

Canarium (*Canarium indicum*) cake as a source of lysine in fermented cassava-copra meal diets with challenzyme for broilers

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ABSTRACT

In a 32-day experiment, the effect of feeding canarium cake replacing of lysine in fermented cassava-copra meal based diets with or without challenzyme enzyme on broiler performance was investigated. Six test diets based on cassava-copra meal were formulated to have similar nutrients content to the commercial feed at starter and finisher phases. Two of the test diets were supplemented with HCL lysine with and without enzyme. In the other four diets canarium cake was fed at 5 and 10% with and without enzyme replacing of HCL lysine. A total of 126, 10-day old, broilers were allotted to 21 floor pens containing 6 birds each and groups of seven diets. The test diets and commercial diet (control) were fed each to birds in 3 replicate pens in a completely randomised design. Feed intake, weight gain, feed: gain, relative weights of carcass and breast muscle were improved on the commercial feed ($P < 0.05$). There were no significant differences in growth and relative weights of carcass and breast muscle among the cassava-copra meal based diets ($P > 0.05$). The relative weight of thighs did not differ among dietary treatments ($P > 0.05$). Drumstick weight was maximised on the canarium diets without enzyme compared to the control ($P < 0.05$). It was concluded cassava-copra meal based diets cannot produce performances comparable to the commercial diet regardless of nutrient balance and enzyme supplementation but canarium cake can replace HCL lysine in the diets without compromising growth and carcass parameters. Further researches into pelleting, enzyme source and concentration are recommended.

KEY WORDS: Alternative lysine sources, Canarium cake, Dietary fibre, Complex enzyme, Broiler performance

INTRODUCTION

High cost of conventional feed ingredients has increased research interest in alternative and affordable sources for poultry feeding. Cassava root is a good source of metabolisable energy (Fetuga and Oluyemi, 1976; Khajareem *et al.*, 1982) which could be used to replace part of the maize in poultry diets but high fibre, low protein and presence of HCN limit its full utilisation in poultry diets (Ochetim, 1992; Buitrago and Luckett, 1999; Chauynarong *et al.*, 2009; Anaeto and Adighibe, 2011; Heuzé *et al.*, 2016b). Adequate processing reduces HCN below toxic levels for animal feeding (10mg/kg) (FAO/WHO, 2013). Fermentation is known to improve protein and reduce fibre contents in the root (Boonnop *et al.*, 2009; Ezekiel *et al.*, 2010; Nebiyu and Getachew, 2011; Gunawan *et al.*, 2015; Yuli *et al.*, 2015).

Copra meal is abundantly available in the Pacific islands. It is moderate in protein (19.6-24.9%), but deficient in essential amino acids, especially lysine (Heuzé *et al.*, 2015). The meal is high in fibre (10-19.7%) with most of the fibre being in the form of non-starch polysaccharides (NPS) (Heuzé *et al.*, 2015; Devi and Diarra, 2017) which impedes feed utilisation by poultry. The utilisation of copra meal by poultry could be improved with appropriate feed formulation, enzyme and amino acid supplementation (Sundu *et al.*, 2009; Sundu and Dingle, 2011; Devi and Diarra, 2017).

Canarium (*Canarium indicum*) is a tropical tree of the family *Burseraceae* which grows in the wild or domesticated in the Melanesian countries of the

South Pacific (Nevenimo *et al.*, 2007; Djarkasi *et al.*, 2017). Canarium kernel is an average source of protein (about 15%) and lysine (1.9-2.7%) (Djarkasi *et al.*, 2011) which might increase with oil extraction. The kernel is high in oil and the oil is a good source of polyunsaturated fatty acids (Nevenimo *et al.*, 2007; Djarkasi *et al.*, 2007; Ogbuagu and Chukuka, 2015). In recent years there has been growing demand for canarium oil on account of its several medicinal properties making the meal available for stock feeding. However, reports on the application of canarium meal in broiler feeding are still limited. The potential of canarium meal as source of dietary lysine in fermented cassava-copra meal based diets with exogenous enzyme for broilers was investigated. Hypothesis:

- i. Broilers will utilise fermented cassava-copra meal with enzyme better;
- ii. Canarium meal can replace HCL methionine in cassava-copra meal based broiler diets.

