Feeding Microbe-Fermented Cassava Tuber Wastes Modulates Gut Microbiota and Faecal Characteristics of Growing Pigs

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Abstract

A four week trial was conducted on 42 weanling pigs to study the effect of feeding differently fermented cassava tuber waste (CTW) diets on faecal parameters and enteric microbial ecology of the experimental animals. Seven different diets were formulated. Three of the diets contained 20% inclusion of cassava starch residues in the unfermented, naturally fermented and microbially fermented form respectively and were designated unfermented cassava starch residues (UFCSR), naturally fermented cassava starch residues (NFCSR) and microbially fermented cassava starch residues (MFCSR). Three other diets, similarly formulated but with 20% inclusion of cassava peels were designated as unfermented cassava peels (UFCP), naturally fermented cassava peels (NFCP) and microbially fermented cassava peels (MFCP) respectively. These six CTW diets replaced 30% of maize in the control diet. The two microbially fermented wastes (MFCSR and MFCP) were processed with a combination of two lactic acid bacteria (*Lactobacillus coryneformis* and *Lactobacillus delbrueckii*) and a fungus (*Aspergillus fumigatus*). The results showed significant differences (P<0.05) in the average faecal output, faecal dry matter, faecal volume, faecal pH and the total bacterial counts, while the faecal density and the total fungal counts were similar (P>0.05) among the seven dietary treatments. The biochemical characterization of bacterial and fungal isolates from the faecal samples revealed that the highest bacterial biodiversity was recorded in both the NFCSR and MFCP diets while the control group had the least. Of the 17 different fungal species, 6 were isolated from the control group while 2 each were isolated from the two unfermented CTW diets (UFCSR and UFCP). The biochemical characterization of the microbial isolates also showed that the bacteria- *Bacillus* spp. and *Escherichia coli* had the highest frequency of occurrence (100%) across treatments, while *Micrococcus luteus* had the least frequency (28.57%). The fungus-*Mycotypha microsporium* had the highest colonizing ability as it was isolated from the faecal samples of pigs in 4 out of the 7 dietary treatments. Conclusively, feeding the CTW diets to pigs could have a profound influence on growth and faecal parameters and by extension on the digestive physiology of the pigs, also these CTW and their methods of processing could modulate the biodiversity of gut microflora in pigs and possibly in any other livestock species.

Keywords: Cassava tuber wastes; Fermentation; Faecal evaluation; Gut microflora

Introduction

The scarcity of conventional feed resources especially in the developing countries of the world has ever continued to challenge livestock producers to find alternative ways of feeding their livestock if animal production and an uninterrupted supply of animal protein are to be sustained. This challenge has become more imperative now since the developed countries are now diverting their abundant grain reserves hitherto used for feeding livestock and as a famine relief package to some hunger-stricken third world countries to the production of biofuel to propel their huge industrial sector. Chauynarong et al. [1] reported that as a result of the increased use of maize for producing ethanol and biofuel, maize prices in the United States of America rose from $2.60 a bushel in 2006 to nearly $4.00 a bushel in 2007. The diversion of excess grains for the production of biofuels in this way has put a lot of pressure on the available conventional feed resources especially in the developing countries thereby forcing their prices to a sky-rocketing level often beyond the reach of the average livestock producer.

As a way of confronting this challenge, the use of alternative feed resources has been canvassed [2-4]. The various by-products of cassava tubers like the peels, the sieveates, the fibre etc. have been used variously in many parts of the world for feeding many species of livestock with promising results [3]. The impact of feed on the digestive physiology of farm animals has been well documented [5,6]. The type of feed and the form in which it is consumed have been known to have a profound influence on growth performance and general well-being of livestock [7]. Feed has been used as prebiotics to beneficially modify gut microbial population [8] and to improve performance and reduce scouring in piglets [9].

The roles of the gut microbiota often times have been described as a mutually symbiotic relationship [10]. These microorganisms have been known to fulfill a host of useful functions including digestion of unutilized carbohydrates, stimulating cell growth, suppressing/repressing the growth of harmful microorganisms, training the immune system to respond only to pathogens and defending their host animals against some diseases [10-12]. To our knowledge, these mutually benefiting roles between the type of feed given to livestock and gut microflora have not been investigated in weanling pigs fed microbially fermented cassava tuber waste diets. Therefore, the objective of this study was to investigate the effects of microbially

fermented CTW diets on growth response, faecal parameters and microbial ecology of weanling pigs.

Materials and Methods

Sources and preparation of cassava tuber wastes

The cassava wastes i.e. the cassava peels (CAP) and the cassava starch residues (CSR) were sourced from Matna Foods Limited, Ogbese, Ondo State, Nigeria. These wastes were prepared according to the methods described by Aro et al. [3] and subsequently used in the formulation of the experimental diets designated as follows: Diet I was the control diet with no inclusion of the CTW.

