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# Evaluation of cassava leaf meal protein in fish and soybean meal-based diets for young pigs

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# Abstract

The unavailability and high cost of traditional ingredients calls for more research into alternative sources for pig feeding in the South Pacific region. The effect of replacing feed protein with cassava leaf meal (CLM) protein in weaner and growing pigs' diets was investigated in two experiments. In experiment 1, three diets in which CLM protein replaced 0, 15 and 30% of feed protein were fed each to five replicate pens of weaner pigs. Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were improved and feed cost of gain reduced (P < 0.05) on 30% while dressing percentage was maximized (P < 0.05) on 15% protein replacement diets. In experiment 2, three diets containing 0, 30 and 45% CLM protein as replacement for feed protein were fed as in experiment 1 to grower pigs. FI and BWG were reduced while FCR and feed cost of gain were increased (P < 0.05) above 30% protein replacement. Dressing percentage assumed the highest value (P < 0.05) on 30% replacement. It was concluded that replacing 30% of feed protein with sun-dried CLM protein will maintain growth and reduce cost of pork production. Efficient use of CLM in the diet will be an alternative way of value addition to this by-product.

Keywords: high feed cost, alternative ingredients, feed processing, pig performance

# 1 Introduction

The scarcity and high cost of conventional feed ingredients such as corn and soybean is a major impediment to commercial pig production worldwide. The situation is further compounded in the South Pacific region where conventional feed ingredients are not grown in the region thus imported at exorbitant prices. Ayalew (2011) reported a 56 to 100 % increase in retail price of commercial pig feeds in Papua New Guinea between 2003 and 2011. There is therefore the need to increase research into locally available feed materials of low economic value in the region.

Locally available energy and protein sources exist in the South Pacific region but their use in commercial feed

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production has not received much attention. Cassava (Manihot esculenta) is a root crop well adapted to many climatic and soil types. Cassava root is a major source of carbohydrates in human diets in many parts of the world. Cassava leaf, a by-product which may make up to 30 % of the root yield at harvest (Ravindran, 1993) is a moderate source of protein. The protein content of the leaves ranges from 16.7 to 39.9% (Ravindran et al., 1987; Ly & Ngoan, 2007) with almost 85% of the protein fraction being true protein (Ravindran, 1993). Cassava leaf protein is deficient in sulphur amino acids but has a good balance of other essential amino acids (Gomez & Valdivieso, 1985). The digestibility of cassava leaf amino acids is reported to be high in rats (Eggum, 1970). Cyanide is the major anti-nutritional factor in cassava products which can be reduced below toxic levels by sun-drying (Ravindran, 1993). Sweet cultivars of cas-

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Open access article licensed under a Creative Commons Attribution 4.0 International License CC BY http://creativecommons.org/licenses/by/4.0 sava which are low in cyanide content are grown in Samoa as pig feed and rarely consumed by Samoans. Currently, the leaves from these cultivars are not utilised in the country, thus making it readily available as stock feed. There are reports on the inclusion of cassava leaf meal in monogastric diets (Phuc *et al.*, 1999), but its use as replacement of feed protein in diets for young pigs is not documented. This study investigated the effects of replacing feed protein with cassava leaf protein on growth, carcass traits and gut weight of young pigs.

#### 2 Materials and methods

## 2.1 Source and processing of cassava products

A sweet cultivar of cassava grown on the University of the South Pacific's School of Agriculture and Food Technology farm was harvested at 7 months after planting and used for the experiment. Cassava leaves (with petiole) and roots were chopped and sun-dried (mean temperature and relative humidity ~28° C and 58 % respectively) separately on a cemented floor for 48 and 72 hours respectively, then ground in a hammer mill to pass through a 2 mm sieve and labelled cassava leaf meal (CLM) and cassava root meal (CRM). Cassava products (CLM and CRM) and fish meal were analysed for proximate composition, CLM and fish meal for amino acid composition and CLM and CRM for hydrocyanic acid content (Table 1) and used in the formulation of the diets in experiments 1 and 2. The research protocols were approved by the animal ethics committee of the University of the South Pacific.

