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Growth, slaughter performance, abdominal visceral organ sizes, and plasma metabolic markers in indigenous, improved, and crossbred growing pigs fed roasted or sprouted cowpea (*Vigna unguiculata*) diets

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Abstract

Growth, slaughter performance, and blood metabolic markers were evaluated in Windsnyer (W), Large White (LW) × Landrace (LR), and three-way crossbred (W × LW × LR) pigs fed control (soybean cake), sprouted, or roasted iso-nutrient, cowpea, maize-based diets. Twelve male pigs of each genotype with initial live weights of 15 ± 2.3 kg, 37 ± 1.4 kg, and 39 ± 1.2 kg (10–11% degree of maturity) were used. The pigs were on the trial diets for eight weeks in a balanced factorial experiment replicated four times. The growth rates were W × LW × LR > LW × LR > W, and control > sprouted cowpeas > roasted cowpea diets. Scaled to pig metabolic (weight $^{0.75}$), feed intake was greatest in the control, followed by sprouted cowpeas and roasted cowpeas, and pig BW was W × LW × LR ≥ LW × LR > W. The feed conversion was control < sprouted cowpeas ≤ roasted cowpea diet. Pigs on the control diet recorded the highest back fat thickness, with the least backfat in LW × LR pigs. The LW × LR pigs had more backfat on roasted than on sprouted cowpeas, whereas W pigs had more backfat on roasted cowpeas. Scaled (% live weight) liver and kidney sizes were largest in W pigs, and the kidney size was larger on roasted cowpeas. Blood marker profiles were aligned to pig growth and slaughter performance, with low total protein, albumin, and alkaline phosphatase activity in LW × LR pigs; sprouted cowpeas caused elevated plasma urea, albumin, and the alkaline phosphatase activity. The W pigs had low plasma creatinine and high cholesterol levels, with elevated cholesterol on sprouted cowpeas. The LW × LR and W × LW × LR genotypes had better growth and slaughter performance than the W pigs; cowpea was inferior to the control diet, with better efficiency on sprouted, rather than roasted, cowpeas. Pig responses to roasting or sprouting cowpeas were considered largely independent of the genotype.

Keywords: anti-nutritional factors, blood metabolites, cowpea processing, Landrace pigs, Large White pigs, native legumes, Windsnyer pigs
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Introduction

Globally, soybean (*Glycine max*) oil cake is considered the conventional, primary plant protein source for stock feeding. However, simulation models predict negative climate-change impacts in most rain-fed, low-technology, arid tropical soybean production systems (Ma *et al.,* 2021; Gong *et al.,* 2022). Despite the dire implications of climate-change on future stock feed supplies, native legume alternatives such as the cowpea (*Vigna unguiculata*) remain marginalized from the mainstream animal nutrition

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research. Unlike the soybean, the cowpea oil content is too low to justify detoxifying expeller extraction to produce high protein oilcake as a byproduct. Therefore, cowpeas are fed whole to pigs, which requires efficient, cost-effective processing technology appropriate to poorly-resourced tropical settings.

Tropical small-holder pig farming systems are typically endowed with a diversity of indigenous, exotic, and the crossed pig genotypes, which likely express unique naturally- (indigenous pigs) or artificially- (improved pigs) acquired nutritional traits of potential production importance (Bovo *et al.,* 2016). Due to feed shortages and cost, pigs in poorly-resourced, extensive systems are often exposed to inefficient diets constituted from unconventional, inferior feedstuffs. The extent to which pigs of different genotypes may adapt to the anti-nutrients, or to nutrient deficiencies typical of such diets is uncertain.

