In Vitro-in Vivo Digestibility and Nitrogen Balance in Indigenous, Exotic and Crossbred Growing Pigs Fed Sprouted or Roasted Cowpea Diets

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Research Article

Keywords: genotype, inhibitor, intake, nitrogen, optimally, balance

Posted Date: August 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-692378/v1

Abstract

The study evaluated effects of processing cowpeas for inclusion in maize-based diets for Windsnyer, Large-White x Landrace, and their 3-way crossbred growing pig genotypes. *In-vitro*, the raw and roasted cowpea diets, sprouting cowpeas decreased (P<0.05) gastric-ileal non-enzymatic (buffer-only) DM digestibility. Roasting increased (P<0.05) the colon enzymatic digestion relative to sprouting. Total ileal and colon *in-vitro* diet DM digestibility were not affected (P>0.05) by cowpea processing. *In-vivo,* Pigs consumed most (P<0.05) feed (g/day/kg BW) in period 1, with significant (P<0.05) genotype X period interaction. Both roasting and sprouting cowpeas reduced (P<0.05) dietary apparent DM digestibility. Pig daily body weight (BW) gain reduced (P<0.05) in period 3 compared to period 1. There was no (P>0.05) treatment effect on feed conversion efficiency. The 3-way crossbred pigs excreted more (P<0.05) urine N (g/day$BW^{0.75}$). Urine N excretion (g/day$BW^{0.75}$**)** peaked (P<0.05) in period 2 (P<0.05), with less (P<0.05) N intake (g/day$BW^{0.75}$), faecal N excretion (g/day$BW^{0.75}$**)** and N balance (g/day$BW^{0.75}$) than in period 3. Significant diet X genotype interaction in faecal N excretion (g/day$BW^{0.75}$**)** resulted from markedly high (P<0.05) in contrast to low (P<0.05) excretion. Significant genotype X period interaction resulted from the numerically (P>0.05) higher urine N excretion. In conclusion*, in-vitro*, sprouting shifted non-enzymatic digestion to the colon, while roasting increased colon fibrolysis, without effect on overall DM digestibility. *In-vivo*, the period of feeding, interpreted to reflect pig maturity, the pig genotype and cowpea processing interacted to influence apparent dietary DM digestibility and N utilization, without significant effect on the conversion efficacy of the maize-cowpea diet.

Introduction

In the advent of climate change, ecologically adaptable native legumes could provide cost-effective, sustainable solutions to increasingly restricted, erratic local commercial plant protein feed supply chains in semi-arid tropical farming systems (Ameen et al., 2005; Adino et al., 2018). The challenge is the wide quantitative and qualitative variation among legume species and their cultivars in protein content (Gilani et al., 2005; Gilani et al., 2011) and in anti-nutritional secondary compounds (Garry et al., 2007; Jezierny *et al.*, 2010). Antinutritional factors (ANFs) may aggravate the inferior protein quality of native legume based diets (Sauvant et al., 2004; NRC, 2012), through impaired digestion (Garry et al., 2007), and by endogenous wastage via damaged gut epithelial cells and secretory protective mucoproteins (Brenes et al., 2004). For maximal dietary efficacy, native legumes require biochemically tailored thermal (Farran et al., 2001) or biological (Malomo et al., 2013) processing, delicately optimally calibrated for effective deactivation of ANFs, without deleterious impact on protein quality. Nutrigenetic studies suggest the pig genotype may influence the efficacy of novel diets, given different nutrient requirements (Fontanesi et al., 2015), consequent to natural adaptive (native pigs) or selection (exotic pigs) genetic differentiation in digestive morphology (Barea et al., 2011), and gut microbiota (Fairbrother et al., 2005; van der Meulen et al., 2010; Rist et al., 2013).

The objective of the study was to examine the effects of roasting versus processing cowpeas on dietary efficacy when included as the protein source in maize based diets fed to Windsnyer (W), Large White x Landrace (LW), and Windsnyer x Large White x Landrace (WxLWxLR) crossbred growing pigs.

