Influence of dietary palm kernel cake on growth performance, carcass composition, meat quality, volatile fatty acids, intestinal bacteria population and villi histology of Cherry Valley ducks

Samsudin[1 *], A.A., Sharmila[1], A., Jahromi[2], M. F., Shokryazdan[2], P., Mohd Nor[1], A.A. and Hendry[1], N.

[1]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, [2]Agriculture Biotechnology Research Institute of Iran (ABRII), East and North-East Branch, P.O.Box 91735, 844 Mashhad, Iran.

*Corresponding author: anjas@upm.edu.my

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Abstract

The effects of feeding palm kernel cake on growth performance, carcass composition, meat quality, volatile fatty acids, intestinal bacteria population and villi histology of Cherry Valley ducks were investigated. A total of 36 one-day old Cherry Valley ducklings were randomly allocated to 3 dietary groups, namely T1: basal diet (control); T2: basal diet + 15% palm kernel cake (PKC) and T3: basal diet + 35% PKC. After 56 d of experiment, results showed that dietary PKC had no significant effect on final body weight and weight gain of ducks. However, ducks fed T3 had higher feed intake and lower feed conversion ratio compared with other treatments. Dressing percentage and weight of liver and heart decreased as the level of PKC increased, while gizzard weight increased. Dietary PKC had no significant effect on the color coordinates, tenderness, drip and cooking losses and pH of muscle. Bacterial population varied significantly among gut sections, except for jejunum. Ducks fed T2 had highest villus height and crypt depth, while the ratio of villus height to crypt depth was the highest for control ducks. Our findings suggest that PKC can be incorporated in the diet of ducks up to 35% to improve their gut health without any adverse effect on growth performance or meat quality.

Keywords: palm kernel cake, Cherry Valley ducks, growth performance, meat quality, bacterial population, volatile fatty acids

Introduction

Palm kernel cake (PKC) is a by-product of palm kernel oil extraction and contains approximately 16-18 % crude protein and 13-20% crude fiber. It is produced in large amount in Malaysia, and attempts have been made to utilize it as a feedstuff in livestock diets (Wan Zahari and Alimon 2004; Chong *et al*,. 2008). The utilization of PKC portends great benefits to the livestock industry because it is relatively cheap, readily available and virtually not competing with man and farm animals (Kperegbeyi and Ikperite, 2011). The utilization of PKC in livestock feed represents a valuable way of converting waste to wealth, and thus enhances the total revenue accruable from the oil palm industry. In spite of the benefits of PKC, its usage in monogastrics is limited due to its high fibre content. However, previous studies by Allen *et al.* (1997) and Fernandez *et al.* (2002) suggested that PKC can be

used to replace commercially available mannan-oligosaccharides as prebiotic to enhance chicken health and immunity. Many studies have examined the efficacy of PKC in poultry diets (Onwudike 1986; Zulkifli *et al.,* 2003; Chong *et al.,* 2008; Mustafa *et al.,* 2014; Jahromi *et al.,* 2015; Sharmila *et al.,* 2015). However, the recommended levels of inclusion vary from one study to another due to variation in the class of poultry, age and sex of birds, as well as the origin and variations in the oil and shell content of the PKC used (Alimon, 2004). Thus, information derived from such studies could not be totally relied upon when utilizing PKC in Cherry Valley ducks. In addition, the impact of dietary PKC on meat quality and gut health and microflora in Cherry Valley ducks is poorly understood and documented. Having such information could offer an opportunity to optimize the utilization of PKC in Cherry Valley ducks. Thus, this study was conducted to examine the consequences of feeding PKC on growth performance, intestinal microflora and morphology, carcass traits and meat quality in Cherry Valley ducks.