2.2 Experiment 1: Replacement of feed protein with cassava leaf meal protein in weaner pigs' diets (14–30kg live weight)

# 2.2.1 Experimental diets

Three pig weaner diets were formulated to meet NRC (2012) recommendations (ME  $\approx$  14 MJ kg<sup>-1</sup>, crude protein  $\approx$  18%, lysine  $\approx$  1.1% and Methionine  $\approx$  0.8%, Table 2). All diets contained maize and cassava root meal as main energy sources. The control diet was devoid of CLM while in diets 2 and 3; feed protein was replaced with CLM protein at 15 and 30% respectively.

### 2.2.2 Pigs and management

Thirty weaner crossbred pigs (Yorkshire  $\times$  Landrace) were weighed individually (14 kg  $\pm$  0.18) and assigned in pairs to fifteen standard concrete floor pig pens. Each of the diets was fed to pigs in 5 pens in a completely

randomized design for a period of 60 days. The diets and drinking water were provided *ad-libitum* throughout the experimental period.

# 2.3 Experiment 2: Replacement of feed protein with cassava leaf meal protein in growing pigs' diets (28–52 kg live weight)

# 2.3.1 Experimental diets

Three pig grower diets were formulated as in experiment 1 except that protein, lysine and methionine contents were reduced to 16, 1 and 0.6% respectively (Table 3). Based on the results of experiment 1 protein replacement levels in this experiment were increased to 30 and 45% in diets 2 and 3 respectively.

#### 2.3.2 Pigs and management

Thirty grower crossbred pigs (Yorkshire  $\times$  Landrace) were weighed individually (28 kg  $\pm$  0.21) and assigned to the same pens as in experiment 1. Each of the diets was fed to pigs in 5 pens in a completely randomized design for a period of 60 days as in experiment 1. Feed and drinking water were supplied *ad-libitum* throughout the experimental period.

## 2.4 Data collection

In both experiments, data were collected on growth (feed consumption, weight gain and feed conversion ratio), carcass traits and gut measurements. Feed consumption data were obtained by difference between quantities fed and left-over in each pen. Pigs were weighed at the beginning and end of the experiment and weight gain calculated by difference. Feed conversion ratio (FCR) was calculated as feed consumed to weight gained. At the end of each experiment all the pigs were stunned electrically, euthanized and dressed, and carcass weighed and expressed as percentages of the live weight. Back fat thickness of the carcass was measured on the last rib at 2 cm from the backbone using a Renco Lean-Meater (Renco Corporation, Minneapolis, MN. 55401 USA). Segments of the gut (stomach, small and large intestines) were weighed full and empty and the content calculated by difference and expressed as percentage of the live weight of the animal.

## 2.5 Chemical analysis

Cassava products and experimental diets were analysed for proximate composition according to AOAC (1990). Dry matter was determined after oven-dying samples at 105°C overnight. Ash was determined by

Constituents	CRM	CLM	Fish meal	Soybean meal
Dry matter	94.8	93.65	95.0	96.9
Crude protein	3.7	25.8	63.2	34.5
Ether extract	2.84	11.4	9.15	18.7
Ash	4.33	9.62	17.1	5.6
Crude fibre	4.6	13.91	1.8	16.4
NDF	10.2	29.55		13.6
Phenylalanine		2.6	1.88	1.69
Histidine		2.1	1.1	0.94
Lysine		1.04	3.02	2.2
Leucine		3.1	3.33	2.58
Valine		3.2	2.58	1.77
Methionine		0.34	1.9	0.56
Arginine		2.9	3.09	2.39
Threonine		2.7	2.14	1.48
Isoleucine		1.12	2.03	1.60
HCN <sup>‡</sup>	12.0	15.03		

**Table 1:** Proximate composition, NDF, amino acid contents  $(g kg^{-1} DM)$  and HCN content  $(mg kg^{-1} DM)$  of Cassava root meal (CRM), Cassava leaf meal (CLM) and fish meal used in the experiment

<sup>†</sup> Values adapted from Devi (2016)

<sup>‡</sup> Fresh cassava root and leaves contained 47.8 and 61.6 mg HCN kg<sup>-1</sup> DM respectively.

