Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 5 Issue 2 February 2023

Research Article

Processing Strategies Modifies Nutrient Composition of Cassava Peel-leaf Blends, Influences Nutrient Digestibility and Nitrogen Balance of Growing Pigs

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DOI: 10.31080/ASVS.2022.05.0602

Received: November 29, 2022

Published: January 17, 2023

Abstract

This study investigated the effect of processing strategies on nutrient composition of cassava peel-leaf blends (CPLB) and impact on apparent nutrient digestibility and nitrogen balance of growing pigs. In-vitro fermentation of CPLB (Cassava peel: Cassava leaf; 5:1) using five spore forming organism was first carried out to evaluate the fermentative efficacy of the microbes. Aspergillus tamarii which had increased ($P < 0.05$) crude protein (10.70%) and reduced ($P < 0.05$) acid detergent fibre (ADF) (28.70%) was selected for solid state fermentation (SSF) of CPLB. The diets formulated for digestibility consists of standard corn-soya based diet (Control) and three other diets were formulated by replacing maize with unfermented CPLB (UCPLB), water fermented CPLB (WCPLB) and microbial fermented CPLB (MCPLB) using Aspergillus tamarii as inoculum. 24 cross bred male pigs of average weight 65.96 kg ± 1.56 were used for the trial. Nutrient composition of test ingredients shows that MCPLB had the highest (11.68%) crude protein. Digestibility of diets shows that crude fibre and NDF digestibility was higher ($P < 0.05$) in pigs fed diet containing UCPLB and WCPLB. In conclusion, fermentation of CPLB improved protein content of the fibrous feed stuff and dietary inclusion of processed CPLB did not cause significant reduction in nitrogen retention in pigs.

Keywords: Pigs; Cassava peel-Leaf Blends; Fermentation; Nutrient Digestibility; Nitrogen Balance

Introduction

The cereal grains especially maize and soyabean constitute the major bulk of commercial feeds for poultry and pigs [1]. In recent times, the price of conventional energy sources such as maize has escalated and highly unpredictable because the local production is far below the demands by man, animals and other alternative channels of usage [2]. The continuous fluctuation and increase in the price of maize coupled with the numerous demands necessitated intensive research into cheap alternative sources of energy yielding ingredients. Pigs has fast growth, short generation interval and high prolificacy with efficient nutrient conversion into high quality meat; therefore it stands as quick source of animal protein and rapid means of correcting animal protein shortage [3]. However, pig performance in terms of weight gain and carcass leanness is a function of the intake level of particular nutrients, particularly energy and protein [4]. Therefore, nutrient supply has to be judiciously manipulated to ensure the production of meat at frugal rates.

Among the available agro industrial by-products and crop residues that can be used in feeding of pigs are cassava peels and leaves [5]. Cassava peels and leaves are under-utilized as they are often left to rot away on farms after harvesting of the roots [6]. Cassava peels had been incorporated in previous studies in the diets of pigs as alternative energy source [7,8]. However, its usefulness is limited by its fibre, cyanide content [9] and low crude protein content [10]. Cassava leaves have good amino acid content comparable to soya bean meal [11], rich in protein, minerals, vitamin (B_1, B_2, and C) and carotenes [12]. Despite these qualities, its utilisation is limited by presence of anti-nutritional factors [13,14] and fibre content [15].

The combination of cassava leaf with cassava peel could complement for the low crude protein of the peel to serve as a sustainable alternative. The efficient use of cassava by-products in pig feeding could also be achieved through manipulative strategies by subjecting it to treatment such as sun drying which is capable of eliminating high percentage of the nutritionally active deleterious factors [14,16] and microbial treatment such as fermentation which enhances release of encapsulated soluble nutrients through fibre break down and enzyme penetration [17]. Fermentation also improves product stability, flavour development and cyanide elimination [18]. Therefore this study investigates the effect of processing strategies on nutrient composition of CPLB and its impact on apparent nutrient digestibility and nitrogen balance of growing pigs.

Materials and Methods

Research ethics declaration

All experimental procedures were executed in accordance with the approved guidelines for Animal Research by Nigeria Institute of Animal Science in Nigeria.

Experimental site

Evaluation of fermentative efficacy of spore forming microorganisms was carried out at the Microbiology laboratory, Department of Microbiology, College of biological sciences while the digestibility trial (using pigs) was conducted at the piggery unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Nigeria. The site is situated in the derived savanna zone of Southwestern Nigeria on Latitude $7^0$9' 39N and $3^0$20' 54E and 76m above sea level [19]. The Mean Annual Rainfall is 1040mm and occurs from March to October, the temperature average is 34^0C throughout the year.