Methods And Materials

Feed processing and experimental diets

Cowpeas were either roasted or sprouted using procedures previously (Lubisi *et al.*, unpublished) designed to optimize nutritive value based on biochemical perturbations detectable by chemical analyses, tannin inhibitor activity and as reflected by non-enzymatic/enzymatic *in vitro* porcine digestibility Lots of 20kg cowpeas were roasted in a cylindrical (L = 1.5 m; Diameter = 0.50 m) manually rotating, cast-iron, gas heated drum in a procedure which involved heating the drum to an initial constant maximal empty interior temperature of 150°C, followed by introduction of cowpeas in a20 minutes roasting to a sampling temperature of 105°C. Bulk grain germination was initiated by 12 hour pre-soaking, followed by 4-day open-air sprouting at ambient conditions. Cowpeas and maize were hammer-milled (Jacobson model P160 Teordrop 10HP) through a 5mm screen, prior to mixing into balanced diets along with amino acid, mineral and vitamin supplements following (Trouw Nutrition South Africa: 28kg pig grower pre mix/tonne) recommendations. Diets were mixed. Mixing was for 20 minutes in 1000 kg lots in a vertical mixer (MORHLANG VERTA MIX 1200VM),

Table 1
Chemical composition and trypsin inhibitor activity of unprocessed, sprouted and roasted Cowpeas

Treatment	Composition (% DM)					TIA (TIU/mg DM)
	Ash	CP	Fat	NDF	ADF	
Raw	421	258	157	373	125	563
[1]Sprouted	491	291	92	367	187	463
[2]Roasted	453	260	131	240	134	226

CP = Crude protein, ADF = Acid detergent fiber, NDF = Nitrogen detergent fiber, TIA = Trypsin inhibitor activity, TUI = Trypsin inhibited units per mg dry matter. [1]Sprouting; 12 hour pre-soaking, 4-day open-air sprouting at ambient conditions; [2]Roasting: cylindrical (L = 1.5 m; Diameter = 0.50 m) manually rotating, cast-iron, gas heated drum, 20 kg cowpeas, initial maximal constant interior drum temperature 150°C, 20 minutes roasting to internal drum temperatures at sampling 105°C.

Table 2
Composition and nutrient levels of the basal diet

Composition	Experimental diets		
	4-day Sprouted cowpea-maize	20-minute Roasted cowpea-maize	Raw cowpea-maize
Dietary Ingredients (% as fed)			
Maize meal	63.6	57.1	56.8
Cowpea meal	33.6	40.1	40.4
*Minerals & Vitamins	2.8	2.8	2.8
Total	100.0	100.0	100.0
Calculated Chemical composition (% DM Basis)			
Ash (%)	4.7	4.7	8.1
ADF (%)	6.5	6.3	5.9
NDF (%)	19.7	25.2	16.5
Fat (%)	2.9	3.0	3.0
Calcium g/kg DM	0.54	0.52	0.54
Phosphorus g/kg DM	0.34	0.34	0.33
Digestible Energy (DE) MJ kg	17.3	17.4	17.2
[2]Metabolizable Energy (ME) MJ kg	15.2	146	14.1
CP (%)	15.0	15.0	15.0
Essential Amino Acids			
Lysine	0.8	1	1
Methionine	0.3	0.2	0.3
*Minerals & Vitamins (supplying per kg of mixture):			
Vitamin A	6500IU		
Vitamin D3	1200IU		
Vitamin E Equivalent	400		
Vitamin K3 (43%)	0.0002g		
Vitamin B1 (Thiamine Mononitra)	0.00015g		
Vitamin B2 80% (Riboflavin)	0.00045g		
Niacin (99.5%)	0.0025g		
Calcium Pantothenate (98%)	0.0012g		
Vitamin B12 (1g/kg)	0.0000003g		
Vitamin B6 (98% pyridoxine HCL)	0.00025g		
Choline (Chloride 60%)	0.019048g		
Folic Acid (96%)	0.00006g		
Biotin (2%)	0.000005g		
L-Lysine 98%	0.00019g		
DL-Methionnine (98%)	0.02g		
Phytase (10 000 FTU/g)	0.005g		
Manganese (Manganese Sulphate 31%)	0.004g		
Zinc (Zinc So4-Mono 35.5%)	0.001g		