Ingredients	T1	T2	T3	T4	T5	T6	T7
Maize	40.00	28.00	28.00	28.00	28.00	28.00	28.00
GNC	28.43	28.43	25.82	24.66	26.95	22.16	21.32
PKC	28.57	20.57	23.18	24.34	23.05	26.84	27.68
UFCSR	0.00	20.00	0.00	0.00	0.00	0.00	0.00
NFCSR	0.00	0.00	20.00	0.00	0.00	0.00	0.00
MFCSR	0.00	0.00	0.00	20.00	0.00	0.00	0.00
UNCP	0.00	0.00	0.00	0.00	20.00	0.00	0.00
NFCP	0.00	0.00	0.00	0.00	0.00	20.00	0.00
MFCP	0.00	0.00	0.00	0.00	0.00	0.00	20.00
Bone meal	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Oyster shell	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vit/min. premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Crude protein	20.10	20.20	20.29	20.26	20.11	20.02	20.06
Energy (MJ/kg)	12.25	11.81	11.90	11.91	12.01	11.92	11.94
Crude fibre	6.23	7.61	7.65	7.62	6.98	7.43	6.76

GNC: Groundnut Cake; PKC: Palm Kernel Cake; UFCSR: Unfermented Cassava Starch Residues; NFCSR: Naturally Fermented Cassava Starch Residues; MFCSR: Microbe-Fermented Cassava Starch Residues; UFCP: Unfermented Cassava Peel; NFCP: Naturally Fermented Cassava Peel; MFCP: Microbe-Fermented Cassava Peel.

T1: The control diet; T2: Diet with 20% UFCSR; T3: Diet with 20% NFCSR; T4: Diet with 20% MFCSR; T5: Diet with 20% UFCP; T6: Diet with 20% NFCP; T7: Diet with 20% MFCP.

Table 1: Gross composition (%) of the experimental diets of the weanling pigs.

Diet II was formulated with 20% inclusion of unfermented cassava starch residues (20% UNCSR). Diet III had 20% inclusion of naturally fermented cassava starch residues (20% NFCSR). Diet IV had 20% inclusion of microbe-fermented cassava starch residues (20% MFCSR). Diets V, VI and VII were formulated with 20% inclusion of

unfermented, naturally fermented and microbe-fermented cassava peels respectively (20% UNCP, 20% NFCP and 20% MFCP). The gross composition of the experimental diets is presented in Table 1.

Experimental animals and their management

Forty-two cross-bred (Large White × Duroc Jersey) weanling pigs of about 9.51 ± 0.32 kg live weight were purchased from a livestock farm near Akure, Ondo State, Nigeria. The animals were housed in individual pens measuring 2 m × 3 m and each was equipped with a fixed concrete feeder and drinker. The floor equally was of hard concrete. The roof was even-span and the height of the wall partition was 1.2 m. The height of the roof from the eave to the ground was 3.5 m, a design that ensures straight through ventilation within the pens. The animals were stabilized for two weeks during which they were given shots of Ivermectin to control both internal and external parasites. They were also given Iron dextran and antibiotic (Oxytec L.A.) injections. The animals were fed the experimental diets and water *ad libitum* during the day throughout the four weeks of the experiment during which records of daily feed intake and weekly weight gain were taken.

Faecal collection

In the last week of the experiment, the animals were transferred to individual metabolic cages to facilitate easy collection of faecal matter. The total faecal output was collected for seven consecutive days and from this the average faecal output per animal was computed. Also freshly voided faeces from each animal was "captured" within this seven days which were used to determine the faecal volume, faecal pH, faecal moisture, faecal dry matter content, faecal consistency and faecal density.

The faecal volume

This was determined by measuring the volume of water displaced by a known mass of freshly voided faeces. 50 g of faecal samples were collected and wrapped in a pre-weighed water proof wrapper (Aluminium foil). This was gently lowered into a graduated measuring cylinder filled with water up to a known volume (initial volume). The rise in volume (final volume) of the water in the cylinder when the faecal sample was gently lowered in was taken as the volume of the faecal sample following the Archimedes principle of floatation. Therefore,

Volume of the faecal sample=Final volume of water in the cylinder-The initial volume.

The faecal density

The faecal density was determined by dividing the faecal mass by its corresponding volume i.e.:

Faecal density=Faecal mass/Faecal volume (gcm^{-3})

The faecal moisture

This was computed as the loss in weight as a result of drying a known weight of faecal sample in the oven at a temperature of 105°C for 24 h until a constant weight was achieved. Thus the percentage faecal moisture content i.e.:

$$\% faecal\ moisture\ content = \frac{W2 - W3}{W2 - W1} \times 100$$

Where W1 is the weight of the Aluminium foil, W2 is the weight of fresh faecal sample+Aluminium foil and W3 is the oven-dried weight of faecal sample+Aluminium foil.

Faecal pH

This was carried out to ascertain whether there is any effect of dietary treatment on the pH of the faecal samples. One gram (1 g) of each fresh faecal sample was weighed and transferred into 20 mL of distilled water, stirred well to make a solution which was left to settle for about 20 min during which the pH electrode was stabilized using pH4 buffer solution. The electrode was inserted into the test solution and the value read off. The stable values were recorded as the pH of the test solutions. All the 42 faecal solution were so treated in turn by removing and washing the pH electrode with distilled water before using it to run the value for a fresh sample.