Evaluation of fermentative efficacy of selected microorganisms and selection of appropriate inoculum

Collection and processing of test ingredients

Fresh cassava leaves (TMS 305) were harvested without petioles. After harvesting, the leaves were spread out-doors on concrete floors under the sun for 3 days until they became crispy while still retaining the greenish colouration. The leaves were turned regularly to prevent uneven drying and possible decay of the leaves. The dried crispy leaves were milled through a 2 mm sieve to produce leaf meal which was stored in plastic bags under room temperature. Fresh cassava peels were collected from cassava processing plant in Igbo-ora, Oyo state. The peels were washed to remove adhering soil particles and spread thinly on a concrete platform to dry to moisture content of 10%. The dried cassava peels were hammer milled through a 2 mm sieve and then bagged for use.

Preparation of cassava peel-leaf blend (CPLB)

A blend of cassava peel meal (CPM) and cassava leaf meal (CLM) was prepared using the Pearson Square method according to Adeyemi., *et al*. [20] by mixing at ratio of 5: 1 (5 parts CPM: 1 part CLM) to form cassava peel-leaf blend (CPLB) with a protein content of 8.83%. Crude protein contribution from individual components in the mix is 81.26% and 18.74% from CPM and CLM respectively. The blend formulated was equally divided into six parts in triplicates. One part of the mix was bagged immediately while the other five parts were fermented with the selected micro-organisms.

Selection of microbes for inoculation

Pure strains of five microorganisms were selected based on their reported fermentative capability. *Rhodotorula* spp, *Candida* spp, and *Pichia* spp [21,22] were selected from the yeast family. *Aspergillus tamarii* and *Aspergillus flavus* [23,24] were selected from the mould family. The microbes were obtained from the Culture Collection Unit of the Department of Microbiology, Federal University of Agriculture, Abeokuta.

Microbial inoculation of CPLB

Fermentation medium was done following standard procedures described by Cooke and Brazis [25] by preparing broth cultures using exactly 3.0g of Yeast extract agar + 0.5g of $MgSO_4$ + 5.0g of glucose mixed with 150mls of distilled water in a conical flask, 20ml each was decanted into 5 units of 100ml conical flasks

and it was cotton-plugged and sealed with paper tape. The conical flask was then autoclaved at 121°C for 15minutes, after which they were brought out to cool. After cooling the yeast medium, the isolates were inoculated with each of the microorganisms at 10.5 × 10^8 spores/g in different conical flasks aseptically and gently shaken for even distribution of inoculum. The conical flasks were lightly cotton plugged to allow for an aerobic condition and then incubated for 24hrs at 37°C. The broth cultures (5 conical flasks) inoculated with the isolates was introduced into the blend samples (initially autoclaved) in conical flasks and corked after pouring. The blends were then mixed with the inoculum for even distribution and placed in a cupboard for 7 days. At the end of 7 days, the fermented materials were sun-dried to 10% moisture content and sampled for crude protein (CP) and fibre fractions analysis.

Aspergillus tamarii was selected due to increased crude protein (10.70%) and reduced ADF (28.70%) composition of CPLB after fermentation (Table 1).

	Unfermented CPLB	**Fermented CPLB**						
		Micro organisms						
Nutrients (%)		*Rhodotorola* spp	*Candida* spp	*Pichia* spp	*Aspergillus tamarii*	*Aspergillus flavus*	SEM	P-value
Crude protein	8.97	8.73^{d}	9.78^{c}	9.94^{c}	10.70^{b}	11.68^{a}	0.26	<0.0001
ADF	37.48	32.93^{b}	32.90^{b}	31.50^{b}	28.70^{c}	35.34^{a}	0.62	0.0002
ADL	25.59	23.04^{a}	22.71^{a}	18.84^{b}	19.33^{b}	22.16^{a}	0.52	0.0002
NDF	37.84	35.34^{a}	34.75^{b}	30.61^{d}	33.54^{c}	34.45^{b}	1.17	0.0009

Table 1: Crude protein and fibre fractions of cassava peel-leaf blends subjected to solid state fermentation with selected microorganisms.

^{abcd} Means on the same row having different superscripts are significantly different (P < 0.05).

SEM = Pooled standard error of means; ADF = Acid detergent fibre; ADL = Acid detergent lignin; NDF = Neutral detergent fibre.

Processing of CPLB for digestibility trial

Unfermented cassava peel-leaf blend (UCPLB)

Prepared by mixing dried cassava peels and dried cassava leaves in ratio 5:1. The blend was bagged immediately without further processing and stored prior to diet formulation.