[1]Composition calculated as DM % of the diet

*Trouw Nutrition, South Africa

Composition	Experimental diets		
	4-day Sprouted cowpea-maize	20-minute Roasted cowpea-maize	Raw cowpea-maize
Copper (Copper So4-25.2% Penta)	0.0125g		
Iodide (Potassium Iodide 76.45)	0.0001g		
Ferrous (ferrous So4-30% Mono)	0.01g		
Selenium (Sodium Selenite 4.5%)	0.00003g		
Limestone Powder	101107.1782g		
Mono Dicalcium Phos. (21%)	0.8g		
Salt	0.6g		
[1]Composition calculated as DM % of the diet			
*Trouw Nutrition, South Africa			

In vitro **digestion**

Diets milled through a 1 mm sieve were digested *in vitro* using the standard 3-step porcine digestion model (Boisen and Fernandez 1993), with modifications to compartmentally partition DM digestibility into non-enzymatic and enzymatic components.

Non-enzymatic digestion;

Samples (0.5g) sealed within Ankom F57 filter bags were suspended in phosphate-buffered enzyme media contained in 250 ml glass digestion jars, which were immersed in a 39^{o} C shaking water bath. Samples were digested in runs/digestive steps in a setup in which digestion jars contained 14 replicates of one treatment, with or without (buffer-only) enzyme (blank 1), with one no-sample (blank 2) filter bag within each jar.

Enzymatic digestion

Pepsin (gastric) digestion; To each digestion jar were added 87.5 ml phosphate buffer (Phosphate buffer for microbiology, APHA, pH 7.2 (Sigma-Aldrich) 17202) (0.1 M, pH 6.0) and hydrochloric acid 35 ml HCl (0.2 M) solutions (Hydrochloric acid puriss. p.a., ACS reagent, reag. ISO, reag. Ph. Eur., fuming, ≥ 37%, APHA ≤ 10 (Sigma-Aldrich) 30721) and the pH adjusted to 2.0 using HCl or sodium hydroxide (NaOH) (Sodium hydroxide BioXtra, ≥ 98% (acidimetric), pellets (anhydrous) (Sigma-Aldrich) S8045) solution. The jars were then placed on CNW Model, WBS 450-B, 39 ^{o} C Water Bath Thermostatic Vibration. A 3.5 ml aliquot of a freshly prepared pepsin solution containing 10 mg/ml pepsin (Pepsin from porcine gastric mucosa powder, ≥ 250 units/mg solid (Sigma) P7000) was then added to the mixture. To prevent bacteria growth, 1.7 ml of a chloramphenicol solution (0.5 g Chloramphenicol ≥ 98% (HPLC) (Sigma) C0378, per 100 ml ethanol) were added to the digestion medium and subsequently digested for 2 h using the stop watch timer.

Pancreatin (small intestine) digestion; Digestion medium pH within jars was adjusted to 6.8 by adding 35 ml of sodium phosphate buffer solution (0.2 M, pH 6.8) and 17.5 ml of NaOH (0.6 M, pH 13.8). A 3.5 ml aliquot of freshly prepared pancreatin solution containing 50 mg pancreatin (Pancreatin from porcine pancreas powder, suitable for cell culture, 4 × USP specifications (Sigma) P3292) was then added to each jar, and digestion was continued for another 5 h.

Viscozyme (colon) digestion; To simulate colon digestion, the medium in each jar was completely discarded and replaced with 218.75 ml of freshly prepared phosphate buffer (0.1 M, pH 4.8). Sample residues from gastic + intestinal digestion were further digested with 1.75 ml Viscozyme (Viscozyme® L cellulolytic enzyme mixture (Sigma) V2010) for 24 h in a freshly prepared buffer with pH of 4.8, and incubated in a shaking incubator for 18 h at 39°C.