Materials and Methods

Feeding trial and sampling procedures

A total of 36 one-day old Cherry Valley ducklings purchased from a local farm in Selangor, Malaysia were used. The ducklings were randomly and equally assigned to 3 dietary groups: T1 (basal diet), T2 (basal diet + 15% PKC) or T3 (basal diet + 35% PKC) and fed for 56 d (Table 1). The experimental diets were *iso*-caloric and *iso*-nitrogenous and were formulated to meet the minimum requirements of ducks as recommended by the National Research Council (NRC, 1994). Each treatment group consisted of triplicates with 4 ducks per replicate. Battery cages of 2 x 1.9 x 1.4 m in height, length and width, respectively, with 6 compartments with an individual compartment size 0.5 x 0.9 x 1.4 m in height, length and width, respectively were used. A commercial diet was offered to all ducks upon their arrival until 14 d. Thereafter, ducks were fed with the experimental diets until they were 56 d old. During the experimental period, feed and water were provided *ad-libitum*, and the weight gain and feed intake of ducks were recorded weekly. At the day 57 the ducks were slaughtered and the needed samples for laboratory analysis were collected.

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

	Dietary treatment		
Ingredient (%)	Control	15% PKC	35% PKC
Corn	65.66	53.44	36.92
PKC	-	15.00	35.00
Soybean meal	21.00	18.80	15.90
Fish meal	3.00	3.00	3.00
Wheat pollard	7.00	4.30	0.40
Palm oil	1.00		6.50
Salt	0.25		0.25
Vitamin	0.05	0.05	0.05
Minerals	0.05	0.05	
Dicalcium phosphate	1.56	1.56	
Limestone	0.35	0.34	0.33
DL-Methionine	0.04	0.05	3.20
L-lysine	0.04	0.05	0.25
Nutrient analysis			
Calculated ME, kcal/kg	2981	2945	2920
DM (%)	88.31	90.67	92.51
CP (%)	16.3	15.4	14.3
EE (%)	3.80	5.23	8.16
CF (%)	2.72	4.43	7.02

Vitamin premix[1] provided per ton of diets: vitamin A 50.00 MIU; vitamin D3 10.00 MIU; vitamin E 75.00g; vitamin K3 20.00g; vitamin B1 10.00g; vitamin B2 20.00g; vitamin B6 20.00g; vitamin 12 0.10g; calcium D-pantothenate 60.00g; nicotinic acid 5.00g; folic acid 5.00g; biotin 235.00g. Mineral premix[2] provided per tones of diets: selenium 0.20g; iron 80.00g; zinc 80.00g; copper 15.00g; potassium chloride 4.00g; magnesium oxide 0.60g; sodium bicarbonate 1.50g; iodine 1.00g and cobalt 1.25g.

Dressing percentage and internal organs weight analysis

At the end of the feeding trial, all ducks were slaughtered after 12 h of feed withdrawal, defeathered and eviscerated. The head, legs and viscera from each duck were removed and carcass was weighed. The dressing percentage was calculated by dividing the carcass weight to live weight of duck multiplied by 100 as described by Rizal (2006). The internal organs such as liver, heart and gizzard were weighed and their weights were expressed as percentage of the carcass weight.

Meat quality analyses - Muscle sampling

The meat quality analyses were assessed based on the *Pectoralis major* muscle of the ducks. The left *Pectoralis major* muscle was dissected from the carcass (n = 36) and divided along the long axis into 2 parts of approximately 60 g each. The samples were kept in the chiller at 4°C and assigned for the determination of drip loss and pH at 24 h. The right *Pectoralis major* muscle was divided along the long axis into 3 parts, kept in the chiller and assigned for the determination of color coordinates, cooking loss and shear force at 24 h postmortem.

Determination of muscle pH

The meat samples were pulverized in liquid nitrogen. About 0.5 g of each pulverized muscle sample at 15 min and 24 h postmortem was homogenized in 2 ml of deionized water in the presence of 5 mM sodium iodoacetate (Merck Schuchardt OHG, Germany) to prevent further glycolysis. The pH of the resultant homogenate was read using a pH meter (Mettler Toledo, USA).