Method	Observation per isolate						
	A	B	C	D	E	F	G
Gram's staining	+	+	-	+	+	+	+
Shape of cells	S	R	R	R	R	S	S
Motility	-	+	+	-	-	-	-
Catalase test	-	+	+	-	-	+	+
Oxidase test	-	-	-	-	-	-	-
Spore formation	-	+	-	-	-	-	-
Indole test	-	-	+	-	-	-	-
Coagulase test	-	-	-	-	-	+	-
Oxidative/fermentation	-/F	O/F	-/F	-/F	-/F	-/F	O/F
Sugar fermentation:							
Glucose	a	a	ag	a	a	a	a
Sucrose	a	a	-	a	a	a	a
Mannitol	a	-	a	a	a	a	a
Lactose	a	-	a	-	-	a	a
Fructose	-	ag	-	a	a	a	a
Galactose	a	ag	a	-	a	ag	a

Probable organisms: A: *Streptococcus faecalis*; B: *Bacillus* spp; C: *Escherichia coli*; D: *Lactobacillus delbrueckii*; E: *Lactobacillus coryneformis*; F: *Staphylococcus aureus*; G: *Micrococcus luteus*.

Observations: +: Positive; -: Negative; S: Spherical; R: Rod; O: Oxidation; F: Fermentation; a: Acid forming; g: Gas forming and ag: Acid and Gas forming.

Table 2: Biochemical characterization of bacterial isolates from the faecal samples of weanling pigs fed microbe-fermented CTW diets.

Faecal consistency

It was carried out to show how well formed or loosely formed the faecal matter was in order to reveal whether the diets provoked a tendency to diarrhoea in the experimental animals. The faecal consistency scoring system of Marquardt et al. [13] which categorized the consistency of faecal matter as normal (0), soft faeces (1), mild diarrhoea (2) and severe diarrhoea (3) was used.

Faecal microbial evaluation

The microbiota of the faecal samples in terms of the bacterial and fungal populations, were isolated using standard procedures. The bacterial evaluation of the faecal samples was carried out in the Microbiology Department of the Federal University of Technology, Akure, Nigeria where the fresh samples were cultured for bacterial growth. Two hundred and fifty-two (252) McCartney or bijou bottles (6 bottles per sample) were used for serial dilution, 42 disposable Petri dishes, nutrient agar, 42 (2 ml) needles and syringes, masking tape, methylated spirit lamp, spatula, bowl, brush, soap, 90% alcohol, cotton wool, electronic weighing balance (Precisa 30000DSCS, Precisa Instrument Ltd., Switzerland) and distilled water were also used. Six labelled bijou bottles for each sample were filled with 9 mL of distilled water, covered and sterilized in an autoclave at 121°C for 15 min, 14 g of nutrient agar was weighed, dissolved in 500 mL of distilled water and autoclaved as well before it was allowed to cool to 50°C. Approximately 1 g of each fresh faecal sample was dissolved into the first bottle labelled "A" for each sample after which 1 mL of the solution was drawn, transferred and mixed with the sterilized water in bottle "B". This was repeated until the sixth bottle for each sample was so treated. 1 mL of the content in each of the sixth bottles was then measured into the corresponding labelled Petri dishes and the agar was poured into each carefully and enough to cover the base and then swirled both clockwise and anticlockwise for thorough mixing of the sample solution with the agar. After solidification of the agar, the culture was incubated in inverted position at 37°C for 18-24 h. All these inoculation were done inside a previously sterilized laminar flow chamber.

The inverted Petri dishes were observed for bacterial growth after this time interval. Seven different colonies based on their growth pattern and morphology in the agar were observed and were later identified *via* characterization through gram staining , motility, spore formation, indole, coagulase, oxidase, catalase and sugar fermentation tests. The bacterial population was determined by counting the number of colonies in each plate. The growth and morphological pattern in agar which is a measure of their physical characteristics include shape, size, chromogenesis, opacity, elevation, surface, edge etc.

Characterization tests

Practical guides as described by Wiley et al. [14] were used in the biochemical characterization of the bacterial isolates.

The Gram stain

This was used to differentiate the Gram positive i.e. those that retain stain firmly after being treated with basic para-rosalinine dyes like crystal violet, followed by iodine and those that do not.

Catalase test

This test uses 30% concentrated H_2O_2 on bacterial colony to confirm catalase presence if bubbles were produced and its absence if bubbles were not produced. Catalase is an enzyme that converts H_2O_2 to water and oxygen in the following reaction:

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2$$
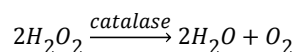
Catalase test is used to differentiate *Streptococcus* (negative to catalase test) from *Staphylococcus* (positive to catalase test) and *bacillus* (positive) from *Clostridium* (negative).

Indole production test

Indole production was confirmed using tryptophan-rich medium to differentiate coliform organisms e.g. *Escherichia* (indole +) from *Enterobacter* (indole -).