After 2nd and 3rd step digestion, dry matter digestibility was estimated gravimetrically by washing filter bags through sequential, gentle rinsing in warm tap water, 95% ethanol, and 99% acetone, and forced air oven-drying at 85 ^{o} C for 18 hours, with the simulated porcine gut compartmental, partial and total DM digestibility defined and calculated as:

Feeding trial

Nine pigs, three each of Windsnyer (W) [11 ± 1.15 kg], Large White x Landrace (LWxLR) [14 ± 1.15 kg], and their 3-way (WxLWxLR) [12 ± 1.15 kg] crossed genotypes were weaned onto a standard starter diet at four weeks, followed by gradual transition to the experimental diets over two weeks. Prior to the trial, pigs received a 1 ml subcutaneous injection of Ivomec vaccine (Reg. No. G2858). The trial was setup in a naturally ventilated house in which each pig was placed within a 57 cm x 118 cm metabolism cage fitted with individual feeders and nipple drinkers, for *ad libitum intake*. Diets were randomly assigned to the

pigs in a 3 × 3 factorial arrangement within a Latin square design replicated three times. The first seven days of each period were used to adapt the pigs to the treatments. Five days was used to measure feed intake, total feces and urine. Collection and sampling was performed between 08:00 – 09:00 hours. A 20 per cent (v/v) HCl was added to urine to prevent N evaporation. Faecal and urine samples were stored at −04°C until analyzed. Frozen faecal samples were dried at 60°C for 48 hours and ground to pass through a 3-mm screen. Nitrogen balance was estimated by the difference between dietary intake and excretion in faeces and urine. Intake and excretion parameters expressed on metabolic body weight ($BW^{0.75}$) basis.

Chemical analyses

Feed samples were hammer-milled through a 1 mm sieve. Dry matter was determined using the AOAC (2000) method 976.050. Ash was determined using the AOAC (2000) method 923.03). Nitrogen was determined using the micro-Kjeldahl method (AOAC, 2000; method 976.05). Ether extract (EE) was determined by soxlet extraction (AOAC, 2000; method 920.39). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Goering and Van Soest (1970).

Statistical analysis

In vitro DM digestibility coefficients were subjected to One-Way ANOVA using the GLM of MINITAB software (Version 17.0) using the model:

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$

Where:

Y_{ij} = Observation

μ = Overall mean

T_i = Effect of the i^{th} processing

ε_{ij} = Random error

In vitro-in vivo, N digestibility and N balance were subjected to factorial ANOVA using the GLM of MINITAB software (Version 17.0) (2014).

$Y_{ijk} = \mu + G_i + D_j + P_k + (G \times D)_{ij} + (G \times P)_{ik} + (D \times P)_{jk} + (G \times D \times P)_{ijk} + \varepsilon_{ijk}$

Where:

Y_{ijk} = Observation

μ = Overall mean of total tract sprouted or roasted Cowpea nutrient digestibility

G_i = Effect of the i^{th} pig genotype

D_j = Effect of the j^{th} diet

P_k = *Effect of the k^{th} period*

$(G \times D)_{ij}$ = Interaction between pig genotype and diet

$(G \times P)_{ik}$ = Interaction between pig genotype and period

$(D \times P)_{jk}$ = Interaction between diet and period

$(G \times D \times P)_{ijk}$ = Interaction between pig genotype, diet and period

ε_{ijk} = Random error

Tukey's test was used to compare means where significant ($P \leq 0.05$) treatment effects occurred, while $0.05 < P < 1$ defined tendency toward significance.

Results

In vitro digestibility

Table 3 shows effects of cowpea processing on partial and total compartmental *in vitro* dietary DM digestibility. Sprouting cowpeas reduced the non-enzymatic (buffer-only) partial dietary DM digestibility in steps 1–2 (gastric-ileal) ($P < 0.05$), which it increased ($P < 0.05$) in step 3. Relative to roasting, sprouting cowpeas reduced ($P > 0.05$) the partial viscozyme DM digestibility, with respective numerical ($P > 0.05$) increase, in contrast to numerical decrease, relative to the control. All other coefficients of DM digestibility were not affected ($P > 0.05$) by cowpea processing.