Determination of drip loss

Drip loss was determined following the method described by Salwani *et al.* (2015). About 30 g of each muscle sample was weighed (W1) at 15 min postmortem, vacuum packed and stored in a chiller maintained at 4 °C. At 24 h postmortem, the samples were gently dabbed dry with clean tissue paper, reweighed and recorded as W2. The percentage drip loss was calculated using the following formula:

Drip loss (%) = (W1- W2)/W1 × 100

Determination of color coordinates

The color coordinates of muscle samples (40 g, 12 mm thickness), were determined by measuring L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) values at 24 h postmortem using a Color Flex spectrophotometer (Hunter Lab Reston, Reston, VA, USA). The colorimeter was calibrated against black and white reference tiles prior to use. Triplicate measurements were taken for each sample and the average was used for the analysis.

Determination of cooking loss

Cooking loss was determined as described by Sabow *et al.* (2015). About 40 g of each sample was weighed and recorded as W1 held in polyethylene bags, vacuum packed and subsequently submerged in 80°C water bath until the core temperature of the muscles reached 80°C. Samples were removed from the water bath and allowed to cool down to room temperature prior reweighing and recorded as W2. The percentage cooking loss was calculated using the following formula:

Cooking loss (%) = (W1- W2)/W1 × 100

Determination of shear force

The samples used for the cooking loss analysis were the same samples used for the shear force analysis. The samples were cut into blocks (2 blocks per sample) of 10 mm in width, 10 mm in height and 15 mm in length. The shear force values were determined using a Volodkevitch bite jaw fitted to a texture analyzer (TAHD plus®, Stable Micro Systems, Surrey, UK). The shearing was consistently conducted perpendicular to the orientation of muscle fibers of each sample block.

Determination of volatile fatty acids (VFA)

The VFAs were measured following the procedure outlined by Wang *et al.* (2005). Briefly, 1 g 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 a new tube, and 0.2 ml metaphosphoric acid was added. The mixture was subjected to another homogenization before it was placed on ice for at least 30 min to allow the protein to settle completely. The samples were centrifuged (10 844 *g*) for 10 min and the supernatants were analyzed using gas chromatography (Agilent 69890N Series Gas Chromatography System from Agilent Technologies, USA) equipped with a flame ionization detector.

Microbial quantification - Digesta sampling

Immediately after the slaughtering of ducks, the intestinal tracts were dissected, and digesta content of duodenum, jejunum, ileum and caecum were removed and stored at -20˚C until further analysis for quantification of bifidobacteria, lactic acid bacteria (LAB), *Enterococcus*, enterobacteria and *E. coli*.

DNA isolation of digesta

DNA was isolated from the digesta using a QIAamp DNA stool kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. At the end, DNA was eluted in 200 µL elution buffer and concentration was determined by a spectrophotometer. The DNA was stored at -20˚C until use for real-time PCR assays.

Quantitative real-time PCR

The populations of bifidobacteria, LAB, *Enterococcus*, enterobacteria and *E. coli* of duodenum, jejunum, ileum and cecal digesta were analyzed by qPCR. Genomic DNA from duodenum, jejunum, ileum and cecal digesta was used as templates for PCR amplification. Absolute quantification of intestinal bacteria was achieved by using standard curves constructed by amplification of known amount of target bacteria DNA. The qPCR master mix was prepared on a total volume of 25 µL using the QuantiFast® SYBR® Green PCR kit (Qiagen Inc., Valencia, USA) consisted of 12.5 µL of 2 × SYBR Green Master Mix, 1 µL of 10 µM forward primer, 1 µL of 10 µM reverse primer, 2 µL of DNA samples and 8.5 µL of nuclease-free water for each reaction. Each sample was analyzed with 6 replication reactions. The targeted intestinal bacterial groups, primer sequences, annealing temperature and literature references in this study are detailed in Table 2. The qPCR assay was performed with BioRad CFX96 real-time PCR system (BioRad, USA) using optical grade plates as follows: the qPCR cycling conditions comprised an initial denaturation at 94˚C for 5 min, followed by 40 cycles of denaturation at 94 ˚C for 20 sec, primer annealing at 50 ˚C for *E. coli*, 58 ˚C for LAB, and 60 ˚C for both bifidobacteria and enterobacteria, respectively, for 30 sec, and extension at 72˚C for 20 sec (Navidshad *et al.* 2012).

