of

Table 3: The bacterial population in the caecal digesta (log 10 CFU/g digesta) fed 20% PKM diets supplemented with or without enzymes.

Bacteria concentration	Treatment groups			*P*
	T1 None	**T2** Xylanase	**T3** Cellulase	
Total bacteria	32.7±3.8[b]	50.0±4.5[a]	23.7±3.5[b]	**
Lactic acid bacteria	7.0±1.0[a]	2.7±0.9[b]	1.0±0.0[b]	**
Coliform bacteria	37.0±11.0[b]	79.7±10.7[a]	17.0±2.7[b]	**

**significantly different at 1% level ($P < 0.01$)
[a,-c]Means with different letter within a row differed significantly.

xylans by certain bacteria such as *Bacteriodes* spp. (Salyers et al., 1977). The coliform bacteria are known to have a higher affinity towards xylobiose and may induce the multiplication of coliform bacteria in xylanase treated group (Salyers et al., 1981).

Xyalanase supplementation in PKM-based diet had increased the numbers of coliforms in caecal contents. This is in contrast with a data reported by Nian et al. (2011). Such effect is difficult to explain, and the difference could be due to the different substrates used in the broiler feed. As described earlier, high numbers of coliform bacteria in the xylanase treated group may be due to the higher affinity of this bacterial group to utilize xylose and xylo-oligomers for their growth.

Reduction of coliform bacteria population observed in caecal contents of bird supplemented with cellulase in the current study could be contributed by few factors. Increase in VFAs production of caeca could have a bacteriostatic effect on the pathogenic bacteria population; thus, may impair their multiplications as reported by McHan and Shotts (1993) and Józefiak et al. (2004). The effect of enzymes on the caecal microbiota is well documented by Bedford (2002). It was proposed that the enzymes supplementation in the diet work during ilaeal and caecal phase, where in the ileum enzymes depressed the bacteria population by accelerating the rate of digestion and absorption, and limiting the amount of substrates for the bacteria, whilst in the caecum, substrate produced (poorly absorbable simple sugar) in the ileum might escape and enters the caecum where it can be fermented by beneficial bacteria and produces the VFAs. The anti-bacterial activity of VFAs is related to the reduction of the caecal pH, in which when the VFAs are in undissociated form, they can easily diffuse across into the cell of bacteria causing internal cell damage to these bacteria (van der weilan et al., 2000).

The addition of exogenous enzyme can indirectly alter the microbial population on the substrate that bacteria use as a carbon source (Choct et al., 1999). The use of enzyme in the diet could increase the fermentation in the caecum, which apparently was due to the lower molecular weight carbohydrates inflowing the caecum (Józefiak et al., 2004). The use of these enzymes appear to influence the intestinal microbiota through two main mechanisms, that is subtraction of fermentable starch and protein through accelerated digestion, and provision of soluble, fermentable oligosaccharides as a result of depolymerisation of insoluble fiber (Choct et al., 1996; Bedford, 1996; Nian et al., 2011). It is also noted that the decrease in the number of caecal pathogenic bacteria in the NSPases supplemented group may be due to the reduction in digesta viscosity (Bedford and Classen, 1993). The reduction in digesta viscosity will eventually increase

Table 4: The VFAs production in caecal contents of T1, T2 and T3 expressed as percentages (%) (Mean ± SE) of the total VFAs in caecal contents.

	Treatment groups			
	T1	**T2**	**T3**	
VFA	None	Xylanase	Cellulase	*P*
Acetic	48.9±2.7[b]	58.6±1.9[a]	55.8±3.6[ab]	NS
Propionic	10.5±1.7[ab]	7.8±0.4[b]	14.1±2.5[a]	NS
Iso-butyric	17.0±1.9[b]	18.7±0.9[ab]	23.5±1.7[a]	*
n-butyric	12.9±1.2[a]	10.8±1.6[a]	18.8±7.9[a]	NS
Iso-valeric	9.6±1.3[a]	6.8±0.7[a]	7.3±2.1[a]	NS
n-valeric	18.9±0.6[a]	7.1±0.9[b]	3.9±0.9[c]	**

*Significantly different at 5% level ($P < 0.05$), **significantly different at 1% level ($P < 0.01$),
NS: Non significant
[a-c]Means with different letter within a row differed significantly

the digesta passage rate, which may have adverse effect to the life cycle of bacteria. However, the changes in the number of bacteria in caecum did not appear to result in detrimental effect on the performance of broiler chickens in the current study.

The amount of VFAs produced from the ceacal content of the broiler fed with different diets is shown in the Table 4. The VFAs product analyzed were acetic acid, propionic, iso-butyric, n-butyric, iso-valeric and n-valeric. The composition of iso-butyric and n-valeric acid produced was significantly different ($P < 0.05$) between the treatment groups. Contrary, the acetic, propionic, n-butyric and iso-valeric acid productions were not significantly different.

The breakdown of crude fiber particularly NSPs fraction via fermentation process in broiler chickens takes place in the caecum where, the type and extent of fermentation depends on the microbial population, and the nature and amount of substrate which enter the caecum (Anisson et al., 1968). The present data showed that, addition of cellulase and xylanase in the PKM-based diets has no significant effect on the percentage of VFAs produced, except for iso-butyric and n-valeric acid produced between the control and enzyme supplemented groups. The increase in acetic acid production in the present study was similar to the result reported by Engberg et al. (2004). No significant effect was observed between the enzyme-supplemented and unsupplemented groups.

Vahjen et al. (1998) and Dunkley et al. (2007) also reported that acetic acid is the primary VFA produced from the caecal fermentation followed by propionate and butyrate by numerous anaerobes. The results is hardly to infer because acetic acid produced cannot easily be correlated with particular substrates or bacterial groups since, proteinous substrates or amino acids as well as carbohydrate fermentation also yield acetate and can be produced by a wide range of intestinal bacteria as a byproduct (Vahjen et al., 1998).

The increase in acetic acid production from enzyme supplemented groups may be explained through a two mechanisms including: i) supplementation of enzyme in PKM-based diets facilitated the breakdown of NSPs to simpler sugar (e.g. xylan to xylose and xylo-oligomers) that might escape from enzymatic digestion, which later may enter the caecum and was fermented by the caecal microflora (Choct et al., 1999; Bedford, 2000); and ii) enterobacteria such as *E. coli* in the caecum are capable of producing substantial amounts of acetate from fermentation of pentoses (xylose and xylo-oligomers) and hexoses (Vahjen et al., 1998).

Conclusion

In conclusion, results obtained in the current study showed that the supplementation of xylanase and cellulase in PKM-based diets had different effects on the growth performance, the number of caecal bacterial population and VFAs produced. The effect of xylanase and cellulase on weight gain of broiler chickens was to be strongly related to the feed intake rather than low number of pathogenic bacteria population in the caeca. Both acetic and iso-butyric acid production was increased with the enzyme supplementation. The increase in the caecal VFAs production may have beneficial effects on broiler chickens health either by enhancing the assimilation of nutrients, providing an extra energy to the broiler chickens and/or by reducing pathogenic bacteria colonization in the caecum.

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