Table 3
In vitro digestibility (% DM Disappearance) of raw, roasted and sprouted cowpea growing pig diets

Cowpea Diets	N	Digestibility					
		Partial				[5]Total	
		[3]Non-enzymatic		[4]Enzymatic			
		Steps 1 + 2	Step 3	Pepsin + Pancreatin	Viscozyme	Steps 1 + 2	Steps 1 + 2 + 3
Raw	14	0.17[a]	0.50[b]	0.50	0.38[ab]	0.67	0.88
[1]Sprouted	14	0.15[b]	0.51[a]	0.52	0.35[b]	0.67	0.86
[2]Roasted	14	0.17[a]	0.48[b]	0.48	0.39[a]	0.64	0.87
SEM		0.984	0.945	0.956	0.406	0.991	0.406
Significance		*	*	ns	*	ns	ns

[abcd] Means within a column with different letter superscripts are significantly different at (P < 0.05); [1]12-hour pre-soaking, 4-day open-air sprouting at ambient conditions; [2]20 kg lots, 20-minutes, L = 1.5 m; Diameter = 0.50 m gas heated manually rotating cast-iron drum, 150°C initial and 105° terminal temperature. [3]Buffer-only spontaneous solubilisation: Step 1-Phosphate buffer (1 M HCl/M NaOH) + HCl (0.2 M); pH = 2.0, 2-hr incubation; Step 2- Sodium phosphate buffer (0.2 M, pH 6.8) + NaOH (0.6 M, pH 13.8); pH = 6.8; 5-hr incubation; Step 3- Phosphate buffer (0.1 M, pH 4.8); pH = 4.8; 18-hr incubation; [4]Partial enzyme action (corrected for buffer-only effects) : 10 mg/ml Pepsin (Pepsin from porcine gastric mucosa powder, ≥ 250 units/mg solid (Sigma) P7000) in step 1 buffer, 3.5 ml aliquot (50 mg) pancreatin (4 × USP specifications (Sigma) P3292) solution in step 2 buffer, 1.75 ml Viscozyme (Viscozyme® L cellulolytic enzyme mixture (Sigma) V2010) solution in step 3 buffer; [5]Summative enzymatic + non-enzymic digestibility, SEM- Standard error of the mean; *Significant at P < 0.05; ns- not significant

In vivo **digestibility**

Tables 4 and 5 shows treatment effects on *in vivo* diet efficacy and N utilization, respectively. Pigs consumed most (P < 0.05) feed (g/day/kg BW) in period 1, with significant (P < 0.05) genotype X period interaction resulting from relative decrease in feed intake by the W type pigs over periods 1–3. Both roasting and sprouting cowpeas reduced (P < 0.05) dietary apparent DM digestibility, with significant (P < 0.05) genotype x period interaction resulting from relatively superior DM digestibility by the W type pigs in period 1. Pig daily body weight (BW) gain reduced (P < 0.05) in period 3 compared to period 1. There was no (P > 0.05) treatment effect on feed conversion efficiency. The LW X LR pigs consumed less (P < 0.05) N (g/day) with the crossbred genotypes excreting more (P < 0.05) faecal N (g/day) than the W type pigs. Significant (P < 0.05) period X diet interaction resulted from relative less faecal N excretion by pigs on the raw diet compared to the processed cowpea diets in periods 2 and 3. The 3-way crossbred pigs excreted more (P < 0.05) urine N (g /day $BW^{0.75}$) than the W type pigs, with better (P < 0.05) N (g /day $BW^{0.75}$) balance. Roasting increased (P < 0.05) urine N excretion (g /day $BW^{0.75}$) in LW X LR pigs, with better (P < 0.05) N (g /day $BW^{0.75}$) balance than the W pigs. Urine N excretion (g /day $BW^{0.75}$) peaked (P < 0.05) in period 2 (P < 0.05), with less (P < 0.05) N intake (g /day $BW^{0.75}$), faecal N excretion (g /day $BW^{0.75}$) and N balance (g /day $BW^{0.75}$) than in period 3, in which pigs also had the least (P < 0.05) growth rate. Significant diet X genotype interaction in faecal N excretion (g /day $BW^{0.75}$) resulted from markedly high (P < 0.05) in contrast to low (P < 0.05) excretion on the sprouted diet by the W and LW X LR genotypes, respectively. Significant genotype X period interaction resulted from the numerically (P > 0.05) higher urine N excretion by LW X LR compared to W pig genotypes in periods 1 and 2, which inverted in period 3, with similarly significant diet X genotype interaction resulting from reduced (P < 0.05) excretion by the W pigs on the roasted diet.