In species such as the pig, blood profiles of intermediary metabolic enzymes and metabolites are considered biomarkers of the animal's nutritional or clinical status (Yang *et al.,* 2011). Compared to large scale, long term, on-farm performance trials, blood biomarkers are considered suitable surrogates to track nutrient utilisation in on-farm or on-station nutrigenomic studies, given the convenience, and the more controlled, rapid, and low-cost application (Montoro *et al.,* 2022). A spectrum of blood metabolite biomarkers for protein and energy utilization and key enzyme signals of digestive, metabolic and metabolite excretory tissue and organ functions have been characterized. Protein metabolic markers include total protein, albumin, urea nitrogen and creatinine, whereas the indicators for energy metabolism include glucose, triglycerides, and cholesterol (Montoro *et al.,* 2022). In pigs, plasma total protein is considered strongly predictive of protein metabolism (Hassan *et al.,* 2020). Plasma albumin and urea correlate to the quantitative, and or qualitative protein adequacy (Akinfala and Tewe, 2001). Creatinine, a product of muscle metabolism, is positively correlated with muscle, particularly striated muscle metabolism (Montero *et al.,* 2022). In mammals, urea is the primary nitrogenous end-product of amino acid catabolism, and the excretory plasma urea nitrogen is predictive of dietary protein quality or animal protein status, with high levels indicating amino acid excess or imbalance (Liu *et al.,* 2015).

Plasma ALP activity is indicative of the enzyme, alkaline phosphatase, which is often used as an indication of disorders of the liver or bones in both animals and humans. Plasma ALP activity is primarily from the liver and bone tissue and is also linked to lipid transport in the intestines (Chinmaya *et al.,* 2017) and is a signal of its metabolism (Liu *et al.,* 2015). Plasma ALP activity is positively correlated to pig weight gain (Pond *et al.,* 1997; Yang *et al.,* 2011; Liu *et al.,* 2015). Amongst others, liver function is routinely tested using blood alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) activities, in combination with bilirubin, total protein, and albumin (Ozer *et al.,* 2008). Elevated ALT and AST activities that are disproportionate to ALP activity and plasma bilirubin, suggest hepatocellular disease, whereas the opposite characterizes cholestasis (Milinković-Tur *et al.,* 2005). Elevated ALT activity also signifies heart and skeletal muscle degeneration (Nathwani *et al.,* 2005). In a healthy subject, albumin degradation should be in equilibrium with its synthesis (Peters, 1996). Albumin catabolism occurs primarily in the muscle and the skin, which respectively account for 40% to 60% of albumin degradation (Juliene, *et al.,* 2004), with as much as 10% of the albumin secreted from the gastrointestinal (GI) and skin secretory epithelia (Juliene *et al.,* 2004). The AST is widely distributed in tissues and organs, with high activity in the liver (Zimmerman *et al.,* 1968), cited by Milinković-Tur *et al.* (2005).

Legume grains contain lectins, tannins, phytase, and proteases inhibitors, which are antinutrients known to impair nutrient intake, extraction and consequently, pig health and productivity (Makinde *et al.,* 1996; Umapathy and Erlwanger, 2008). Processing options include either biological or thermal processing methods. Thermal methods are effective in destroying thermolabile antinutrients, though over-processing may reduce nutrients, particularly protein digestibility (Drulyte and Orlien, 2019). Depending on the duration (Ehirim *et al.,* 2018), progressive germination metabolism during sprouting results in the double advantage of antinutrient reduction, coupled to an improvement in the nutrient profile.

In the current study, the effects of roasting and sprouting cowpeas were compared to test if chemical effects specific to the method or efficacy of processing may uniquely influence the pig response to cowpea-based diets by affecting nutrient intake, absorption, and differential channelling of substrates to alternative metabolic pathways, sufficiently to alter the profile of intermediate, or the final plasma metabolites, to provide insight into potentially genotype-discriminated nutritional phenotypes (Bovo *et al.,* 2016). Accordingly, pig growth, slaughter performance parameters, and visceral abdominal organ weights were determined in combination with measurements of plasma enzyme metabolite biomarkers for protein and energy metabolism to evaluate the effects of feeding different growing pig genotypes with diets in which soybean cake was totally replaced by roasted or sprouted whole cowpeas. Indigenous (Windsnyer, W), commercial type (Large White × Landrace, LW × LR), and the crossbred (Windsnyer × Large White × Landrace, W × LW × LR) were considered for their socio-economic

importance in local production systems, with the three-way cross representing common, non-descript, indigenous-exotic crossbred pig genotypes.