MATERIALS AND METHODS

Study area

The study was conducted at Lunga area (Latitude = 09.42687° S and Longitude = 160.03125° E), approximately 7 km east of Honiara. The location is of tropical climate with average temperature of 27°C, and 83% humidity that changes very little all year around (Tomahawk, 2018).

Fermentation of cassava root

Saccharomyces cerevisiae (yeast) was cultured in a sterilised cylinder vessel containing 20% sucrose (w/v), 4% urea (w/v) and 10 g yeast extract/l and incubated at room temperature for 60 hours (Boonnop *et al.*, 2009) prior to inoculation. Cassava roots from a seven-month old sweet variety was purchased from local cassava growers in the study area and thoroughly washed under tap water. Cassava roots with peel were sliced and loaded in a large fermentation vessel (200 l capacity) at 50% (w/v) with 12.5% inoculum. About 37.5% water

was added to keep the roots submerged. The vessel was then covered with loosen lid to allow dispersion of carbon dioxide during subsequent 60 hours of fermentation. After fermentation, the whole cassava root was pounded and subjected to 3-4 days sun-drying. It was then stored in well shielded containers at room temperature until needed for diet formulation. Samples of fermented cassava root, copra, fish and canarium meals were analysed for proximate composition and amino acid profile. Fermented cassava root was also analysed for cyanide content (Table 1).



Figure 1: Sliced cassava roots



Figure 2: Cassava root fermentation



Figure 3: Pounded cassava root meal



Figure 4: Sun-dried cassava root meal

Experimental diets

Six experimental diets based on fermented cassava and copra meal were formulated at starter and finisher phases (Table 2) to have similar ME, crude protein, lysine and methionine contents to the commercial broiler feed (control) from Riverina. Two of the experimental diets were formulated to contain HCL lysine. Canarium meal was included in the other diets in place of HCL lysine at 5 and 10%. The experimental diets were supplemented with or without challenzyme 1309A, a complex enzyme from Beijing Challenge Bio-Technology Co. Ltd. China.

Experimental birds and managements

In total, 126, 10-day old, Cobb broiler chicks were weighed and assigned to 21 floor pens (1 x 1.5 m) of similar weights (273 ± 15 g). Each of the diets was fed to 3 replicate pens in a completely randomised design from 10 d to 42 d. The starter diet was fed from day 10-21 d and the finisher diets from 22-42 d. The lighting programme consisted of 20 hours light. Feed and clean drinking water were provided *ad-libitum* throughout the duration of the experiment.

Table 1. Proximate composition (g/kg DM), amino acid content (g/100 g protein) and metabolisable energy (MJ/kg) of canarium cake, fermented whole cassava root meal, copra and fishmeal

Constituents	Canarium meal	Copra meal	Fish meal	Fermented cassava
Crude Protein	296	18.20	578	18
Crude fibre	112	118	<1	21
Crude fat	436	115	160	4
Ash	74	50	206	11
HCN (mg/kg)	NT	NT	NT	<0.0005
ME (MJ/kg)	20.5	12.8	13.85	11.97
Histidine	0.64	0.32	1.80	0.03
Arginine	1.20	0.70	2.50	0.06
Threonine	1.10	0.58	3.00	0.06
Valine	1.90	0.98	3.50	0.08
Lysine	2.95	0.60	4.70	0.09
Isoleucine	1.20	0.57	2.60	0.06
Leucine	2.40	1.20	5.10	0.10
Phenylalanine	1.60	0.73	2.60	0.06
Methionine	0.71	0.27	1.70	0.01
Tryptophan	0.45	0.14	0.55	0.02

NT: not tested.

Data collection

Weighed quantities of feed were fed and leftover weighed to account for feed-intake by difference. Weight gain was monitored by weekly weighing and feed conversion ratio calculated by dividing the feed intake by the weight gain in each pen. At the end of the experiment, two birds having the closest weight to the pen mean were selected and fasted overnight and euthanised by decapitation. Slaughtered birds were scaled in hot water (55°C) for 2 minutes, plucked and eviscerated. The weight of carcass and cuts (breast, thigh and drumsticks) were expressed as relative weights of the live chicken. Abdominal fats and organs (caeca, small intestine, pancreas, liver, gizzard, and heart) were also weighed and expressed as relative weights.