Sugar fermentation and oxidation tests

These tests were performed to determine the aerobic (those that utilize sugar in the presence of oxygen) and anaerobic organisms (those that utilize sugar in the absence of oxygen) using glucose, sucrose, mannitol, lactose, fructose and galactose. These sugar fermentation tests are accompanied by acid and gas production with the use of appropriate indicators. Change of the sugar solution from blue to yellow indicate acid production and an inverted Durham tube which shows a space at the bottom of the tube is indicative of gas production.

Coagulase test

This test uses human plasma and was carried out to differentiate pathogenic from non-pathogenic *Staphylococcus* through their coagulating ability.

Oxidase test

This was carried out to differentiate enteric bacteria (all negative) using 1% aqueous solution of tetramethyl-phenylenediame hydrogen chloride as reagent with which the culture was streaked to observe their oxidative ability. If colonies develop pink colour that successfully turns dark red, purple and black within 10-30 min, then they are oxidase positive. The various biochemical methods used in the characterization of the bacteria isolates are as given in Table 2.

Evaluation of faecal fungi

This was carried out in the Diagnostic Unit of the Department of Animal Production and Health Laboratories of the Federal University of Technology, Akure, Nigeria. 4.5 g of Potato Dextrose Agar (PDA) was diluted in 500 mL of distilled water and about 0.1 g chloramphenicol was added to inhibit the growth of other microorganisms in the culture. Following agar sterilization and cooling, the sterile sample solutions (9 mL each) were inoculated with 1 g of faecal samples from the animals and serially diluted; the sample solution in the sixth bottle was used as in the bacteria culture. The culture was placed in a sterilized inoculating chamber for five days to incubate at room temperature. The population of the fungi was determined by counting the number of visible colonies in each plate as done for bacterial evaluation. Identification of the different species of fungi was done at the Microbiology Department of the Federal University of Technology, Akure, Nigeria.

Results

Growth response

Table 3 shows the growth response of the experimental animals to the differently fermented CTW diets. The final weights were similar (P>0.05) in all the treatments but ranged from 13.66 kg in pigs fed with the MFCP diet to 14.60 kg in those fed with the NFCSR diet. The highest (P<0.05) weight gain was recorded in NFCP diet (6.28 kg) while the MFCP had the least value (5.36 kg). The NFCP that had the highest weight gain also had the second highest feed intake (22.11 kg) while the MFCP with the least weight gain had the least total feed intake (15.33 kg). The best feed conversion to flesh and the best efficient feed users were the animals on the UFCSR diet.

Parameters	Control	UFCSR	NFCSR	MFCSR	UFCP	NFCP	MFCP	± SEM
Initial wt(kg)	8.93	8.60	8.43	8.43	8.60	8.30	8.30	0.37
Final wt(kg)	14.53	14.60	13.91	14.03	14.52	14.58	13.66	0.41
Total wt gain(kg)	5.60^{bc}	6.00^{ab}	5.48^{bc}	5.60^{bc}	5.92^{b}	6.28^{a}	5.36^{c}	0.20
Weekly wt gain(kg)	1.40	1.50	1.37	1.40	1.48	1.57	1.34	0.11
Daily wt gain(g)	200.00	214.29	195.71	200.00	211.43	224.29	191.43	20.00
Total feed intake(kg)	20.89^{ab}	15.84^{cd}	23.73^{a}	15.79^{cd}	18.53^{bc}	22.11^{a}	15.33^{d}	0.33
Weekly feed intake(kg)	5.22^{ab}	3.96^{cd}	5.93^{a}	3.95^{cd}	4.63^{bc}	5.53^{a}	3.83^{d}	0.13
Daily feed intake(kg)	0.75^{ab}	0.57^{cd}	0.85^{a}	0.56^{cd}	0.66^{bc}	0.78^{a}	0.55^{d}	0.02
FCR	3.73	2.64	4.33	2.82	3.13	3.52	2.86	0.36
EFU	0.23	0.38	0.23	0.35	0.32	0.28	0.35	0.04

a,b,c,d: Means in the same row but with different superscripts are statistically (P<0.05) different. UFCSR: Unfermented Cassava Starch Residues; NFCSR: Naturally Fermented Cassava Starch Residues; MFCSR: Microbe-Fermented Cassava Starch Residues; UFCP: Unfermented Cassava Peel; NFCP: Naturally Fermented Cassava Peel; MFCP: Microbe-Fermented Cassava Peel

Table 3: Growth response of weanling pigs fed microbe-fermented CTW diets.