Table 2: The sequence of primers used targeting bifidobacteria, LAB, *Enterococcus*, enterobacteria and *E. coli*

Target bacteria	Sequence 5'-3'	Annealing temperature (˚C)	References
Bifidobacteria	**F**- GGGTGGTAATGCCGGATG **R**- TAAGCCATGGACTTTCACACC	60	Bartosch et al. (2005)
LAB	**F**- CATCCAGTGCAAACCTAAGAG **R**- GATCCGCTTGCCTTCGCA	58	Wang et al. (1996)
Enterococcus	**F**- CCCTTATTGTTAGTTGCCATCATT **R**- ACTCGTTGTACTTCCCATTGT	60	Navidshad et al. (2012)
Enterobacteria	**F**- CATTGACGTTACCCGCAGAAGAAGC **R**-CTCTACGAGACTCAAGCTTGC	60	Navidshad et al. (2012)
E.coli	**F**- GTGTGATATCTACCCGCTTCGC **R**-AGAACGCTTTGTGGTTAATCAGGA	50	Frahm and Obst (2003)

Ileal histological analysis

Approximately 5 cm of ileum sample was subjected to histological examination. The ileum samples were sectioned 1 to 2 cm, flushed and kept in a neutral buffered formalin solution until further morphometric analysis. The samples were then cut into 2 parts from each segment for cross and length section of the intestinal surface. Each segment was fixed in 10% formalin. Intestinal samples were excised and dehydrated for 16 h in a tissue processing machine, then embedded in paraffin wax. Each section was cut in 4-µm thickness and fixed onto glass slides, heated at 57°C until samples were dried. The samples were stained with haematoxylin and eosin, mounted with cover slips. The villus height was measured as the distance between the crypt mouth and the tip of villi. The crypt depth was measured as the distance between the basement membrane and the mouth of crypt using a microscope and read by PC Life Science Olympus software (Olympus Soft Imaging Solutions).

Statistical analysis

The experiment followed a completely randomized design model. Data obtained from all parameters were subjected to General Linear Model (GLM) procedures of SAS (SAS Institute). The differences between the means of groups were separated by Duncan multiple range test.

Results and Discussion

Feed intake and growth performance

The feed intake and growth performance characteristics of ducks fed graded levels of PKC are shown in Table 3. There was no significant difference in terms of body

weight, weight gain, feed intake and feed conversion ratio (FCR) between the ducks receiving 15% PKC and the ducks of the control group. Ducks fed 35% PKC had higher (P<0.05) feed intake and FCR compared to those fed 15% PKC and the control diet. However 35% of PKC had no effect (P > 0.05) on the final body weight and weight gain of ducks. In this regard, the relationship between live-weight gain, and the associated feed cost is of paramount importance. The use of corn in poultry feed is expensive and would increase the cost of production. Moreover, it is known that the cost of feeding represents 70% of the total variable cost of poultry production. Inclusion of 35% PKC in the ducks feed can be accepted as a least cost ration that would partially replace corn in the commercial diet and would help farmers to increase their revenue. The increase in feed intake with higher level of PKC could be attributed to the lower digestibility of the nutrients in PKC due to its high fibre content. Thus, the ducks ate more PKC to satisfy their nutrient requirements. These observations are in tandem with those of earlier trials (Zulkifli *et al.,* 2003; Zulkifli *et al.,* 2007; Chong *et al.,* 2008) in chickens. This observation is also consistent with that of Okeudo *et al.* (2006) who observed that dietary supplementation of up to 30% PKC had no effect on the final body weight and weight gain in broiler chickens. However, they observed that birds fed 45% PKC had reduced body weight.