Chemical analysis

The test ingredients and fish meal were analysed for proximate composition according to AOAC (1990). Dry matter was determined after 24 h in a forced-air oven (103°C). Nitrogen concentration was analysed by

Kjeldahl method (AOAC 1990, ID 954.01) and CP was calculated as nitrogen · 6.25. Total fat, ash and fibre contents were analysed according to AOAC (1990, ID 42.05, 920.39 and 962.09 respectively). Amino acid profile was determined using the Standard AAA hydrochloric acid hydrolysis followed by RP-HPLC separation using AccQ-Tag derivatisation (AOAC 1990, ID 994.12). The cyanide content of the UCRM was determined according to Zitnak (1973).

Statistical Analysis

Data collected on growth, carcass measurements and organ weights were subjected to one-way analysis of variance (ANOVA) (Steel and Torrie, 1980) of the GLM in SPSS (SPSS for Windows, version 22.0; IBM Corp., Armonk, NY, USA). Pen was the experimental unit for feed intake while data on weight gain, carcass and organ measurements were collected on individual birds. Treatments means were compared using LSD and significant differences reported as 5% level of probability.

Table 2. Ingredient composition of experimental starter diets (g/kg as fed)

Ingredients	Starter phase (10-21 d)				Finisher diets (22-42 d)			
	HCL lysine	HCL lysine + enz.	5% canarium	10% canarium	HCL lysine	HCL lysine + enz.	5% canarium	10% canarium
Cassava root	580	579.6	584.5	584.1	592	591.6	595	594.6
Copra meal	271	271	218	168	293.5	293.5	242	192
Canarium meal	-	-	50	100	-	-	50	100
Fishmeal	120	120	120	120	70	70	70	70
Challengzyme	0	0.4	0	0.4	0	0.4	0	0.4
Peanut oil	5	5	5	5	20	20	20	20
Limestone	15	15	15	15	15	15	15	15
Salt	3.5	3.5	3.5	3.5	4	4	4	4
Lysine	1.5	1.5	0	0	1.5	1.5	0	0
Methionine	1	1	1	1	1	1	1	1
Premix	3	3	3	3	3	3	3	3
Calculated composition (g/kg)								
ME (MJ/kg)	12.82	12.81	12.89	12.96	13.12	13.11	13.24	13.26
Crude Protein	226.3	226.2	227.2	229.6	199.1	199.	200.2	202.5
Crude Fibre	54	54	51.5	49.2	57	57	54.6	52.2
Lysine	14.3	14.3	12.9	13	12.1	12.1	10.6	10.7
Methionine	4.6	4.6	4.6	4.6	3.9	3.9	3.9	3.8
5% canarium + enz.			584.1	584.1			595	594.6
10% canarium + enz.			168	168			192	192
5% canarium + enz.			50	50			50	50
10% canarium + enz.			100	100			100	100
5% canarium + enz.			584.1	584.1			595	594.6
10% canarium + enz.			168	168			192	192
5% canarium + enz.			50	50			50	50
10% canarium + enz.			100	100			100	100

Enz: enzyme

RESULTS AND DISCUSSION

Chemical composition

Although there is literature on the composition of canarium nut (Nevernimo *et al.*, 2007; Djarkasi *et al.*, 2007), documented reports on the composition of the meal after oil extraction are still limited. The lower ME, higher crude protein (29.6%) and fibre (11.2%) contents of canarium cake used in this study (Table 1) compared to values for the kernel was attributed to the higher concentration of energy in the oil and the inverse relationship between fat and protein contents. The lysine content (2.95%) in canarium cake was slightly higher than the range (1.91- 2.73%) observed by Djarkasi *et al.* (2011) in the kernel. The higher fat in canarium meal (43.6%) was reflected by a slight increase in the energy content of diets containing this ingredient.