Materials and Methods

The experimental protocols for the management and care of animals were approved by the Ethics Committee of the University of Venda (SARDF/17/ANS/07/0412).

The study was conducted at the Pig Research Unit of Agricultural Research Council, Animal Production Institute, Irene, South Africa. The Agricultural Research Council-Irene Research Station is located at 25° 55' South: 28° 12' East. The location is on the Highveld region of South Africa, at an altitude of 1525 m above sea level.

Cowpeas (*Vigna unguiculata L. Walp)* intended for a feeding trial were sourced from local bulk retailers, from which cracked, and weevil-bored grains were manually cleaned off, after which representative analytical samples were obtained.

Cleaned cowpeas intended for sprouting were first sterilized by 30-min treatment in 2.5% aqueous sodium hypochlorite, followed by thorough rinsing. The sterile grain was soaked overnight (12 h) in tap water, followed by 4-d sprouting, thinly spread on steel screens, with intermittent irrigation to prevent drying. Sprouting was terminated by exposure to peak, daily, hot sun-drying by spreading0 on black plastic sheeting on an open concrete surface.

Another lot of the cleaned cowpeas was roasted in a gas-heated, continuously manually-rotated cylindrical (1.5 m length, 0.50 m diameter) cast-iron drum. The roasting involved pre-heating the empty drum to 150 ºC constant initial temperature, and roasting of 20 kg cowpea batches for 20 min to a terminal 105 °C grain temperature.

Maize based test diets were formulated using roasted or sprouted cowpeas (Table 1) as the primary plant protein source. A commercial, maize-soybean mix (Meadow Feeds, Pty Ltd, Delmas, South Africa, Product V23515, containing per kilogram of feed: crude protein, 150 g minimum; crude fat, 25 g minimum; crude fibre, 80 g maximum; moisture, 120 g maximum; calcium 6–10g; phosphorus, 5 g/kg minimum; total lysine, 8.5 g minimum) served as the control against which iso-nutrient cowpea diets were formulated to meet minimum nutrients recommended for commercial growing pigs (National Research Council, 2012). To prepare the different test diet mixes, cowpeas and maize were hammer-milled (Jacobson model, P160, Teordrop 10HP) through a 3-mm screen. Ingredients were then blended into the balanced dietary composites by 20-min mixing in 1000 kg lots in a vertical mixer (MORHLANG VERTA MIX 1200VM; Mohrlang Manufacturing, Colorado, USA). The diets are presented in Table 2.

Feed and dietary dry matter were determined by oven-drying 2-g samples at 105 °C for 48 h (AOAC, 2000 method 976.050); ash by heating 2-g samples at 550 ºC overnight in an electric furnace (AOAC, 2000 method 923.03); nitrogen using the micro-Kjeldahl method (AOAC, 2000 method 976.05); ether extract (EE) using Soxhlet extraction (AOAC 2000 method 920.39); and neutral (NDF) and acid (ADF) detergent fibre according to the method of Van Soest *et al*. (1991). Samples for amino acid analyses (AOAC, 2000; method 923.03) were hydrolysed in 6N HCl for 24 h at 110 °C, for determination using an Automatic Analyzer (L-8800 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan). After cold performic acid oxidation overnight and hydrolysis with 7.5N HCl for 24 h at 110 °C, methionine was analysed as methionine sulfone (AOAC, 2000, method 999.13) and tryptophan after LiOH hydrolysis for 22 h at 110 °C using High Performance Liquid Chromatography (Agilent 1200 Series, Santa Clara, CA, USA) (Method 988.15). To determine minerals, samples were subjected to acid digestion, followed by determination of calcium using atomic absorption spectrophotometry (Brand GBC, Mod. Avanta PM) (AOAC, 2000; method 968.08) and phosphorous by colorimetry (Clesceri *et al.,* 1989; method 4500-P).