Faecal parameters	Control	UFCSR	NFCSR	MFCSR	UFCP	NFCP	MFCP	± SEM
Output (kg/wk)	2.16^{ab}	1.47^{d}	2.17^{ab}	1.94^{cd}	1.75^{cd}	2.46+	1.63^{cd}	0.11
Dry matter (kg/wk)	1.00^{a}	0.58^{c}	0.87^{ab}	0.69^{bc}	0.69^{bc}	0.96^{a}	0.96^{a}	0.06
% dry matter	46.48^{a}	39.41^{b}	39.67^{b}	35.51^{b}	39.59^{b}	39.07^{b}	34.95^{b}	1.80
% moisture	53.52^{b}	60.59^{a}	60.33^{a}	64.49^{a}	60.41^{a}	60.93^{a}	65.05^{a}	1.80
Volume (cm^3)	2.58^{ab}	1.65^{b}	2.55^{ab}	3.62^{a}	1.86^{ab}	2.80^{ab}	2.02^{ab}	0.43
Density (gcm^{-3})	0.84	0.89	0.90	0.81	0.87	0.89	0.81	0.08
pH	4.52^{b}	4.80^{ab}	4.87^{a}	4.98^{a}	4.75^{ab}	5.02^{a}	4.87^{a}	0.09
Consistency	0.50	0.83	0.83	0.83	0.83	0.83	1.00	0.21
Bacteria (× 10^6)	45.33^{c}	57.33^{bc}	49.00^{c}	102.67^{ab}	83.00^{abc}	150.00^{ab}	165.00^{a}	25.57
Fungi (× 10^6)	5.00^{c}	2.67^{cd}	4.00^{c}	11.33^{b}	4.67^{c}	1.00^{d}	14.67^{a}	3.01

a,b,c,d: Means in the same row but with different superscripts are statistically (P<0.05) different. UFCSR: Unfermented Cassava Starch Residues; NFCSR: Naturally Fermented Cassava Starch Residues; MFCSR: Microbe-Fermented Cassava Starch Residues; UFCP: Unfermented Cassava Peel; NFCP: Naturally Fermented Cassava Peel; MFCP: Microbe-Fermented Cassava Peel.

Table 4: Faecal evaluation and microbial population parameters of weanling pigs fed microbe-fermented CTW diets.

Isolates	Control	UFCSR	NFCSR	MFCSR	UFCP	NFCP	MFCP	Freq	% Occ.
S. faecalis	+	+	+	+	+	-	+	6	85.71
Bacillus. spp.	+	+	+	+	+	+	+	7	100.00
E. coli	+	+	+	+	+	+	+	7	100.00
S. aureus	-	+	+	-	+	+	+	5	71.43
L. delbrueckii	-	-	+	+	-	-	+	3	42.86
L. coryneformis	-	-	+	+	-	+	+	4	57.14
M. luteus	-	-	-	-	+	+	-	2	28.57
Total isolate/treatment	3	4	6	5	5	5	6		

Freq: Frequency of Occurrence; % Occ.: Percentage of Occurrence; UFCSR: Unfermented Cassava Starch Residues; NFCSR: Naturally Fermented Cassava Starch Residues; MFCSR: Microbe-Fermented Cassava Starch Residues; UFCP: Unfermented Cassava Peel; NFCP: Naturally Fermented Cassava Peel; MFCP: Microbe-Fermented Cassava Peel.

Organisms: *S. faecalis* (*Streptococcus faecalis*), *Bacillus*. spp. (*Bacillus species*), *E. coli* (*Escherichia coli*), *S.aureus* (*Staphylococcus aureus*), *L. delbrueckii* (*Lactobacillus delbrueckii*), *L. coryneformis* (*Lactobacillus coryneformis*), *M. luteus* (*Micrococcus luteus*).

Table 5: Bacterial isolates from the faecal samples of weanling pigs fed dietary inclusion of microbe-fermented cassava tuber wastes (CTW).

Faecal evaluation

Table 4 shows the result of faecal evaluation and microbial population in the faecal samples of pigs on the seven dietary treatments. The largest faecal output per week (2463.33 g) was observed in pigs fed NFCSR diet while the least faecal output (1468.33 g) was recorded in the UNCSR diet. The largest and least faecal outputs were therefore recorded among the CTW diets while the control diet was intermediate among them. Also faecal output showed statistical significant differences among the treatment means. The dry matter content of the faeces in the control diet had the highest value and was significantly higher (P<0.05) than the values obtained for the CTW diets except for the NFCP and MFCP diets. The least dry matter content was observed in the UFCP diet. The moisture content was

highest in the MFCP diet but had similar (P>0.05) value with all other CTW diets and was significantly different (P<0.05) from the control diet which had the least faecal moisture content.

The faecal volume gave values that ranged from 1649.87cm^3 in the UFCSR diet to 3619.97 cm^3 in the MFCSR diet. The faecal volumes of these two dietary treatments were statistically different form each other but both were similar to other dietary treatments. The faecal density gave similar (P>0.05) values across all dietary treatments but the NFCSR had the highest faecal density (0.90 gcm^{-3}) while the MFCSR and the MFCP (the two diets whose CTW were fermented with selected microbial inoculums) had the lowest faecal density (0.81 gcm^{-3}). The bacterial population was highest (165.00 × 10^6 CFU) in the MFCP and lowest (45.33 × 10^6 CFU) in the control diet. The bacterial

count in the NFCSR gave similar (P>0.05) values with the control diet while that of MFCSR, though numerically lower, had similar (P>0.05) value with the NFCP.