Despite the high fibre (7.6-38.4%) reported in cassava peel (Heuzé *et al.*, 2016a; Dayal *et al.*, 2018), fermentation reduced the fibre content of whole cassava to as low as 2.1% suggesting possible breakdown of complex structures during fermentation. Adeleke *et al.* (2017) also reported reduced fibre in fermented cassava roots and attributed this to the ability of fermenting microorganisms to secrete oxidising and hydrolysing enzymes, produce abundant organic acids and degrade crude fibre. Boonnop *et al.* (2009) reported up to 21% protein in cassava root meal fermented with *Saccharomyces cerevisiae* against the 1.8% observed in this study. This wide difference could not be thoroughly explained, but the cultivar of cassava, age of maturity, agronomic practices and analytical methods which have been reported to affect the composition of cassava products (Corbishley and Miller, 1984; Manano, *et al.*, 2017) may be possible sources of variations. Cassava root fermentation in this study reduced the HCN content far below the safe concentration (10mg/kg) in processed cassava root for animal feeds (FAO/WHO, 2013). Boonnop *et al.* (2009), also found significant reduction in HCN concentration from 3.4 to 0.5mg/kg cassava chips fermented by *Saccharomyces cerevisiae*.

Growth performance

During the starter phase (Table 3) feed intake did not differ between HCL lysine supplemented and commercial diet fed groups ($P>0.05$). There was no significant difference ($P>0.05$) in feed intake among the HCL lysine supplemented and 5% canarium meal

with or without enzyme but increasing canarium meal to 10% depressed feed intake regardless of enzyme supplementation ($P<0.05$). Body weight gain and feed: gain were improved on the commercial diet ($P<0.05$) but their values did not differ among the cassava-copra meal diets ($P>0.05$). During the finisher phase (22-42 d) and the overall feeding period (10-42) birds fed the commercial diet consumed more feed and converted their feed into weight better than the cassava-copra meal fed groups ($P<0.05$). Thus, control (commercial feed) diet gave the best results for both the starter and finisher birds. The results of other six diets were lower and almost similar to each other.

Several factors including metabolisable energy, fibre content, amino acid balance, feed particle size and anti-nutritional factors (Peter and Abel, 2006; Classen, 2017) are known to influence feed intake in poultry. The slight increase in metabolisable energy content of canarium cake based diets may explain the lower feed intake in broilers fed 10% canarium with or without enzyme during both phases of growth. The finer particle size of cassava root meal in this study might explain the relatively lower intake of the test diets. Several studies (McKinney and Teeter, 2004; Amerah *et al.*, 2008; Chewning *et al.*, 2012; Morgan and Choct, 2016) have shown increased feed intake in broilers fed pelleted diets due to improved palatability, reduced selective feeding and feed wastage. The reduced feed intake in poultry fed mash cassava diets has been mainly attributed to dustiness (Morgan and Choct, 2016). Despite similarities in nutrient content, the experimental diets were slightly higher in fibre than the control diet. The adverse effect dietary fibre on feed intake (Sundu *et al.*, 2009; Heuzé *et al.*, 2015) and amino acid digestibility (Classen, 2017) may also be implicated in the reduced growth performance on the test diets compared to the control commercial diet.

Contrary to the findings of Sundu *et al.* (2005; 2006). Addition of exogenous enzyme showed no improvements in daily feed intake and weight gain in this study. It is possible that the level of enzyme inclusion was probably low to cause sufficient hydrolysis of dietary fibre in the test diets. The comparable weight gains ($P>0.05$) between diets containing HCL lysine and canarium without enzyme in both starter and finisher diets suggests that canarium can replace HCL lysine without compromising broiler growth.