Table 4
Utilization of raw, roasted and sprouted cowpea-maize diets among different pig genotypes

Treatments			DM Digestibility	Average daily feed intake		Average daily gain	Feed conversion ratio
				g/day/kg BW	g/day/ kg $BW^{0.75}$	(g/day/ kg BW)	
Period	[1]Genotype	Cowpea Processing					
1	LW x LR	Roasted	0.85	121.1^{ab}	390.1	333.3	3.36
	W	Raw	0.88	128.0^{a}	314.6	416.6	1.73
	W x LW x LR	Sprouted	0.82	127.1^{ab}	339.9	388.9	2.60
2	LW x LR	Sprouted	0.83	96.3^{abcd}	303.3	444.4	2.43
	W	Roasted	0.81	88.2^{d}	230.6	444.4	2.10
	W x LW x LR	Raw	0.89	111.1^{abc}	340.0	444.4	2.73
3	LW x LR	Raw	0.90	95.2^{bcd}	373.5	361.1	3.29
	W	Sprouted	0.84	85.6^{d}	388.0	305.6	3.40
	W x LW x LR	Roasted	0.86	90.2^{cd}	354.6	361.1	2.96
Genotype		LW x LR	0.86	104.1	355.6	379.6	2.80
		W	0.85	100.5	311.1	388.9	2.41
		W x LW x LR	0.86	109.1	344.8	398.1	2.82
Diet		Raw	0.89^{a}	111.3	342.7	407.4	2.35
		Roasted	0.84^{b}	99.4	325.1	379.6	2.81
		Sprouted	0.83^{b}	103.3	343.7	379.6	2.86
Period		1	0.85	125.0^{a}	348.2	379.6^{ab}	2.62
		2	0.85	98.5^{b}	291.3	444.4^{a}	2.42
		3	0.85	90.0^{b}	372.0	342.6^{b}	2.98
SEM			0.006	2.16	17.60	15.40	0.118
P-value							
		G	0.593	0.258	0.380	0.888	0.536
		D	0.003	0.076	0.813	0.703	0.983
		P	0.345	0.000	0.060	0.040	0.057
		G x D	0.503	0.000	0.058	0.101	0.067
		P x D	0.675	0.593	0.555	0.750	0.33
		G x P	0.013	0.276	0.378	0.667	0.494
		G x D x P	0.413	0.343	0.862	0.620	0.047

[ab] Treatment means within treatment with different letter superscripts are significantly different at (P < 0.05).

[1]W: Windsnyer, LW = Large White; LR = Landrace

BW: Live body weight

[2]Metabolic body weight

SEM- Standard error of the mean

*Significant at P < 0.05; ns- not significant

Table 5
N utilization from raw, roasted and sprouted cowpea-maize diets by different pig genotypes

Treatments							N Utilization				
					Digestibility		g N/day				
		Intake	Faecal Excretion	Urine Excretion	Faecal/Urine Excretion ratio	Balance	Intake	Faecal Excretion	Urine Excretion	Faecal/Urine Excretion ratio	Balance
Period	Genotype	Cowpea Processing									
1	[2]LW x LR	Roasted			0.85		73.4	31.0	3.0	1:10	39.3
	[1]W	Raw			0.88		95.0	35.3	2.4	1:15	58.0
	[3]W x LW x LR	Sprouted			0.83		67.2	27.3	2.5	1:11	38.2
2	[2]LW x LR	Sprouted			0.83		50.0	25.0	2.0	1:12	23.4
	[1]W	Roasted			0.82		85.4	43.1	2.5	1:17	39.4
	[3]W x LW x LR	Raw			0.90		70.2	25.4	2.1	1:12	44.1
3	[2]LW x LR	Raw			0.90		75.3	28.4	2.3	1:12	45.1
	[1]W	Sprouted			0.85		91.5	52.2	2.1	1:25	37.5
	[3]W x LW x LR	Roasted			0.90		80.1	28.0	3.3	1:8	49.3
	SEM				0.006		3.78	1.96	0.131		2.55
	Diet										
	Raw				0.89		80.2	29.2	2.1[b]	1:14	49.3[a]
	Roasted				0.85		79.4	34.4	3.4[a]	1:10	43.1[ab]
	Sprouted				0.84		69.2	34.4	2.2[ab]	1:16	33.4[b]
	SEM				0.006		29.45	16.91	1.38		20.60
	Genotype										
	[1]W				0.88		90.4[a]	43.1[a]	2.3	1:19	45.1
	[2]LW X LR				0.86		66.1[b]	28.0[b]	2.3	1:12	36.4
	[3]W x LW x LR				0.86		72.4[ab]	26.2[b]	2.5	1:10	44.2
	SEM				0.006		29.45	16.91	1.38		20.60
	Period										
	P1				0.853		78.4	31.3	2.6	1:13	45.3
	P2				0.850		68.1	31.1	2.4	1:15	35.3
	P3				0.872		82.3	36.2	2.2	1:14	44.4
	SEM				0.00		3.78	1.96	0.131		2.55
	P Values										
	D				0.815		0.438	0.517	0.027		0.054
	G				0.586		0.045	0.004	0.588		0.313
	P				0.345		0.309	0.463	0.373		0.266
	D X G				0.503		0.623	0.478	0.557		0.549