Water fermented cassava peel-leaf blend (WCPLB)

Prepared by mixing dried CPLB (5:1) with water (in ratio 1:1, kg: Lt) in plastic drums. The blend was mixed thoroughly to ensure all portions of the blend come in contact with water. After mixing, the wet blend was placed in black polythene bags and tied properly to create an anaerobic environment within the bags. The bags were left for 7 days in order to ensure proper fermentation of the contents. On the seventh day, the bags were opened and the ingredients were sundried and stored prior to diet formulation.

Microbial (*Aspergillus tamarii*) fermented cassava peel-leaf blend (MCPLB)

Pure strains of *Aspergillus tamarii* was used as inoculums. Spores of *Aspergillus tamarii* used for SSF of the CPLB was prepared by following standard protocols described by Murray., *et al*. [26]. Spore suspension (inoculum) was prepared at an inoculum size of 10.5 × 10^8 spores/g of CPLB. The wet blend was mixed properly and placed into black polythene bags which were tied properly to create an anaerobic environment within the bags. The bags were stored and left for 7 days in order to ensure proper fermentation of the contents. On the seventh day, the bags were opened and the ingredients were sundried and stored prior to diet formulation.

Chemical analysis of samples

Proximate composition of samples from CPM, CLM, UCPLB, WCPLB, MCPLB and dried faecal samples was determined using the

method described by Association of Official Analytical Chemists (AOAC) [27] and fibre fractions were carried out according to the standard method by McCleary [28] respectively (Table 2). All analysis done was on dry matter basis. NDF (assayed without a heat stable amylase and expressed inclusive of residual ash), ADF (expressed inclusive of residual ash), and Lignin (determined by solubilisation of cellulose with sulphuric acid) and crude protein (total nitrogen × 6.25). Gross energy was estimated using the adiabatic bomb calorimeter (Model 1261; Parr Instrument Co., Moline, IL, USA). The cyanogenic glycosides of the samples were done using the method described by Vetter [29]. Nitrogen content in feed, faeces and urine was determined according to AOAC [26] procedure.

Nutrient composition (%)	CPM	CLM	UCPLB	WCPLB	MCPLB
Crude protein	4.46	27.78	8.97	8.11	11.68
Ether extract	1.81	4.93	0.76	1.66	0.61
Crude fibre	14.23	17.70	13.00	12.24	12.87
Ash	5.51	8.08	7.73	9.06	7.56
Carbohydrate	84.25	44.57	57.39	56.03	60.79
Total cyanide (mg/kg)	4.03	1.82	1.37	1.32	1.28
ADF	25.04	26.14	37.48	23.55	26.96
ADL	13.67	14.10	21.59	20.23	20.23
NDF	24.26	21.32	37.84	35.62	26.19
Gross energy (Kcal/g)	3575.70	3011.01	3980.80	3918.95	3825.34

Table 2: Chemical composition of samples.

UCPLB: Unfermented cassava peel-leaf blends; WCPLB: Water fermented cassava peel-leaf blends; MCPLB: Microbial fermented cassava peel-leaf blends

ADF = Acid detergent fibre; ADL = Acid detergent lignin; NDF = Neutral detergent fibre.

Experimental diets

Four experimental diets were formulated for the study (Table 3). A standard soyabean-maize based diet (Control; Diet 1) was formulated according to National Research Council (NRC) requirement for growing pigs NRC [30]. Three additional experimental diets were formulated such that UCPLB (Diet 2), WCPLB (Diet 3) and MCPLB (Diet 4) replaced maize at 50% inclusion level in the control diet. Pigs in each treatment group were fed with their respective experimental diets.

Experimental design and digestibility trial

Digestibility trial was carried out to determine the apparent nutrient digestibility of experimental diets fed to pigs. Twenty-four pigs of average weight 35.96 kg ± 1.56 were used in conducting the trial. Pigs were randomly assigned to four dietary treatments. Each treatment has six replicates consisting 1 pig per replicate. A completely randomized design was used in allotting the pigs to 24 individual cages. Each was housed separately in appropriate metabolic cages fitted with individual feed troughs, water trough and facility for separate droppings collection. The pigs were acclimatized for 7 days prior to the commencement of 5 days metabolic trial (faeces and urine collection). Known weight of feed was fed to the pigs housed in individual metabolic cages and offered in wet mash form (feed: water = 1: 1) in two equal meals per day (8:00 a.m. and 4:00 p.m.). Drinking water was given *ad libitum*. Faeces were collected daily and oven dried (60°C); at the end of the sampling period, faeces from each animal were pooled and thoroughly homogenized and sampled for proximate and fibre fraction analysis.