Table 2: Ingredients composition (or	n as-fed basis) and analysed composition of the diets in experiment 1.
	Level of protein replacement (%)

Ingredients (g kg <sup><math>-1</math></sup> DM)	Level of protein replacement (%)					
	0	15	30			
Corn	234	227.8	222.1			
CRM	350	341.7	333.1			
Wheat middling	117	114	111			
Fish meal	92	87	82			
Soybean meal	184	174	164			
CLM	0	32	64			
Salt	6	6	6			
Lysine HCl	3	3.3	3.5			
DL-Methionine	1.5	1.7	1.8			
Premix <sup>†</sup>	2.5	2.5	2.5			
Coral sand	10	10	10			
Analysed composition (% DM)						
Dry matter	91.33	90.78	90.65			
Crude protein	18.08	17.97	18.03			
Ether extract	11.74	11.98	11.79			
Ash	10.30	9.80	9.84			
Crude fibre	7.62	8.86	9.20			
NDF	21.16	26.09	27.06			
Calculated composition (%)						
ME $(MJ kg^{-1})$ <sup>‡</sup>	14.62	14.30	14.12			
Lysine	1.10	1.08	1.08			
Methionine	0.79	0.77	0.78			

<sup> $\dagger$ </sup> Biomix provided per kg diet: Vit A: 10,000 IU; Vit D<sub>3</sub>: 2,000 IU; Vit E: 23 mg; Niacin: 27.5 mg; Vit B<sub>1</sub>: 1.8 mg; B<sub>2</sub>: 5mg; B<sub>6</sub>: 3 mg; B<sub>12</sub>: 0.015 mg; K<sub>3</sub>: 2 mg; Pantothenic acid: 7.5 mg; Biotin: 0.06 mg; Folic acid: 0.75 mg; Choline Chloride: 300 mg; Cobalt: 0.2 mg; Copper: 3 mg; Iodine: 1 mg; Iron: 20 mg; Manganese: 40 mg; Selenium: 0.2 mg; Zinc: 30 mg; Anti-oxidant: 1.25 mg.

<sup>‡</sup> calculated according to Wiseman (1987) as: ME =  $3951 + 54.4 \times EE - 88.7 \times CF - 40.8 \times Ash$ Where EE: ether extract and CF: crude fibre; CRM: Cassava root meal; CLM: Cassava leaf meal

Ingredients (g kg <sup><math>-1</math></sup> DM)	Level of protein replacement (%)				
	0	30	45		
Corn	254.9	240.7	233.8		
CRM	318.7	361	350.6		
Wheat middling	191.2	120	116.4		
Fish meal	70.7	68.5	67.5		
Soybean meal	141.5	137.5	134.4		
CLM	0	48.8	73.5		
Salt	6	6	6		
Lysine HCl	3	3.3	3.5		
DL-Methionine	1.5	1.7	1.8		
Premix <sup>†</sup>	2.5	2.5	2.5		
Coral sand	10	10	10		
Analysed composition (% DM)					
Dry matter	93.5	92.9	93.1		
Crude protein	16.04	15.98	16.01		
Ether extract	8. 98	9.24	9.45		
Ash	7.22	7.45	7.80		
Crude fibre	8.54	8.88	8.9		
NDF	25.39	27.24	28.23		
Calculated composition (%)					
ME $(MJ kg^{-1})^{\ddagger}$	14.17	14.07	14.05		
Lysine	1.02	1.01	1.01		
Methionine	0.62	0.59	0.60		

Table 3: Ingredients composition (on as-fed basis) and analysed composition of the diets in experiment 2.

<sup> $\dagger$ </sup> Biomix provided per kg diet: Vit A: 10,000 IU; Vit D<sub>3</sub>: 2,000 IU; Vit E: 23 mg; Niacin: 27.5 mg; Vit B<sub>1</sub>: 1.8 mg; B<sub>2</sub>: 5mg; B<sub>6</sub>: 3 mg; B<sub>12</sub>: 0.015 mg; K<sub>3</sub>: 2 mg; Pantothenic acid: 7.5 mg; Biotin: 0.06 mg; Folic acid: 0.75 mg; Choline Chloride: 300 mg; Cobalt: 0.2 mg; Copper: 3 mg; Iodine: 1 mg; Iron: 20 mg; Manganese: 40 mg; Selenium: 0.2 mg; Zinc: 30 mg; Anti-oxidant: 1.25 mg.