The study was performed in a customized, open, trial house in which pigs were in individual 1.2 m × 1.4 m clear-view steel crates, from which feed and water were freely dispensed from individual self-feeders and nipple drinkers *ad libitum*. The pigs were bred at the Agricultural Research Council-Irene Pig Breeding Unit. They were selected from litters born by naturally-serviced sows. The pigs were weaned at 28 d onto a commercial weaner diet. Previously, for the same pig populations, Kanengoni *et al*. (2014) estimated 300–350 kg mature weights for the LW × LR genotype, and 100 to 150 kg for the Windsnyer-type pigs. These estimates guided the weight selection of 36 males, 12 each of Windsnyer (W) (15 ± 2.3 kg), Large White (LW) × Landrace (LR) (37 ± 1.4 kg), and W × L × LR crossed (39 ±1.2 kg) pigs. The selected pigs were all within an estimated 10–11% degree of maturity. The pigs were further blocked by weight within breed into three-pig groups, within which they were randomly allocated

to dietary treatments for a live-weight-balanced, 3 × 3 factorial experiment, replicated four times. Early in the trial, some pigs showed signs of exposure to elevated ammonia levels, which was promptly addressed by optimizing ventilation. As a precaution, all pigs received treatment for potential mild pneumonia. The feeding trial lasted for 8 weeks.

Table 1 Chemical composition and trypsin inhibitor activity of raw, sprouted, and roasted cowpeas (dry matter basis)

Components	Maize	Cowpeas		
		Raw	Sprouted[1]	Roasted[2]
Trypsin inhibitor activity (Units mg $^{-1}$)	-	5.6	4.6	2.3
Dry matter (g kg $^{-1}$)	924	934	933	934
Organic matter (g kg $^{-1}$)	984	945	953	893
Ash (g kg $^{-}$1)	16	55	47	41
Crude Protein (g kg $^{-1}$)	78	258	291	260
Ether Extract (g kg $^{-1}$)	35	157	92	131
Acid detergent fibre (g kg $^{-1}$)	29	125	187	134
Neutral detergent fibre (g kg $^{-1}$)	103	373	367	240
Amino Acids (g/100g)				
Tryptophan	0.70	0.29	0.23	0.30
Arginine	0.43	1.38	1.97	1.84
Serine	0.40	0.80	1.27	1.19
Aspartic acid	0.37	1.34	2.67	2.59
Glutamic acid	1.30	2.48	3.77	3.81
Glycine	0.29	0.65	0.93	0.91
Threonine	0.30	0.53	0.87	0.86
Alanine	0.59	0.65	1.13	0.97
Tyrosine	0.27	0.27	0.61	0.9
Proline	0.75	0.69	1.13	0.95
HO-Proline	0.02	0.04	0.08	0.06
Methionine	0.14	0.22	0.32	0.32
Valine	0.37	0.80	1.18	1.12
Phenylalanine	0.2	0.94	1.37	1.3
Isoleucine	0.29	0.73	1.03	1.03
Leucine	1.01	1.25	1.82	1.76
Histidine	0.37	0.62	1.11	1.32
Lysine	0.31	1.38	1.68	1.93

[1]Sprouting; 12-h pre-soaking in water, 4-d 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 min roasting to 105 °C terminal grain temperature

Table 2 Ingredient and chemical composition of maize–cowpea test diets fed to growing pig genotypes

Components	**[1]Sprouted cowpeas**	**[2]Roasted cowpeas**
Ingredients (% as fed)		
Maize	61.2	55.4
Sprouted Cowpea	36.0	-
Roasted Cowpea	-	42
Soybean Meal	-	-
NaCl	0.57	0.51
Mineral & Vitamin Mix	2.23	2.09
Total	100.0	100.0
Analysed chemical (g kg $^{-1}$) and calculated energy (MJ kg^{-1}) composition		
Crude protein	155.0	155.0
Crude fibre	21.0	20.8
Nitrogen detergent fibre	195.3	164.4
Acid detergent fibre	84.9	72.1
Ash	27.3	27.8
Crude fat (ether extract)	24.7	24.8
[3]Metabolisable energy	14.1	14.3
Calcium	5.4	5.4
Phosphorus	3.4	3.4