Urine collection

Total collection technique as described by Khan., *et al*. [31] was used for urine collection. Total volume of urine voided was measured and 10% fraction was retained daily in plastic bottles containing 10ml of 10% H_2SO_4 solution to prevent ammonia nitrogen loss and maintain pH below 3.0. Retained fraction were stored at -4^{0}C and bulked for each animal within the collection period (5 days).

Ingredients (%)	Control diet	UCPLB	WCPLB	MCPLB
Maize	50.00	25.00	25.00	25.00
UCPLB	0.00	25.00	0.00	0.00
NCPLB	0.00	0.00	25.00	0.00
MCPLB	0.00	0.00	0.00	25.00
Soyabean meal	15.00	15.00	15.00	15.00
Groundnut cake	9.00	9.00	9.00	9.00
Palm kernel cake	4.00	4.00	4.00	4.00
Wheat offal	7.00	7.00	7.00	7.00
Corn bran	10.00	10.00	10.00	10.00
Limestone	1.00	1.00	1.00	1.00
Bone meal	3.00	3.00	3.00	3.00
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Vitamin-mineral premix ‡	0.30	0.30	0.30	0.30
Salt (NaCl)	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Determined nutrients				
Gross energy (Kcal/Kg)	3978.17	3969.81	3824.14	3908.44
Digestible energy (Kcal/kg) †	3156.10	3184.64	3135.16	3060.27
Protein (%)	18.28	18.28	18.19	18.55
Fat (%)	3.76	3.44	3.53	3.42
Fibre (%)	4.36	5.46	5.38	5.37
Ash (%)	2.24	2.88	3.01	2.86
Carbohydrate	57.96	53.7	58.24	54.23

Table 3: Gross composition of experimental diet.

‡ Vitamin-mineral premix supplied per kilogram of complete diet: 100 mg of Fe as FeSO4; 100 mg of Zn as ZnSO4;20 mg of Mn as MnO; 10 mg of Cu as CuSO4; 0.30 mg of I as CaI; 0.30 mg of Se as Na2Se O3; 5,506 IU of vitaminA; 551 IU of vitamin D3; 33 IU of vitamin E; 3.6 mg of vitamin K; 5.5 mg of riboflavin; 25 mg D-pantothenic acid;33 mg of niacin; 27µg of vitamin B12; 1.7 mg of folic acid; 220µg of biotin, and 120 mg of choline.

UCPLB: Unfermented cassava peel-leaf blends; WCPLB: Water fermented cassava peel-leaf blends; MCPLB: Microbial fermented cassava peel-leaf blends; †Calculated using the method of Adeola [31].

Calculations

Efficiency of nitrogen (N) utilization was calculated as: N output / N intake.

Nitrogen output (g/d) = N intake – N excretion (Faecal N + Urinary N)

The values of nitrogen intake (NI), nitrogen output in faeces (NOF) and nitrogen output in urine (NOU) were calculated by multiplying nitrogen levels of diets, faeces and urine, by feed intake, excreted faeces and urine, respectively.

Total output (TNO = NOF + NOU), Nitrogen retention (NR = NI - TNO) and net protein utilization (NPU = NR/NI) were subsequently calculated according to Adeola [32].

The apparent nutrient digestibility was estimated using the formula below:

$$\text{Apparent nutrient digestibility} = \frac{[(F_i \times N_f) - (E \times N)]}{(F_i \times N_e)} \times 100$$

Where F_i and *E* is the quantity of feed intake and excreta output (g DM) during metabolic trial. The N is the nutrient, N_f is the nutrient composition in feed while N_e is the nutrient composition in excreta voided (g DM).

Statistical analysis

Data obtained from this study were analyzed using one-way analysis of variance (ANOVA) of Statistical Analysis Software (SAS) [33]. Differences between significant mean values were separated using Tukey's Test in SAS Software and significance were based on a probability of $P < 0.05$.

Results and Discussion

Fermentative efficacy of selected microbes

Fermentation of CPLB with selected microorganism significantly ($P < 0.05$) affected crude protein and fibre fractions of CPLB (Table 1). CPLB inoculated with *Rhodotorola* spp had the least ($P < 0.05$) (8.73%) crude protein (CP) while CPLB inoculated with other micro-organism recorded increased ($P < 0.05$) CP however *Aspergillus flavus* had the highest ($P < 0.05$) crude protein (11.68%). The increase in CP of CPLB fermented with this organism confirms ability of these microbes in synthesizing various enzymes including amylases and proteases thereby enhancing nutrient availability [34]. *Aspergillus tamarii* significantly ($P < 0.05$) reduced acid detergent fibre (ADF) (28.70%) and acid detergent lignin (ADL) (19.33%) content which suggest the efficient capacity to degrade fibrous constituent of CPLB. This is in agreement with the report of Mathivanan., *et al*. [35]; Villena and Gutierrez-Cornea [36] that *Aspergillus* spp produces ligno-cellulolytic enzyme (cellulases and xylanases) essential in fibre break down.