<sup>‡</sup> calculated according to Wiseman (1987) as:  $ME = 3951 + 54.4 \times EE - 88.7 \times CF - 40.8 \times Ash$ Where EE: ether extract and CF: crude fibre; CRM: Cassava root meal; CLM: Cassava leaf meal

burning the samples in a furnace at  $500^{\circ}$  C. Nitrogen was determined using the Kjeldahl apparatus and crude protein calculated as nitrogen × 6.25 (feed factor). Samples were hydrolysed in acid (6 molL<sup>-1</sup> HCl, 115°C, for 23 h) and amino acids analysed by chromatography using the AAA 400 analyser (INGOS, Prague, CZ). Fat extraction was carried out in the Soxhlet apparatus and neutral detergent fibre according to van Soest *et al.* (1994). Hydrogen cyanide (HCN) was determined by boiling and titrating samples in 0.1N AgNO<sub>3</sub> and entrapping HCN in KOH (AOAC, 1990).

# 2.6 Statistical analysis

Analysis of variance (ANOVA) (Steel & Torrie, 1980) was carried out on data collected using the GLM of SPSS (Statistical Package for Social Sciences, version 22). Pen was the experimental unit for feed consumption data while weight gain, carcass and gut weight were measured on individual pigs. Treatment means were compared using the Bonferoni test and significant differences between means were reported at 5 % level of probability.

# **3** Results

#### 3.1 Chemical analysis

Results of chemical analysis are presented in Table 1.With the exception of methionine the protein of CLM used in the study had a good supply in essential amino acids. The concentration of HCN was higher in cassava leaf than the root. Sun-drying reduced HCN content in cassava root and leaf meals by 74.9 and 75.6% respectively. The cost of CLM (US\$ 0.14 per kg DM) was mainly from labour (harvesting and drying) and grinding.

#### 3.2 Performance of the pigs in experiment 1

Growth, carcass and gut content data of the pigs in experiment 1 are presented in Table 4. Feed intake (FI), body weight gain (BWG) and FCR were significantly improved and feed cost of gain reduced (P < 0.05) on the 30% protein replacement diet (6.4% dietary CLM). Daily intake of essential nutrients (ME, lysine and methionine) was also increased in the group fed 30% protein replacement diet. Crude fibre and neutral detergent fibre increased linearly as CLM level increased in the diet.

The pigs were slaughtered at an average weight of 30 and 50 kg in experiments 1 and 2 respectively. These weights are within the preferred slaughter weight range of 25 to 55 kg in Samoa. A higher dressing percentage (P < 0.05) was recorded on the 15 % protein replacement (3.2 % dietary CLM). Per cent dressing did not differ between the control and 30 % protein replacement diets (P > 0.05). There was no significant dietary effect on back fat thickness. Pigs fed the CLM-based diets recorded higher digesta content in the stomach compared to the control fed group (P < 0.05). Digesta in the small and large intestines of the pigs was not affected by the diet (P > 0.05).

# 3.3 Performance of the pigs in experiment 2

From the results of the performance of the pigs in experiment 2 (Table 5) feeding CLM above 30% protein replacement or 4.88% dietary CLM significantly reduced FI and BWG and increased FCR and feed cost of gain (P < 0.05). The best FCR and lowest feed cost of gain were observed on the 30% protein replacement diet. The reduced FI on the 45% protein replacement diet resulted in significantly lower daily intake of lysine and methionine on this diet (P < 0.05). There was no significant dietary effect on ME intake of the pigs (P > 0.05).

Dressing per cent assumed the highest value (P < 0.05) on 30% replacement but did not differ ((P > 0.05) between the control and 45% protein replacement (7.35% dietary CLM) diets. Back fat thickness and digesta content of the different segments of the gut were not affected by dietary treatment (P > 0.05).

# 4 Discussion

#### 4.1 Chemical analysis

Poor methionine content in CLM has earlier been reported (Roggers & Milner, 1963; Eggum, 1970). The crude protein and crude fibre contents of cassava root

and leaf meals used in this study fall within the ranges of 1-3% (Buitrago A., 1990; Salcedo et al., 2010) and 16.7-39.9% (Allen, 1984) reported for cassava root and leaf meals respectively. The higher fibre content of CLM was reflected by a higher fibre in the diets containing CLM compared to the control. The high HCN in CLM compared to cassava root in this study supports earlier findings by Yeoh & Chew (1976) who reported six times more HCN in cassava leaf compared to the root. The significant reduction in HCN in the dried products (root and leaves) is in agreement with earlier reports (Gomez & Valdivieso, 1985; Ravindran, 1993; Ly & Ngoan, 2007). The latter authors reported a 51 % reduction of HCN in cassava leaves after 24 hours of sun-drying. The higher HCN reduction in CLM in this study may be attributed to the duration of sun-drying (48 hours) and chopping which might have increased the activity of endogenous linamarase on cyanogenic glycosides. HCN content of both sun-dried CRM and CLM was below the threshold level of  $50 \text{ mg kg}^{-1}$  reported for growing pigs (Bolhuis, 1954).