Table 3: Growth performance characteristics of ducks fed different levels of PKC (Means ± SE)

Treatment	Body weight (g)	Weight gain (g)	Feed intake (g)	FCR
Control	2521.67 ± 15.89	1881.00 ± 13.57	6351.67 ± 32.59[b]	2.52 ± 0.01[b]
15% PKC	2509.00 ± 17.57	1863.33 ± 14.83	6402.33 ± 28.95[b]	2.55 ± 0.02[b]
35% PKC	2543.33 ± 17.05	1886.00 ± 13.57	7044.00 ± 41.28[a]	2.77 ± 0.02[a]

[ab] Means with different letter within a column differ significantly (P < 0.05)

Dressing percentage and internal organ weight

As shown in Table 4, the dressing percentage of ducks decreased (P< 0.05) as the level of PKC increased in the diet. This finding is in contrast to that of Okon and Ogunmodede (1996) who observed that the dressing percentage of broiler chickens increased as the level of PKC was increased in the diet. On the other hand, Okeudo *et al.* (2006) who observed that broiler chickens fed 30% PKC had higher dressing percentage compared to those fed 0 and 45% PKC. This discrepancy between the results of different studies could be due to some factors such as different classes of poultry, different sources of PKC included in the diet and different conditions of rearing.

Ducks fed the control diet had higher (P< 0.05) liver weight compared with those fed PKC. The ducks of control and the 15% PKC groups had similar heart weight which was higher (P < 0.05) than those fed 35% PKC diet. The weight of gizzard increased (P< 0.05) as the level of PKC increased in the diet. Similarly, gizzard weight increased in response to dietary PKC in broiler chickens (Okuedo *et al.,* 2005; 2006). Onwudike (1986) pointed out that increase in the weight of gizzard could be related to the higher level of crude fiber and the grittiness of PKC in which the birds had to adapt themselves to digest the gritty and fibrous feed. This finding is also supported by Branion (1963) where the size of gizzard will be affected by the amount of work required by the muscular wall of gizzard to grind the feed offered.

Table 4. Dressing percentage (%) and organ weight (%) of ducks fed different levels of PKC (Means ± SE)

Treatment	Dressing percentage	Liver	Heart	Gizzard
Control	70.74± 0.04[a]	29.50± 0.27[a]	8.74± 0.01[a]	44.31 ± 0.22[a]
15% PKC	70.21± 0.03[b]	28.77± 0.10[b]	8.71 ± 0.01[a]	46.99 ± 0.39[b]
35% PKC	68.83± 0.12[c]	28.44± 0.03[b]	8.49± 0.02[b]	53.78 ± 0.36[c]

[abc] Means with different letter within a column differ significantly (P < 0.05)

Meat quality

Dietary PKC had no effect (*P*> 0.05) on the physicochemical properties of *Pectoralis major* muscle in Cherry valley ducks (Table 5). The muscle pH was not influenced by dietary PKC. The pH is a reliable indicator of the potential quality of meat (Adeyemi and Sazili, 2014). The pH observed in all treatments fell within the range of the normal ultimate pH of meat of about 5.4 to 5.7 (Tougan *et al.*, 2013; Adeyemi and Sazili, 2014). The color coordinates, drip and cooking losses and shear force values of *Pectoralis major* muscle in Cherry valley ducks was not influenced by dietary PKC. This could be attributed to the similar pH values between the treatments. Similarly, Okuedo *et al.* (2006) did not observe changes in the meat quality of broiler chickens fed graded levels of PKC.