Chemical composition of UCPLB, WCPLB and MCPLB

The test ingredients show differences in nutrient composition and fibre fraction (Table 2). The CP content of MCPLB (11.68%) in the current study was higher when compared to UCPLB (8.97%). The increase in CP of MCPLB might be attributed to secretion of certain extracellular enzymes such as linamarases and cellulose enzymes by the microorganisms into the fermentative product during enzymatic breakdown [37] and ability of the microorganisms to also synthesize amino acids [38]. It can also be associated with the structural content of the fungi which are proteinous in nature. This corroborated the finding of Adeleke., *et al*. [39] who reported increased CP content of microbial fermented cassava peel. The crude fibre content of WCPLB (12.24%) and MCPLB (12.87%) is reduced compared to UCPLB (13.00%). This reduction is as a result of the ability of the fermenting microorganisms to degrade the crude fibre due to secretion of hydrolyzing and oxidizing enzymes leading to conversion of the insoluble carbohydrate complex in the waste into utilizable compounds [40]. The carbohydrate content of WCPLB (56.03%) is lower compared to that contained in UCPLB (57.39%) and MCPLB (60.79%). The decrease can be attributed to the utilization of the carbohydrate nutrient as source of energy by the fermenting microorganisms for growth and metabolism due to conversion of oligosaccharides to simple sugars [41]. The value of carbohydrate is observed to be higher compared to other nutrients and this suggests a good energy source for animal feed. The total cyanide content of MCPLB (1.28 mg/kg) and NCPLB (1.32 mg/kg) is lower compared to that of UCPLB (1.37 mg/kg) which is in tandem with the report of Adeleke., *et al*. [39] who reported decrease in cyanide content due to fermentation of cassava peel. The cyanide reduction is a response to the synergistic effect of hydrogen cyanide evaporation during drying, diffusion of vacuole-bound cyanogenic glycosides and loss of cyanogenic glycosides on hydrolysis by linamarase enzyme [42].

The Acid detergent fibre (ADF), Acid detergent lignin (ADL) content of NCPLB (23.55% and 20.23%) and MCPLB (26.96% and 20.23%) are lower compared that of UCPLB (37.48% and 21.59%). This is similar to the findings of Aderemi and Nworgu [43] who reported decrease in ADF and ADL content of solid state fermented cassava peel using *Aspergillus niger* as inoculum. The reduction in the fibre fractions is associated with degrading activity of cellulolytic bacteria through hydrolysation or saccharification [43]. Chesson [44] also stated that breakdown of cell wall polysaccharide require the action of enzymes such as glucan hydrolases and glycosidases which is able to reduce oligosaccharides to their monomeric components.

Nutrient digestibility

The digestibility was similar (P>0.05) for dry matter (DM), CP, ether extract (EE), ash, NDF, ADF and gross energy (Table 4). This indicates that the differently processed CPLB were able to supply similar nutrients comparable to the control diet irrespective of the processing methods used. The similarities in the digestibility suggest that processing of the ingredients has influenced nutrient availability of diets. The result of the current study shows that processed CPLB had digestibility value ranges of 78-80% and 50-59% for crude protein and energy respectively. This contradicts the report of Madalla., *et al*. [45] who stated that CLM had 45% and 44.17% for CP and gross energy digestibility. Pigs fed diet containing UCPLB and WCPLB showed increased (P < 0.05) CF and NDF digestibility compared to those fed control diet. The increased in CF digestibility observed may be due to higher fibre content of the diet. Huu and Khammeng [46] reported that fibre digestibility of yeast fermented cassava pulp (FCP) increased as inclusion of FCP increases in the diet of pigs. Ziemer., *et al*. [47] also reported that fibre digestibility increased when total dietary fibre increased. The energy digestibility was similar (P > 0.05) across treatments in the present study. This diverges from the report of Bakker [48] and Acosta., *et al*. [49] that implicated dietary fibre as a poor source of energy due to low digestion. The improvement in nutrient digestibility of diets containing CPLB being comparable to the control diet in this study is associated with processing effect on test ingredient which is also evident in the similar energy utilisation in the diets.