Table 3. Growth performance of starter broilers (10-21d) fed canarium meal as source of lysine in fermented cassava-copra diets

Diets	10-21 d		22-42 d			10-42 d			
	Feed intake (g/day)	Weight gain (g/day)	Feed: gain	Feed intake (g/day)	Weight gain (g/day)	Feed: gain	Feed intake (g/day)	Weight gain (g/day)	Feed: gain
HCL lysine	76.04 ^{ab}	12.60 ^b	6.07 ^a	103.47 ^b	31.28 ^b	3.31 ^{ab}	93.17 ^b	23.81 ^{bc}	3.92 ^a
HCL lysine + enzyme	70.37 ^{bc}	13.99 ^b	4.99 ^a	97.99 ^{bc}	30.95 ^b	3.17 ^b	87.63 ^{bc}	24.17 ^{bc}	3.62 ^{ab}
5% canarium	66.43 ^{bcd}	14.58 ^b	4.59 ^{ab}	92.36 ^{bcd}	31.28 ^b	2.95 ^c	82.67 ^{cd}	24.60 ^b	3.36 ^{ab}
10% canarium	56.28 ^d	11.43 ^b	5.31 ^a	86.55 ^d	27.74 ^{bc}	3.12 ^b	75.20 ^d	21.21 ^{bc}	3.56 ^{ab}
5% canarium + enzyme	63.45 ^{cd}	13.18 ^b	5.70 ^a	87.46 ^{cd}	25.08 ^c	3.50 ^a	78.47 ^{cd}	20.32 ^c	3.93 ^a
10% canarium + enzyme	55.56 ^d	14.28 ^b	3.89 ^{ab}	86.53 ^d	27.78 ^{bc}	3.12 ^{bc}	74.93 ^d	22.38 ^{bc}	3.35 ^b
Commercial starter (control)	82.92 ^a	38.06 ^a	2.18 ^b	141.22 ^a	72.79 ^a	1.95 ^d	119.37 ^a	58.90 ^a	2.04 ^c
SEM	3.974	1.725	0.830	3.686	1.671	0.093	3.33	1.416	0.187
P value	0.002	0.000	0.074	0.000	0.000	0.000	0.000	0.000	0.000

a, b, c, d: Means in the same column with different superscripts are significantly different (P = 0.05); SEM: Standard error of the mean.

Carcass measurements

The results of carcass measurements (Table 4) showed improved carcass and breast weights on the control diet (P<0.05). Carcass and breast weights did not differ amongst cassava-copra meal based diets (P>0.05). The relative weight of thighs was not affected by dietary treatment (P>0.05). Heavier drumsticks were observed on the canarium-based diets without enzyme compared to the control (P<0.05). The improved carcass

parameters (dressing percentage and breast meat may be attributed to better nutrient utilisation on the commercial compared to the test diets. As mentioned earlier, the higher in fibre content of the test with resultant lower amino acid digestibility might be implicated in the trend of carcass development observed. The beneficial effect of amino acid availability, particularly methionine, on carcass development is well known (Yalcin *et al.* 1999; Corzo *et al.*, 2005; Rakangtong and Bunchasak, 2009).

Table 4. Effect of canarium meal as source of lysine in cassava-copra diets on the relative weight of carcass and major cuts of broiler (% live weight)

Diets	Parameter			
	Carcass	Breast	Thighs	Drumsticks
HCL lysine	73.03 ^b	15.92 ^b	12.28	10.55 ^{bc}
HCL lysine + enzyme	72.32 ^b	16.41 ^b	11.73	10.55 ^{bc}
5% canarium	72.00 ^b	14.64 ^{bc}	12.18	11.22 ^{ab}
10% canarium	71.86 ^b	13.03 ^c	11.95	11.88 ^a
5% canarium + enzyme	70.69 ^b	14.40 ^{bc}	11.97	9.86 ^c
10% canarium + enzyme	72.12 ^b	14.19 ^{bc}	12.02	11.30 ^{bc}
Commercial feed (control)	78.14 ^a	23.77 ^a	12.09	10.05 ^c
SEM	0.93	0.934	0.514	0.307
P value	0.002	0.000	0.992	0.004

a, b, c: Means in the same column with different superscripts are significantly different (P=0.05); SEM: Standard error of the mean.

CONCLUSION

These findings reveal that cassava-copra meal based diets fed as mash will not produce performance comparable to the commercial broiler feed regardless of nutrient balance and enzyme addition. However,

canarium cake has potential as source of HCL lysine in cassava-copra meal based diets. More research into feed pelleting, canarium cake level, enzyme source and concentration as well as cost-benefit analysis is recommended.

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