Table 5: Physicochemical properties (%) of *Pectoralis major* muscle of duck fed different levels of PKC (Means ± SE)

Parameter	Dietary treatment		
	Control	15% PKC	35% PKC
pH	5.76 ± 0.05	5.79 ± 0.05	5.73 ± 0.05
Drip loss	4.62 ± 0.12	4.81 ± 0.15	4.33 ± 0.14
Cooking loss	33.23 ± 0.12	33.47 ± 0.13	32.98 ± 0.17
Shear force	1127.81±125.36	1288.16±145.07	942.17±103.01
Colour coordinates			
Lightness (L*)	36.56±0.94	34.84±1.25	37.17±1.00
Redness (a*)	11.81±0.26	12.08±0.49	12.44±0.65
Yellowness (b*)	13.57±0.44	12.99±0.47	14.15±0.31

Volatile fatty acids

The VFA profiles of the duodenal, jejuna, ileal and cecal digesta of ducks fed graded level of PKC are shown in Table 6. Based on the results, in most of the cases, acetic acid was the most abundant VFA produced followed by propionate, *iso*-butyrate, *n*-butyrate, *iso*-valerate and *n*-valerate. These observations are consistent with those of Dunkley *et al.* (2007) and Vahjen *et al.* (1998) who reported that acetate was the primary VFA produced followed by propionate and butyrate from the fermentation of feedstuffs by laying hens and broiler chickens in cecal contents. Although, it was reported that the breakdown of crude fiber via fermentation process in poultry took

place mainly in the cecum (Anisson *et al.* 1968), the current results suggest that substantial fermentation of dietary fibre also takes place in the duodenum, jejunum and ileum. Dietary PKC had no effect ($P > 0.05$) on the concentration of acetate in the duodenal and jejuna digesta samples. Contrarily, the amount of acetate in ileal and cecal digesta samples increased ($P< 0.05$) for the group receiving 35% of PKC in comparison to the other groups. The control ducks had higher ($P< 0.05$) concentration of propionate in the duodenum and jejunum compared with those fed other diets. The concentration of iso-butyrate in the ileal and cecal digesta samples increased as the level of PKC increased in the diet. These observations could be attributed to the increase in the fiber content of the diet as the level of PKC increased in the diets. Generally, only trace amounts of *n*-butyrate, *iso*-valerate and *n*-valerate were detected in the digesta samples of ducks fed 35% PKC. The changes in VFA pattern could be due to the changes in the gut microflora induced by dietary PKC. It has been reported that the production of acetic, propionic, and butyric acids by gut microbiota, especially LAB, as the major fermentation products, is an important defence mechanism for limiting colonization of pathogenic bacteria, such as *Salmonella* sp. and *E. coli* (Dunkley *et al.*, 2007).

Table 6: Effects of different levels of PKC on intestinal VFAs (mMol/mL) of ducks (Mean ± SE)

		Dietary treatments		
Part of GIT	VFA	Control	15% PKC	35% PKC
Duodenum	Acetate	129.72±51.92	118.10±16.62	112.48±19.08
	Propionate	210.64±13.94[a]	86.45±22.39a[b]	69.60±0.00[b]
	Iso-butyrate	ND	26.96±6.35	8.51±0.00
	n-butyrate	ND	ND	ND
	Iso- valerate	ND	ND	ND
	n- valerate	ND	ND	ND
Jejunum	Acetate	260.96±28.68	191.34±105.04	176.58±49.33
	Propionate	138.81±4.57[a]	60.39±9.08[b]	68.41±13.56[b]
	Iso-butyrate	67.58±1.56	58.90±18.00	21.76±1.60
	n-butyrate	ND	ND	17.36
	Iso- valerate	ND	ND	23.60
	n- valerate	ND	ND	9.52
Ileum	Acetate	229.61±98.32[b]	224.62±20.11[b]	529.14±13.29[a]
	Propionate	111.10±29.17	270.02±183.64	250.36±76.66
	Iso-butyrate	27.74±5.58[b]	85.17±0.00[a]	92.29±4.97[a]
	n-butyrate	ND	14.23±0.00	37.14±24.07
	Iso- valerate	ND	ND	ND
	n- valerate	ND	ND	8.88±0.00
Cecum	Acetate	212.99±40.55[b]	251.75±22.36[b]	403.30±8.75[a]
	Propionate	69.17±9.24	317.60±32.47	527.58±390.86
	Iso-butyrate	36.89±5.04[c]	62.73±15.73[b]	118.80±42.05[a]
	n-butyrate	ND	ND	44.26±0.00
	Iso- valerate	ND	ND	39.87±0.00
	n- valerate	ND	ND	5.41±0.00