Parameters	Control	UCPLB	WCPLB	MCPLB	SEM	P value
Digestibility (%)						
Dry matter	74.76	80.71	80.35	78.30	0.96	0.095
Crude protein	88.19	86.20	82.54	79.28	1.43	0.112
Ether extract	78.65	77.50	69.92	61.43	4.62	0.570
Crude fibre	33.99^{b}	54.71^{a}	57.86^{a}	47.06^{ab}	3.30	0.028
Ash	47.48	63.55	53.03	46.57	4.52	0.110
Nitrogen free extract	85.46	89.64	90.27	90.91	0.94	0.153
NDF	57.18^{c}	68.77^{ab}	71.06^{a}	60.31^{bc}	2.04	0.025
ADF	69.35	78.85	79.62	75.68	1.61	0.079
ADL	45.34^{ab}	50.81^{a}	50.54^{a}	35.38^{b}	5.11	0.022
Gross energy	53.30	59.61	55.64	50.40	1.91	0.402
Nitrogen balance (g/d)						
Urine output	2.55	2.85	2.52	2.83	0.15	0.846
Urine N output	9.92	10.61	9.38	8.38	1.45	0.967
Urine gross energy (Mcal/d)	0.40	0.42	0.38	0.34	0.06	0.967
N Intake	45.17^{a}	35.60^{d}	39.39^{c}	40.60^{b}	0.65	0.001
N in faeces	5.33	5.47	6.88	8.09	0.55	0.251
N output	15.25	16.08	16.25	16.46	1.49	0.994
N Retained	29.92	23.52	23.14	25.57	1.67	0.983
N Output/N Intake	0.34	0.41	0.41	0.42	0.04	0.884
Urine N/N Intake	0.22	0.27	0.24	0.21	0.04	0.963
N in faeces/ N intake	0.12	0.14	0.17	0.21	0.01	0.112
N Retained/N intake (NPU)	0.66	0.59	0.58	0.57	0.04	0.884

Table 4: Effect of cassava peel-leaf blends on apparent nutrient digestibility and nitrogen balance of growing pigs.

[ab]Means on the same row having different superscripts are significantly different (P < 0.05).

UCPLB: Unfermented cassava peel-leaf blends; NCPLB: Naturally fermented cassava peel-leaf blends; MCPLB: microbial fermented cassava peel-leaf blends; SEM = Pooled standard error of means.

Nitrogen balance

Nitrogen balance of pigs revealed that apart from nitrogen intake from diet, other parameters were not significantly ($P > 0.05$) affected (Table 4). The nitrogen intake reduced ($P < 0.05$) with inclusion of CPLB in which pigs fed diet containing UCPLB had the least ($P < 0.05$) nitrogen intake. The reduction in nitrogen intake is associated with the impact of dietary fibre level as crude fibre has been reported to reduce the digestion of other dietary nutrients [50]. However, pigs fed diet containing MCPLB had higher ($P < 0.05$) nitrogen intake than those fed diet containing UCPLB and those fed diet containing WCPLB. The better nitrogen intake observed with pigs fed MCPLB diet is due to increase in nitrogen content of the fermented ingredients as a result of nitrogen from microbial origin [51]. Other parameters such as nitrogen retained, nitrogen output and net protein utilisation were not significantly ($P > 0.05$) affected which suggests equal utilisation of nitrogen from the diets.

Conclusion

The subjection of CPLB to solid state fermentation using spore forming microbes as inoculum increased crude protein content of the fibrous feedstuff. Inclusion of UCPLB and WCPLB in diet of pigs improved NDF and ADL digestibility. Dietary inclusion of MCPLB resulted in higher nitrogen intake compared to UCPLB and WCPLB.

Acknowledgements

This work was supported by the Grant provided by Tertiary Trust Fund (TETFUND) Nigeria with Grant No. TETFUND/Serial No. 9/2017 and the assistance provided by the Department of Microbiology, College of Biological Sciences towards the success of this work is highly appreciated.

Conflict of Interest

The authors declared no conflict of interest.

Bibliography

1. Adesehinwa AOK., *et al*. "Growing pigs fed cassava peel based diet supplemented with or without Farmazyme® 3000 proenx: Effect on growth, carcass and blood". *African Journal of Biotechnology* 10.14 (2011): 2791-2796.

2. Afolayan SB. "Evaluation of sweet potato meal as a source of energy in the diets of chickens". Ph.D. Thesis, Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria (2010).

3. Ekwe OO., *et al*. "Effects of Sun-dried Cassava Peels Supplementation on the Performance of Weaner Pigs". *Asian Journal of Applied Sciences* 4 (2011): 794-800.

4. Njoku CP., *et al*. "Influence of feed quantity offered on linear body measurements, nutrient digestibility and back fat composition of finishing pigs". *The Pacific Journal of Science and Technology* 14.1 (2013): 387-396.