## 4.2 Performance of the pigs in experiment 1

The increased fibre content with increasing CLM was probably the reason for increased feed intake of pigs fed the CLM-containing diets to compensate for energy on these diets. Contrary to these results, Kallabis & Kaufmann (2012) reported reduced feed intake and weight gain in growing pigs fed diets containing lower fibre level (7.3%) compared to about 9% fibre in the diets containing CLM in this experiment (8.86-9.2% CF). Pig age, fibre source and composition, feed processing and diet composition have all been reported to affect the utilisation of dietary fibre by pigs (Low, 1993). The higher feed intake on CLM diet resulted in higher daily intake of essential nutrients (ME, lysine and methionine) compared to the control. This pattern of amino acids intake may explain the improved growth performance of pigs fed the 30% CLM diet. Edwards & Campbell (1993) observed a positive relationship between amino acid intake and body protein accretion in pigs. Ly et al. (2012) also reported improved performance of pigs fed ensiled cassava leaf protein diets supplemented with dl-methionine and l-lysine. Methionine is involved in growth and maintenance of body protein and also known for detoxification of HCN in-vivo (Tewe, 1992). The linear reduction in feed cost and the improved FCR with CLM inclusion were the main reasons for lower feed cost of gain observed on these diets.

The improved dressing percentage observed on the 15% protein replacement diet could not be explained. The role of dietary methionine (Corzo *et al.*, 2006;

Parameters	Level of protein replacement (%)					
	0	15	30	s.e.m	P value	
Initial weight (kg/pig)	14.2	14	14.1	0.047	0.529	
Feed intake (kg/pig/day)	1.0 <sup>b</sup>	1.06 <sup><i>ab</i></sup>	1.15 <sup><i>a</i></sup>	0.036	0.008	
Body weight gain (kg/pig/day)	$0.28^{b}$	0.29 <sup><i>ab</i></sup>	0.35 <sup>a</sup>	0.018	0.007	
Feed conversion ratio (feed: gain)	3.57 <sup>a</sup>	3.66 <sup><i>a</i></sup>	3.29 <sup><i>b</i></sup>	0.091	0.011	
Cost of kg feed (US\$)	0.62	0.52	0.47	n.a.	n.a.	
Feed cost per kg gain (US\$)	2.21 <sup><i>a</i></sup>	1.90 <sup><i>ab</i></sup>	1.5 <sup>b</sup>	0.168	0.005	
Daily nutrient intake						
$ME (MJ kg^{-1})$	14.62 <sup>b</sup>	15.16 <sup>b</sup>	16.24 <sup>a</sup>	0.287	0.036	
Lysine (g)	11 <sup>b</sup>	11.45 ab	12.42 <sup><i>a</i></sup>	0.342	0.028	
Methionine (g)	7.9 <sup><i>b</i></sup>	8.2 <i>ab</i>	9.0 <sup><i>a</i></sup>	0.263	0.023	
Carcass traits and gut content (% live weight)						
Dressing per cent	64.5 <sup>b</sup>	68.3 <i>ª</i>	63.8 <sup><i>b</i></sup>	1.143	0.004	
Back fat thickness	11.2	10.8	11.4	0.172	0.150	
Digesta in the stomach	$0.8^{b}$	1.3 <sup><i>a</i></sup>	1.0 <sup><i>a</i></sup>	0.121	0.001	
Digesta in the small intestine	0.5	0.5	0.5	0.008	0.063	
Digesta in the large intestine	0.6	0.6	0.6	0.021	0.052	

**Table 4:** Growth performance, carcass traits and gut content of pigs fed cassava leaf protein as replacement for feed protein (14 to 30 kg body weight)

s.e.m: standard error of the mean; <sup>*a*</sup>, <sup>*b*</sup>: means within the row bearing different superscripts are significantly different (P=0.05); n.a: not analysed