[abcd] Treatment means within treatment with different letter superscripts are significantly different at ($P < 0.05$).

[1]W: Windsnyer, LW = Large White; LR = Landrace

Treatments		N Utilization			
	Digestibility	g N/day			
P X D	0.675	0.156	0.014	0.718	0.606
G X P	0.013	0.755	0.511	0.082	0.174
D X G X P	0.563	0.452	0.393	0.094	0.164

[abcd] Treatment means within treatment with different letter superscripts are significantly different at (P < 0.05).

[1]W: Windsnyer, LW = Large White; LR = Landrace

BW: Live body weight

[2]Metabolic body weight

SEM- Standard error of the mean

[*]Significant at P < 0.05; ns- not significant

Discussion

The *in vitro assay suggested* roasting cowpeas increased colon fibrolysis while sprouting shifted non-enzymatic cowpea digestion (likely the spontaneous solid macromolecular disintegration) to the 3rd step, effects which implied increased, readily fermentable substrate for lower pig gut microbes. Unfortunately, despite insignificant effect on DM digestibility, the gravimetric analyses excluded molecular insight into the nature of the apparent biochemical shifts which could have important implications on pig nutrition.

In contrast to *in vitro* digestion, roasting and sprouting compromised total *in vivo* apparent DM digestibility, which could reflect either disparate digestion and, or, the result of increased endogenous faecal excretion. Compared to a commercial product such as Viscozyme, greater diversity of pig gut microbial fibrolytic activity (Fushai et al., 2019) should confer higher true digestibility of fibrous plant embryonic secondary tissue in cowpea sprouts, and of crosslinked compounds in over processed roasted cowpeas. Tendency (P = 0.053) toward pig genotypic x diet interaction on DM digestibility was consistent with numerically greater Windsnyer pig capacity to digest sprouted cowpea dietary fibre. The fibrolytic advantage of the indigenous pig gut was previously explained by genomic evaluation of faecal microbiota (Kanengoni et al., 2015), which might present a mechanism for broader tolerance of feed ANF's present in their more complex native diet. Unfortunately, in this study, neither of *in vitro/in vivo* evaluation measured ileal protein or amino acid digestibility, to predict processing effects on protein quality (Mosenthin et al., 2000; Święch, 2017). Though growing pigs are considered highly N efficient, as high as 40% on standard diets (Rotz, 2004), in the current study, N balance was low (16.4 ± 1.0 %), despite high (98%) DM digestibility, which implied inferior dietary amino acid profiles, and consequently, low efficiency of tissue utilization (Smiricky et al., 2002). In previous studies, both sprouting (Urbano et al., 2005) and thermal processing (Doblado et al., (2007) of cowpeas improved protein digestibility, though excessive heating reduced digestibility, reflecting negative effects of non-enzymatic (Maillard) reactions between the reducing sugars and proteins, and thermally induced amino acid cross-linking (Tuśnio et al., 2017). El-Jasser (2011) reported as high as 75–79% *in vitro* protein digestibility of sprouted cowpea. However in current study DM digestibility was high from 86–88 % for raw 88%, sprouted 86% and roasted 87% cowpea.