5. Sriroth K., *et al*. "Processing of cassava waste for improved biomass utilization". *Bioresource. Technology* 71 (2000): 63-69.

6. Akinfala EO and Tewe OO. "Supplemental effects of feed additives on the utilization of whole cassava plant by growing pigs in the tropics". *Livestock Research for Rural Development* 16 (2004): 1-10.

7. Nwakpu PE., *et al*. "Performance of wearner pig fed diet containing different proportion of derided cassava peel and whole maize". *Tropical Journal of Animal Science* 2 (1999): 81-87.

8. Akinola OS., *et al*. "Cassava root peel as a replacement for maize in diets for growing pigs: effects on energy and nutrient digestibility, performance and carcass characteristics. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, 114.2 (2013): 159-166.

9. Adesehinwa AOK., *et al*. "Response of growing pigs to cassava peel based diets supplemented with avizyme® 1300: growth, serum and hematological indices". *Bulgarian Journal of Agricultural Science* 14.5 (2008): 491-499.

10. Okpako CE., *et al*. "Proximate composition and cyanide content of cassava peels fermented with *Aspergillus niger* and *Lactobacillus rhamnosus"*. *Journal of Agriculture and Environment* 6.2 (2008): 251-255.

11. Bui Huy Nhu Phuc., *et al*. "The nutritive value of sun-dried and ensiled cassava leaves for growing pigs". *Livestock Research for Rural Development* 8.3 (1996): 26-33.

12. Bokanga M. " Processing of cassava leaves for human consumption". *Acta Horticulturae* 375.18 (1994): 203-208.

13. Ngudi DD., *et al*. "Cassava cyanogens and free amino acids in raw and cooked leaves". *Food and Chemical Toxicology* 41 (2003): 1193-1197.

14. Cardoso AP., *et al*. "Processing of cassava roots to remove cyanogens". *Journal of Food Composition and Analysis* 18 (2005): 451-460.

15. Oni AO., *et al*. "Chemical composition and nutritive value of four varieties of cassava leaves grown in South-Western Nigeria". *Journal of Animal Physiology and Animal Nutrition* 95 (2010): 583-590.

16. Nguyen THL., *et al*. "Ileal and total tract apparent crude protein and amino acid digestibility of ensiled and dried cassava leaves and sweet potato vines in growing pigs". *Animal Feed Science and Technology* (2011).

17. Achinewhu SC., *et al*. "Physicochemical properties and garification (gari yield) of selected cassava cultivars in Rivers State, Nigeria". *Plant Foods for Human Nutrition*, 52.2 (1998): 133-140.

18. Motarjemi Y. "Impact of small scale fermentation technology on food safety in developing countries". *International Journal of Food Microbiology* 75.3 (2000): 213-229.

19. Google Earth. "Earthmaps". Online map finder (2021).

20. Adeyemi OA., *et al*. "Performance of growing rabbits fed diets containing fermented and unfermented cassava leaf: peel meal mix as replacement for maize". *Malaysian Journal of Animal Science* 17.1 (2014): 61-72.

21. Walker GM and Stewart GG. "Review. Saccharomyces cerevisiae in the production of fermented beverages". *Beverages* 2.3 (2016): 1-12.

22. Aidoo KE., *et al*. "Occurrence and function of yeasts in Asian indigenous fermented foods". *FEMS Yeast Research* 6.1 (2006): 30-39.

23. Ferreira G., *et al*. "Production of xylanolytic enzymes by Aspergillus tamarii in solid state fermentation". *FEMS Microbiology Letters* 173 (1999): 335-339.

24. Kranthi VS., *et al*. "Production of Protease by Aspergillus flavus Through Solid State Fermentation Using Different Oil Seed Cakes". *International Journal of Microbiology Research* 3.1 (2012): 12-15.

25. Cooke WB and Brazis AR. "Occurrence of molds and yeasts in dairy products". *Mycopathologia Et Applied Mycologia* 35 (1968): 281-289. International Dairy Federation. Standard Methods ISO/DIS 6611.

26. Murray PR., *et al*. "Manual of Clinical Microbiology". (Ed.), 8th Ed., American Society for Microbiology, Washington, D.C. (2003).

27. Association of Official Analytical Chemists (AOAC), Determination of moisture, ash, protein and fat. 18th edn. Washington, USA (2005).

28. McCleary BV. "An integrated procedure for the measurement of total dietary fibre (including resistant starch), non-digestible oligosaccharides and available carbohydrates". *Analytical and Bioanalytical Chemistry* 389 (2007): 291-308.