[abc] Means with different letter within a row differ significantly ($P < 0.05$)
ND: Not detected

Bacterial population

The results of bacterial quantification for different parts of the intestine are detailed in Table 7. Dietary supplementation of PKC affected the target intestinal bacteria in different parts of the intestine. In the duodenum, ducks receiving PKC showed reduction ($P< 0.01$) in the number of bifidobacteria (22.8% and 19.9% reduction for 15% and 35% PKC, respectively) and enterobacteria (26.1% and 34.5% reduction for 15% and 35% PKC, respectively) when compared to ducks in the control group. However, the population of LAB did not show any significant difference among the three treatment groups, and the populations of *E. coli* and *Enterococcus* sp. did not seem

to follow the changes of PKC level in the diet. In the jejunum, dietary treatment had no significant effect on the population of any of the target bacteria. However, numerically, population of all of the quantified bacteria increased with increasing amount of PKC in the diet. In the ileum, populations of bifidobacteria and *E. coli* did not show any significant difference among the three treatment groups. On the other hand, 35% PKC decreased the population of *Enterococcus* sp. and enterobacteria. In terms of LAB population, ducks fed 15% PKC diet showed the least amount of LAB (2.95 $\log_{10}$ copy number) in comparison to ducks fed the control diet (4.02 $\log_{10}$ copy number) and the ducks which received 35% PKC (4.39 $\log_{10}$ copy number). In the cecum, by feeding 35% of PKC, the populations of *E. coli* and enterobacteria were reduced by 8.62% and 9.66%, respectively, in comparison to the control diet. However, the populations of bifidobacteria, LAB and *Enterococcus* sp. did not show any significant difference among the three treatment groups. The reduction in the population of pathogenic bacteria in cecum might be due to the VFAs produced from the fermentation process of feed that had bacteriostatic effect on some enteric bacteria, and had no effect on the beneficial bacteria, such as *Lactobacillus* sp. (Nisbet, 2002). Our results are in accordance with Sundu *et al.* (2006), where inclusion of PKC in the diets of poultry reduced the population of pathogenic bacteria and increased the population of non-pathogenic bacteria in the intestine.

Several researchers have reported that PKC can also be used to replace commercial oligosaccharide as prebiotic in poultry diet (Allen *et al.*, 1997; Fernandez *et al.*, 2002; Jahromi *et al.*, 2015). The use of oligosaccharides, such as fructo-oligosaccharides (Waldroup *et al.*, 1993), and mannose based carbohydrates, either as mannan-oligosaccharides (MOS) (Lyons, 2002) or mannose (Oyofo *et al.*, 1989) to improve the immune system of animals is well accepted (Sundu *et al.*, 2006). However, the mode of β-mannan or MOS of PKC in improving poultry gut health is not totally clear. Fernandez *et al.* (2002) proposed that in the cecum, β-mannan or MOS of PKC may be fermented to the simpler form of sugars, such as mannose, thus promoting the proliferation of the beneficial bacteria, such as *Bifidobacterium* sp. and enhancing the production of lactic acid, consequently prevents the growth of pathogenic bacteria, such as *Salmonella* and *E. coli* (Okumura *et al.*, 1994; Wang and Gibson, 1993). Mannan-oligosaccharides can also be bound to the cell wall of *E. coli* and *Salmonella* sp. and keep them away from the intestinal binding sites. The pathogenic bacteria-oligosaccharide complex then can harmlessly pass through the digestive tract and out of the animal body, resulting in the elimination of colonization of these particular bacteria as the digesta flows out (Sundu *et al.*, 2006; Spring *et al.*, 2000; Pettigrew, 2000).