**Table 5:** Growth performance, carcass traits and gut content of pigs fed cassava leaf protein asreplacement for feed protein (28 to 55 kg body weight)

Parameters	Level of protein replacement (%)					
	0	30	45	s.e.m	P value	
Initial weight (kg/pig)	28.3	28.3	28.3	0.012	0.529	
Feed intake (kg/pig/day)	1.6 <i>ª</i>	1.6 <i>a</i>	1.5 <sup>b</sup>	0.016	0.005	
Body weight gain (kg/pig/day)	0.5 ab	0.6 <i>a</i>	$0.4^{b}$	0.044	0.000	
Feed conversion ratio (feed: gain)	3.4 <sup><i>ab</i></sup>	2. 8 <sup><i>b</i></sup>	3.7 <sup>a</sup>	0.218	0.011	
Cost of kg feed (US\$)	0.5	0.5	0.4	n.a.	n.a.	
Feed cost of kg gain (US\$)	1.7 <i>ª</i>	1.3 <sup>b</sup>	1.6 <sup>a</sup>	0.109	0.004	
Daily nutrient intake						
$ME (MJ kg^{-1})$	23.0	23.1	21.8	0.614	0.056	
Lysine (g)	16.5 <sup>a</sup>	16.6 <sup><i>a</i></sup>	15.7 <sup>b</sup>	0.242	0.008	
Methionine (g)	10.0 <sup><i>a</i></sup>	9.7 <sup>ab</sup>	9.3 <sup>b</sup>	0.174	0.013	
Carcass traits and gut content (% live weight)						
Dressing per cent	68.8 <sup>b</sup>	79.4 <sup>a</sup>	66.9 <sup><i>b</i></sup>	3.176	0.018	
Back fat thickness	14.5	14.4	14.5	0.023	0.101	
Digesta in the stomach	1.5	1.5	1.4	0.167	0.061	
Digesta in the small intestine	0.9	0.9	0.9	0.017	0.059	
Digesta in the large intestine	0.9	1.0	1.1	0.052	0.064	

s.e.m: standard error of the mean; <sup>*a*</sup>, <sup>*b*</sup>: means within the row bearing different superscripts are significantly different (P=0.05); n.a: not analysed

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Wang *et al.*, 2010) and lysine (Grisoni *et al.*, 1991; Nasr & Kheiri, 2011) in reducing body fat deposition and the similarity of the diets in these amino acids may be a reason for the pattern of back fat deposition observed in both experiments. Heavier digesta in the stomach of pigs fed CLM included diets may be due to slower digesta transit time due to higher fibre content. Jørgensen *et al.* (1996) observed that pigs adapt to high fibre diets by increasing gut weight.

# 4.3 Performance of the pigs in experiment 2

When the level of protein replacement in the diet exceeded 30% (11.38% crude fibre and 27.24% NDF) feed intake was reduced probably on account of the higher dietary fibre. Similar results have been reported by Len et al. (2008) who found that feed intake was reduced in Mong Cai × Yorkshire and Landrace × Yorkshire crossbred pigs when dietary fibre increased from 6.4 to 10%. Souza da Silva et al. (2012) also observed lower feed intake in growing pigs on high fibre diets as a result of gut fill. This reduced feed intake with the resultant lower intake of lysine and methionine may explain the lower weight gain, poorer FCR and higher feed cost of gain on the 45% compared to the 30% protein replacement diet. Low availability of CLM methionine (Eggum, 1970) could be implicated in the reduced performance above this level of protein replacement.

Like in experiment 1 the reason for the pattern of dressing percentage in this experiment was not understood. The similarities observed in digesta contents of different gut segments is probably the result of improved feed utilisation on the control and 30 % replacement and low feed intake on the 45 % replacement diets.

Based on the results of experiments 1 and 2, it is concluded that replacement of 30% feed protein with sun-dried cassava leaf meal protein in fish and soybean meal-based diets will maintain the growth performance of young pigs and reduce dependence on expensive feed resources. More research in the effects of age of cassava leaves, processing methods and diet composition on pig performance and pork quality is needed. In addition to feed cost reduction, the substitution will add value to cassava foliage in the study area.

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