29. Vetter J. "Plant Cyanogenic Glycosides". *Toxicon* 38 (2000): 11-36.

30. NRC. Nutrient Requirements of Poultry 9th ed. National Academic Press, Washington, D.C., USA (1994).

31. Khan M A., *et al*. "Techniques Measuring Digestibility for the Nutritional Evaluation of feeds. Review". *International Journal of Agriculture and Biology* 5.1 (2003): 91-94.

32. Adeola O. "Digestion and balance techniques in pigs". In: Swine Nutrition (2nd Ed. A. J. Lewis and L. L. Southern). CRC Press, Washington, DC (2001): 903-916.

33. SAS. SAS/STAT User's Guide Sas Institute Incorporation, Cary, North Carolina. USA (2000).

34. Moreira FG., *et al*. "A thermostable maltose-tolerant α-amylase from *Aspergillus tamari*". *Journal of Basic Microbiology* 44 (2004): 29-35.

35. Mathivanan R., *et al*. "Feeding of fermented soybean meal on broiler performance". *International Journal of Poultry Science* 5 (2006): 868-872.

36. Villena G K and Gutierrez-Correa M. "Production of lignocellulolytic enzymes by *Aspergillus niger* bio-films at variable water activities". *Journal of Biotechnology* 10 (2007): 124-140.

37. Akinyele B J., *et al*. "Bioconversion of Selected Agricultural Wastes and Associated Enzymes by *Volvariella volvacea*: An Edible Mushroom". *Research Journal of Microbiology* 6 (2011): 63-70.

38. Jokotagha OA and Amoo IA. "Effect of fermentation on the nutritive value of *Aspergillus niger* and *Aspergillus fumigatus* fermented *Hura crepitans* seed flour". *Greener Journal of Physical Sciences* 2 (2012): 85-88.

39. Adeleke B S., *et al*. "Effect of fermentation of cassava peels". *Asian Journal of Plant Science and Research* 7.1 (2017): 31-38.

40. Obueh H O and Ikenebomeh MJ. "Bioethanol production and compositional changes during fermentation of cassava processing wastes from a local cassava mill". *International Journal of Current Research in Biosciences and Plant Biology* 1.4 (2014): 43-51.

41. Omafuvbe BO., *et al*. "Chemical and biochemical compositon changes in African locust beans *Parkia biglobosa* and melon (*Citrullus vulgaris*) seeds during fermentation to condiments". *Pakistan Journal of Nutrition* 3 (2004): 140-145.

42. Padmaja G. "Cyanide detoxification in cassava for food and feed use". *Critical Review in Food Science and Nutrition* 35.4 (1995): 299-339.

43. Aderemi FA and Nworgu FC. "Nutritional Status of Cassava Peels and Root Sieviate Biodegraded with *Aspergillus niger*. *American.-Eurasian Journal of Agriculture and Environmental Science* 2.3 (2007): 308-311.

44. Chesson A. "Mechanistic models of forage cell wall degradation". In: H.G.Jung, D.R. Buxton, R.D. Hatfield, and J. Ralph (Ed.) Forage Cell Wall Structure and Digestibility. p347. ASA- CSSA-SSSA, Madison, WI (1993).

45. Madalla N., *et al*. "Evaluation of ground - sundried cassava leaf meal as protein source for Nile tilapia *Oreochromis niloticus* (L) juvenile's diet". *Tanzania Journal of Agricultural Sciences* 15.1 (2016): 1-12.

46. Huu HL and Khammeng T. "Effect of yeast fermented cassava pulp (FCP) on nutrient digestibility and nitrogen balance of post-weaning pigs". *Livestock Research for Rural Development* 26 (2014): 8.

47. Ziemer CJ., *et al*. "Effects of feeding fiber fermenting bacteria to pigs on nutrient digestion, fecal output, and plasma energy metabolites". *Journal of Animal Science* 90 (2012): 4020-4027.

48. Bakker GCM. "Interaction between carbohydrates and fat in pigs: impact on energy evaluation of feeds, Bakker, PhD Thesis". Wageningen University, Wageningen, the Netherlands (1996): 31-67.

49. Acosta J A., *et al*. "Digestion and nitrogen balance using swine diets containing increasing proportions of coproduct ingredients and formulated using the net energy system". *Journal of Animal Science* 95.3 (2017): 1243-1252.

50. Milgen JV. "Nutrient flow models, Energy Transactions and Energy Feed Systems". In: R.M. Gous, T.R. Morris, and C. Fisher, editors, Mechanistic modelling in pig and poultry production. CABI, Wallingford, UK. (2006): 143-162.

51. Xiao J., *et al*. "Gut function-enhancing properties and metabolic effects of dietary indigestible sugars in rodents and rabbits". *Nutrients* 7.10 (2015): 8348-836.