Significant difference should be expected between dietary DM, protein and amino acid (AA) digestibility, particularly in diets in which the legume feed contains significant antinutritional factors (Kumar et al., 2006; Kayembe, 2013). Gut protein extraction for assimilation in pig tissues occurs with variable wastage via the urinary or faecal routes (Ball et al., 2013), with complex gut-systemic exchange of endogenous protein, AA and urea, which is dependent on diet quality (Ball et al., 2013) and intake (Ball et al., 2013). In the present study, the impact of processing on protein quality was complex and overall unexpected. Roasting and sprouting both depressed cowpea protein quality as reflected by the N balance. While roasting significantly increased the urinary N, sprouting induced numerically low N intake, greater faecal wastage, and significantly lower relative N balance when expressed on body weight, but not on metabolic body weight basis. Imbalance in amino acids supplied for protein synthesis for growth and other functions results in catabolism of excess amino acids, with excess N converted to urea, which increase its excretion in urine (Ball et al., 2013). On the other hand, protein indigestion in the upper tract diverts N to colon fermentation (Bindelle et al., 2009). With adequate fermentable energy supply, colon bacteria assimilate both endogenous and dietary N, to lock it and shift excretion from urea in urine to microbial protein in faeces (Bindelle et al., 2009). If energy is deficient, increased colon protein fermentation may also produce toxic metabolites (Tuśnio et al., 2017). Potentially toxic byproducts of protein fermentation include ammonia, amines and N-nitroso compounds (Bindelle et al., 2009). Yang et al., (2007) reported no detrimental effects on the growth of pigs from faba bean legume dietary inclusion levels of 20 to 37%. Genotype X diet X period interaction suggested superior relative FCR for Windsnyer, Land race x Large white and Land race x Large white genotype on the raw, sprouted and roasted cowpea diet, whereby pigs gained more weight while consuming lesser on period 2, which could be an indication of greater tolerance of the ANFs contained in the cowpea. Umapathy and Erlwanger (2008) reported depressed growth and feed intake in 30% raw dietary cowpea-fed pigs, in contrast to pigs fed thermally processed cowpea diets. Rats fed cooked cowpea gained more weight than those fed uncooked cowpea (El-Jasser, 2011).

Conclusion And Recommendation

It is concluded that roasting and processing do not improve the protein quality in maize based diets, though factors such as the pig genotype, in relation to gut maturity, may be important influences on diet efficacy, including N utilization. Validatory performance trials are recommended.

Declarations

Contributions

M.W. Lubisi; designed research, conducted experiment, analyzed data and wrote manuscript, F. Fushai; designed research and contributed analytical tools, R.S Thomas; contributed experimental animals & J.J. Baloyi; contributed analytical tools. All authors read and approved the manuscript before submission.

Acknowledgements

Financial support for this research was from National Research Foundation (NRF) and University of Venda Research Grant, not to forget Agricultural Research Council (ARC) for providing facility.

Statement of Animal Rights

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of the University of Venda (PROJECT NO:SARDF/17/ANS/07/0412), and the Animal Ethics Committee of the Agricultural Research Council-Animal Production Institution and approved and Department of Agriculture Gauteng according to Meat Safety Act, 2000 (Act No. 40 of 2000).

Conflict of interest declaration

We wish to confirm that there are no known conflict of interest associated with the publication of this manuscript and there has been no significant financial support for this work that could have influenced its outcome. We also confirm that this manuscript has been read and approved by all authors and that the order of authors listed in the manuscript has been approved by all of us.

Data availability

Data will be available on request as soon as it is required.

Declaration

I Mfanuzile Welcome Lubisi, hereby declare that (*IN VITRO-IN VIVO* DIGESTIBILITY AND NITROGEN BALANCE IN INDIGENOUS, EXOTIC AND CROSSBRED GROWING PIGS FED SPROUTED OR ROASTED COWPEA DIETS) manuscript submitted by me is not been submitted to any journal for review, and that in execution, it entirely my own work and that all reference material contained therein has been duly acknowledged.

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