[1]Sprouting; 12-h pre-soaking in water, 4-d 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 min roasting to 105 °C terminal grain temperature
[3]Metabolizable Energy (ME) MJ kg $^{-1}$: = 1.000 × DE – 0.68 × CP (Noblet and Perez, 1993; Equation 43), where DE (digestible energy) MJ kg $^{-1}$ = 4.168 - 9.1 × Ash + 1.9 × CP + 3.9 × EE - 3.6 × NDF (Noblet and Perez, 1993; Equation 23)

Pigs were weighed at the beginning of the experiment and weekly thereafter to the end of the experimental period. Feed spillage was returned to the trough except when fouled. The 3-mm hammer milling effectively excluded significant refusals. Feed intake was recorded per pen, and corrected for any fouled spillage or refusals, to calculate the average daily feed intake (ADFI). Average daily weight gain (ADG) was scaled to unitary (kg) live weight and the feed: gain ratio (FCR) was calculated weekly. The pigs were slaughtered at the Agricultural Research Council-Irene Research Station abattoir. The pigs were slaughtered in accordance with the abattoir standard protocols, whereby the animal was stunned with an electrical stunner set at 220 V and 1.8 A current flow for 6 s and was exsanguinated within 10 s of stunning. Upon slaughter, the heart, liver, kidneys, spleen, stomach, and lungs were removed and weighed. The hot carcass weight was recorded after dressing, and the cold carcass weight and back fat thickness were measured after 24-h at 3–4 °C cold storage.

Blood samples were collected at slaughter from the severed anterior *vena cava*, into heparin tubes, placed in ice for 4–6 h prior to centrifuging at 3000 × *g* for 15 min at 4 °C for plasma separation. The plasma was frozen to -18 °C for storage prior to metabolite analysis. An IDExx analyser with Catalyst Dx Chemistry Analyzer was used to determine glucose, urea, creatinine, total protein (TP), albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, cholesterol, and triglycerides (TG).

The growth performance, organ data, and plasma metabolites were subjected to analysis of variance (ANOVA) for a 3 × 3 factorial experiment using the generalised linear model (GLM) of MINITAB software (Version 17.0) (2014) based on the model:

$$Y_{ijk} = \mu + G_i + D_j + (G \times D)_{ij} + \mathcal{E}_{ijk} \quad (1)$$

where:

Y_{ijlk} = l^{th} Observation
μ = Overall mean
G_i = Effect of the i^{th} pig genotype

D_j = Effect of the jth diet
$(G \times D)_{ij}$ = Interaction between pig genotype and diet
ε_{ijkl} = Random error

Tukey's post-hoc test was used to compare different (α ≤0.05) means.

Results and Discussion

Dietary and pig genotype effects on the pig growth performance indices are presented in Table 3. The pig genotypes differed in initial weight in the order W < LW × LR < W × LW × LR, which persisted to the terminal live weight, consistent with the similar (*P* >0.05) growth rates of the W × LW × LR and LW × LR, which was greater than for the W pigs (*P* <0.05). Pigs which were uniformly (*P* >0.05) distributed in initial weight within the dietary treatments were differentiated (*P* >0.05) in final weight in the order, control > sprouted cowpeas > roasted cowpeas diet as a result of different daily weight gains in the order, control > sprouted cowpeas ≥ roasted cowpeas diet (*P* <0.05). Feed intake was in the order W × LW × LR ≥ LW × LR > W, and control > sprouted cowpeas ≥ roasted cowpea diet. The FCR was similar across the genotypes, and in the order, control < sprouted cowpeas ≤ roasted cowpea diets. Genotype × diet interaction was significant for back fat thickness, which, while low (*P* <0.05) for the LW × LR treatment, and for the sprouted cowpea compared to the control diet (*P* <0.05), was highest for W × LW × LR pigs on the sprouted cowpea diet, similar to W and LW × LR on the control diet, with declining (*P* <0.05) ack fat thickness in the order, W and W × LW × LR pigs on the roasted cowpea diet, W pigs on the spouted cowpea diet, and the LW × LR on the sprouted and the roasted cowpea diets (*P* <0.05). The diets did not affect the dressing percentage (P >0.05). The W pigs had low cold and warm dressed percentages (*P* <0.05). Dietary and pig genotype effects on visceral organ sizes are presented as percentages of the slaughter weight of the animals in Table 4. The W pigs had enlarged (*P* < 0.05) livers, kidneys, spleens, and lungs, whereas pigs on the roasted cowpeas had enlarged kidneys (*P* <0.05).