Isolates	Descr.	Ctrl	UFCSR	NFCSR	MFCSR	UNCP	NFCP	MFCP	Freq	% Occ
Mycotypha racemosum	Green	+	-	-	-	-	-	-	1	3.70
Mycotypha microsporum	BG	+	-	+	+	-	-	+	4	14.82
Aspergillus fumigatus	Brown	+	-	-	-	-	-	-	1	3.70
Itersonilia perplexans	WS	+	-	-	-	-	-	-	1	3.70
Streptothrix atra	White	+	-	-	-	-	-	-	1	3.70
Sporobolomyces salmomcolor	RW	+	-	+	+	-	-	-	3	11.11
Oidiodendron griseum	WC	-	+	-	-	-	-	-	1	3.70
Trichosporonoides oedocephalis	DG	-	+	-	-	-	+	+	3	11.11
Glicocladium deliquesces		-	-	+	-	-	-	-	1	3.70
Halosporangium parium	WR	-	-	+	-	-	-	-	1	3.70
Aspergillus spp.	WB	-	-	-	+	-	-	-	1	3.70
Penicillium italicum	DG	-	-	-	+	-	-	-	1	3.70
Articulospora inflata	White	-	-	-	-	+	+	-	2	7.41
Syncephalastrum racemosum	WEG	-	-	-	-	+	+	-	2	7.41
Geotrichum albidum	White	-	-	-	-	-	+	-	1	3.70
Botryotrichum piluliferum	Grey	-	-	-	-	-	+	+	2	7.41
Penicillium notatum		-	-	-	-	-	+	-	1	3.70
Total Isolate/sample		6	2	4	4	2	6	3		

Descr.: Description; Ctrl: Control treatment; UFCSR: Unfermented Cassava Starch Residues; NFCSR: Naturally Fermented Cassava Starch Residues; MFCSR: Microbe-Fermented Cassava Starch Residues; UFCP: Unfermented Cassava Peels; NFCP: Naturally Fermented Cassava Peels; MFCP: Microbe-Fermented Cassava Peels; WS.: White and Spreading; WC.: White and Circular; WR.: White Rhizoid; WB.: Whitish-Brown; WEG: White-Edge-Green; D: Green; DG: Dense-Green; BG: Blue-Green; RW: Red-White; (+): Isolate present; (-): Isolate absent.

Table 6: Fungal species isolated from the faecal samples of the weanling pigs fed microbe-fermented cassava tuber waste (CTW) diets.

The fungal population gave values that were not statistically different (P>0.05), but the highest fungal load was observed in the MFCP and the lowest in NFCP. The two unfermented CTW had the least fungal load. The pH of all the faecal samples fell within the acidic range. The control diet gave the most acidic faeces (pH 4.52) while the NFCP diet had the least acidic faeces (pH 5.05).

Bacterial ecology/biodiversity

The distribution of the bacterial isolates from the faecal samples of the experimental animals is as shown in Table 5. Seven bacteria species were isolated from the pig's faecal samples. The control treatment had the least bacterial biodiversity as only 3 bacteria species were isolated from it while both the NFCSR and MFCP had the highest bacteria biodiversity with 6 of the 7 bacterial species isolated from them. The percentage distribution of all the seven bacterial isolates across all the treatments showed that both *Bacillus* spp and *E. coli* were isolated

from all the seven dietary treatments (100% distribution). *Streptococcus faecalis* was isolated 6 out of the 7 dietary treatments (85.71% distribution), *Staphylococcus aureus* from 5 out of the 7 dietary treatments (71.43% distribution), *Lactobacillus coryneformis* from 4 out of 7 treatments (57.14% distribution), *Lactobacillus delbrueckii* in 3 out of 7 treatments (42.86% distribution) while *Micrococcus luteus* was found in only two of the 7 treatment samples (28.57% distribution).

Fungal ecology/biodiversity

The fungal species isolated from the faecal samples of the weanling pigs are as shown in Table 6. Seventeen different fungi were isolated from the faecal samples with the control and NFCP diets having the largest fungal biodiversity (6 of the 17 fungal isolates were characterized from each of these two diets), 2 fungi each from UFCSR and UFCP diets, 4 fungi each from NFCSR and MFCSR diets while 3

different fungi were isolated from the MFCP diet. The two unfermented CTW diets had the least fungal biodiversity. The fungus with the highest percentage of occurrence (14.82%) across all the treatments was *Mycotypha microsporium* which occurred in 4 out of the 7 treatment samples. *Sporobolomyces salmomcolor* and *Trichosporonoides oedocephalis* appeared thrice each with 11.11% of occurrence respectively.

Discussion

Growth response

The growth responses of the experimental animals to the differently fermented CTW diets revealed that all but one (NFCSR) of the CTW diets performed numerically better than the control diet as far as the growth response criteria like weight gain, feed conversion ratio and efficiency of feed utilization were concerned. The poor response of pigs fed the control diet to these growth parameters could be as a result of relatively low prevalence and diversity of resident microbiota in their gut as reflected in their faecal matter. In fact, there is accumulated evidence in literature that a decline in microbial diversity in the colon is associated with digestive instability/disturbance [7,15,16]. Better growth response in the animals fed microbially fermented CTW diets could have resulted from greater availability of microbe-fermented products like the short chained fatty acids (SCFA) which the animals used as additional sources of energy and nutrients [10,17,18].