Table 7. Bacterial population (log_{10} of copy number/g sample) of ducks fed different levels of PKC (Means± SE)

Intestinal section	Target bacteria	Treatment diet		
		Control	15% PKC	35% PKC
Duodenum	Bifidobacteria	5.56±0.06[a]	4.29±0.10[b]	4.45±0.31[b]
	LAB	2.43±0.12[a]	2.69±0.23[a]	2.73±0.12[a]
	Enterococcus	3.29±0.11[ab]	3.65±0.29[a]	2.95±0.17[b]
	Enterobacteria	2.26±0.05[a]	1.67±0.04[a]	1.48±0.06[b]
	E. coli	2.95±0.15[a]	1.80±0.29[b]	2.52±0.13[ab]
Jejunum	Bifidobacteria	4.79±0.29[a]	4.60±0.30[a]	4.84±0.36[a]
	LAB	2.86±0.15[a]	3.44±0.34[a]	3.51±0.14[a]
	Enterococcus	3.57±0.21[a]	3.94±0.20[a]	4.32±0.78[a]
	Enterobacteria	1.99±0.09[a]	2.09±0.07[a]	2.56±0.40[a]
	E. coli	2.45±0.37[a]	3.25±0.14[a]	3.42±0.65[a]
Ileum	Bifidobacteria	5.47±0.41[a]	5.02±0.41[a]	4.79±0.13[a]
	LAB	4.02±0.07[ab]	2.95±0.45[b]	4.39±0.17[a]
	Enterococcus	6.47±0.05[a]	5.79±0.08[ab]	5.42±0.45[b]
	Enterobacteria	4.32±0.03[a]	4.22±0.02[ab]	3.34±0.52[b]
	E. coli	5.44±0.03[a]	5.55±0.04[a]	4.58±0.83[a]
Cecum	Bifidobacteria	7.51±0.61[a]	7.81±0.19[a]	7.53±0.14[a]
	LAB	5.07±0.02[a]	5.49±0.19[a]	5.56±0.16[a]
	Enterococcus	5.82±0.02[a]	5.79±0.12[a]	5.94±0.11[a]
	Enterobacteria	4.97±0.12[a]	4.82±0.15[ab]	4.49±0.11[b]
	E. coli	6.38±0.04[a]	6.38±0.19[a]	5.83±0.13[b]

[ab:] Means with different letter within a row differ significantly ($P < 0.05$)

Ileal histology

Table 8 shows the effects of feeding different levels of PKC on the villus height, crypt depth and the ratio of villus height to crypt depth of ileal samples. The villus height and crypt depth were higher ($P<0.05$) in ducks fed 15% PKC compared to those fed other diets. However, the control ducks had the highest ($P< 0.05$) ratio of villus height to crypt depth compared with those fed other diets. The present observations contradict the findings of Mustafa *et al.* (2002), Sklan *et al.* (2003) and Rahim *et al.* (2007) who observed that high crude fiber content in the diet decreased villus height of poultry.

Table 8. Effect of different levels of PKC on ileal morphology of ducks (Mean ± SE)

Treatment	Villus height (µm)	Crypt depth (µm)	Villus height: Crypt depth
Control	808.95±8.84[b]	127.95±2.79[b]	6.37±0.19[a]
15% PKC	853.01±8.84[a]	138.69±2.51[a]	6.20±0.15[b]
35% PKC	789.29±12.29[b]	111.06±2.58[c]	7.17±0.22[b]

[abc] Means with different letter within a column differ significantly (P < 0.05)

Conclusion

This study demonstrated that PKC, when supplemented at 15 or 35% in the diet of Cherry Valley ducks, did not have detrimental effect on the growth performance and meat quality. However, 35% dietary PKC increased feed intake and FCR of the ducks. Dietary PKC at both 15 and 35% improved intestinal microflora of the ducks by reducing the number of pathogenic bacteria, particularly *E. coli*. Hence, PKC at the level of 15% in the diet can be considered as a good feedstuff for ducks, because of its beneficial effect on gut health, while having no adverse effect on growth performance and meat quality. In the future, probably more intense studies on the effect of PKC as a feedstuff and also as a prebiotic supplement on different hosts should be carried out.

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