Bhandari et al. [7] reported a higher average daily gain of between 236-289 g and a better gain to feed ratio (efficiency of feed utilization) of between 0.44-0.66 for weanling pigs in their 3 week experiment on raw potato starch. The better growth performance/response in their experiment might not be unconnected with a lower dietary fibre (1.87-2.13) and the more digestible nature of the raw potato starch in their diets. The better feed efficiency in the microbially fermented diets could also have come from enzymes produced by the microflora which improved nutrient uptake, reduced luminal viscosity and increased animals' overall growth performance [19].

Faecal evaluation

The faecal parameters like faecal output, faecal dry matter, faecal moisture content, faecal pH, faecal volume and faecal bacterial and fungal counts gave statistical significant differences among the treatment means. The faecal pH for instance was significantly higher in the control diet than in the CTW diets. This could be as a result of the physiological adjustment of the animals' gut function in the alteration of pH (lowering of intestinal tract acidity) towards the proliferation of micro-organisms in the caecal and colonic section of the gastro-intestinal tract because the acidity of the stomach as well as bile secretion in the duodenum and proximal intestine would hinder the proliferation of these gut flora [20]. The highest dry matter content recorded in the control diet could have resulted from a lower rate of fermentation of resistant starch and dietary fibre in the colon of these animals as a result of non-inclusion of microbial inoculums in the ration formulation. The fact that the UFCSR with the best feed efficiency had the lowest faecal dry matter content pointed to the highly fermented nature of the CTWs in the distal gastro-intestinal tract where most of the carbohydrates that escaped enzymatic digestion in the intestine were digested to yield short chain fatty acids (SCFA) which were directly made available to the animals as additional sources of energy and nutrients [11].

Also, the better saccharolytic fermentation of the CTWs in the hind gut might not be unconnected with their different carbohydrate constituents [21] and the varying ability of the colonic microbiota to produce enzymes that could break them down [22]. The two unfermented CTW diets (UFCSR and UFCP) had the lowest faecal volume and these happened to be directly related to their respective faecal output. Since the fermented CTW products had higher faecal volume and output than the unfermented ones, the discrepancy observed could therefore mean that fermentation had effects on the physico-chemical properties like hydration, gelation, water binding and water retaining ability of these CTWs [23-25]. These variations in the physico-chemical properties of the CTWs could also explain differences in the faecal dry matter and moisture content observed among the dietary treatments.

The faecal density gave similar (P>0.05) values across all the treatments but interestingly, the animals fed with two of the CTWs that were selectively treated with specific microorganisms had the lowest faecal density (0.81 gcm^{-3}). The significance of this is that fermentation with lactic acid bacteria like the species used for these two CTWs i.e. *Lactobacillus delbrueckii* and *Lactobacillus coryneformis* in consortium with the fungus- *Aspergillus fumigatus* had the tendency of increasing colonic faecal volume thereby making more surface area of the digesta available for microbial degradation. Buddington and Weiher [26] reported that the addition of fermentable carbohydrates increased dog's intestinal length with more surface area. This increase in the volume of the digesta and by extension in the surface area provides evidence for interaction among the digesta, the resident microbiota and gastro-intestinal characteristics. Since density is one of the environmental factors (gastro-intestinal characteristics) affecting species diversity, these authors opined that identifying the key environmental factors that control the composition of the GIT microbiotic community is one of the several question research needs to answer. Also, in their study with neonatal mice, Hill and Cowley [27] concluded that the change in the density of colonic digesta will enable the GIT bacteria modify their environment i.e. their ecological niche for optimum growth and development.

The faecal bacteria counts were significantly (P<0.05) higher in the CTW diets than in the control. This could have been as a result of the more fermentable nature of carbohydrates from roots and tubers than those from grains [21]. Zeoula et al. [6] reported that dry matter effective degradability (DMED) of cassava by-products is higher than those of corn, also Dian et al. [28] reported that the dry matter soluble fraction and DMED presented a linear increase when the proportion of cassava by-products replacing corn in the diets increased. The gut microbiotic community thus found the CTW diets a more optimum medium for their proliferation. Their higher population in the pre-fermented CTW products also supports the view that pre-fermented products present a more conducive environment for intestinal microflora population to flourish because prior fermentation would have released readily utilizable nutrients for the micro-organisms to directly work upon [29]. The statistically different bacterial population in the different diets also revealed the influence of diets at altering or changing the composition and metabolic activities of intestinal microbiota [30].

The fungal population of the faecal matter showed that the highest counts (11.33 × 10^6 and 14.67 × 10^6 CFU) were recorded in the two selectively inoculated CTWs i.e. MFCSR and MFCP respectively. Just as it was observed under the faecal bacterial population, the fungal population of the faecal matter of these weanling pigs showed that

there is dietary effect on fungal population. Fungal growth is most ill-favoured in the NFCP and UFCSR diets. The fungal population across treatments was observably low when compared with bacterial population. The plausible explanation is that there could be a form of competitive exclusion against the fungi by the bacteria [31]. Competitive exclusion for substrates, production of anti-microbial metabolites that inhibit pathogens and competition for attachment sites are all factors that could affect microbial population in the gastro-intestinal tract (GIT) [32]. Guarner and Malagelada [11] reported on another mechanism for the repression of pathogenic organism called the "barrier effect" by which harmful organisms are prevented from colonizing the gut by the helpful gut flora. These two mechanisms might have prevented the proliferation on fungi in the gut of these weaning pigs.

The faecal consistency scores ranged from 0.50 in the control diet to 1.00 in the MFCP diet. These scores, according to Marquardt et al. [13] range from zero (normal) to 3 (severe diarrhoea). All values fell between 0 (normal) and 1 (soft faeces) indicating either the absence of or mild diarrhoea among the experimental animals which called for a word of caution in the use of the MFCP at least for this category of pigs. This point is also elucidated by the dismal growth response of pigs that fed on this diet. The possible explanation of this observation is the dietary modulation of the gut microbiota towards the proliferation of micro-organisms with diarrheagenic propensities [29].

Bacterial ecology/biodiversity of the pigs' gastro-intestinal tract

Bhandari et al. [7] in their classification of bacterial isolates from the colon digesta of weanling pigs categorized the bacteria into 6 Phyla made up of 13 Classes, 1 Subclass and 13 Orders using culture-independent molecular method of terminal restriction fragment length polymerase (T-RFLP) chain reaction of 16S rDNA gene. The number of isolates identified by these authors was more than those reported here because this current study used the culture-dependent total plate count. The most diversified organisms are the *Bacillus* spp. and *E. coli* having a proportional microbial distribution or percentage occurrence of 100%. They were followed by *Streptococcus faecalis* (85.71%) and *Staphylococcus aureus* (71.43%) while *Micrococcus luteus*, found only in the faeces of pigs that fed on UFCP and NFCP diets had the least bio-diversity (28.57%). The bacteria that were selectively used to ferment the CTW i.e. *L. delbrueckii* and *L. coryneformis* were only found at levels of 42.86% and 57.14% in the faecal matter of the pigs respectively.

The higher percentage of occurrence of *Bacillus* spp., *E. coli*, *S. faecalis* and *S. aureus* in the faecal samples of the weanling pigs gave credence to the classification of these four micro-organisms under the normal microbiota of the vertebrate GIT [14]. The lactic acid bacteria that were selectively used in the fermentation of CTWs in this study appeared to have a rather very low percentage occurrence despite the fact that they were the candidate organisms used for the fermentation of the sterile substrates (CTWs). The very low occurrence of these lactic acid bacteria could be as result of unfavourable pH of the colonic digesta to these organisms which are known to proliferate optimally at a pH of 4.4-4.6 [14]. The very low percentage of occurrence of *M. luteus* was also not unexpected because a higher percentage could have been an aberration judging from the fact that this organism is an obligate aerobe that inhabits soil, water or mammalian skin [14]. Its isolation from the faecal samples in only two of the 7 experimental treatments and from the only unsterilized (NFCP) and unfermented

(UFCP) cassava peel diets gave pointers to its extraneous origin in the faeces and is suggestive of its contamination with the faeces during the act of defaecation.

Fungal ecology/biodiversity

The dietary treatment with the highest gastro-intestinal fungal biodiversity was the control treatment where 6 different fungi were isolated from the faecal samples of pigs that fed on this diet. The diets with the least fungal isolates were the two unfermented CTW diets (the UFCSR and UFCP diets). Interestingly, the fungal inoculum (*Aspergillus fumigatus*) that was selectively added to ferment the CTW was only isolated from the faecal samples of pigs fed with the control diet and not in those of pigs fed with the *Aspergillus fumigatus* inoculated diets. Literature reported that fungi and protozoa also make up part of the gut flora and that the currently known genera of fungi in the gut flora include *Candida*, *Saccharomyces*, *Aspergillus* and *Penicillium* (Wikipedia). It then means that *Aspergillus fumigatus* is one of the normal gut micro-organisms, but its absence from the faecal samples of pigs in the two treatments which it was selectively used to inoculate would have been caused by its obliteration from the gut micro-environment through competitive exclusion and "barrier effect" [11,31] earlier on mentioned. The various types of fungi found in the gut of these weanling pigs need further research clarification to ascertain whether they could be included as normal gut flora or whether they are just extraneous contaminants in the faeces.

Conclusion

This trial lent support to the dietary modulation postulate of the gut micro-environment of vertebrates as observed in the experimental pigs. The variation in types (biodiversity) and population (colony forming units) of micro-organisms in the faecal matter revealed that the different types of feed given to these animals played a major role in dictating the type and number of organisms that were found in the faeces. The faecal parameters investigated also gave significant statistical differences alongside the dietary treatments applied. The possibilities of exerting health benefits, growth promoting effects and treating some gastro-intestinal disturbances by using the diet as a management tool has therefore been highlighted in this trial. Hence, it could be concluded that the digestive physiology and the variants of gut microbiota of pigs and possibly of any mammalian species, is dependent on the type of feed/food consumed and the various processing methods such feed/food had undergone.

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