Dietary and pig genotype effects on the measured plasma parameters are presented in Table 5. Total protein and albumin were higher (*P* <0.05) in the W × LW × LR compared to LW × LR pigs, whereas plasma ALP activity was higher (*P* <0.05) in LW × LR compared to W × LW × LR pigs. Plasma urea and cholesterol were elevated (*P* <0.05) in pigs on the sprouted cowpea diet. Both the sprouted and roasted cowpea diets increased (*P* <0.05) plasma albumin, with decreased (*P* <0.05) ALP activity. The W pigs had low plasma creatinine and cholesterol (*P* <0.05). Plasma glucose, globulin, triacylglycerols, alanine aminotransferase (AST), and aspartate aminotransferase (ALT) activities were not affected by the treatments (*P* >0.05).

In the present study, different pig genotypes were differentiated in growth performance and slaughter parameters, with breed influences on scaled liver and kidney sizes, creatinine, cholesterol, plasma total protein and albumin, whereas the diet affected the scaled feed intake, FCR, scaled kidney size, cholesterol urea, albumin, and ALP profiles, with genotype × diet interactions for back-fat. The literature is scant on studies which similarly combine the measurement of growth, slaughter performance, and effects on scaled organ sizes with the chemical profiling of plasma biomarkers to evaluate effects of sprouting versus roasting whole cowpeas for total dietary replacement of expeller soybean cake for the spectrum of pig genotypes used in this study. Comparability of findings from plasma biomarker-based studies is further complicated by the multiple factors which may influence metabolism, such as animal age, weight, growth stage, or the feeding level or period (Montoro *et al.,* 2022).

In the present study, growth rate was in the genotype order, LW × LR ≥ W × LW × LR > W, feed intake in the order, W × LW × LR ≥ LW × LR > W, with higher backfat of the W compared to LW × LR pigs. These were consistent with the improved genetics of the exotic pigs and with expected heterosis in the three-way crossbreed (Len *et al.,* 2008; Jiang *et al.,* 2011). Measured by pig growth rate and feed intake, both cowpea diets were inferior to the commercial control diet. Slower growth on the cowpea diets was likely the result of intrinsically inferior cowpea protein quality compared to soybean cake (Khattab, 2009; Frota *et al.,* 2017). However, if processing was suboptimal, residual anti-nutrients could have impaired digestion, caused endogenous protein wastage, and importantly, unpalatable tannins could have induced low intake (Umapathy and Erlwanger, 2008). Pigs consumed more of the control diet compared to the cowpea diets.

Table 3 Growth and slaughter performance of indigenous, exotic, and crossbred growing pigs fed a standard (control), and roasted or sprouted cowpea diets

Treatment		Slaughter Weight (kg)	Feed intake		Weight gain (kg/day)	FCR	Back Fat (mm)	Dressing %	
			(kg/day)	kg/ $LW^{0.75}$				Warm	Cold
Genotype	**Diets**								
	Control	65.6	2.19	0.67	0.69	3.41	8.25^{cde}	77.8	75.9
LW × LR	Sprouted Cowpeas	59.7	1.85	0.61	0.46	5.61	7.50^{de}	77.1	75.0
	Roasted Cowpeas	55.1	1.77	0.62	0.35	6.58	6.50^{e}	78.1	75.9
	Control	34.2	1.45	0.72	0.42	3.43	11.00^{ab}	73.8	69.8
W	Sprouted Cowpeas	29.4	1.20	0.66	0.26	4.61	9.25^{cde}	71.1	67.5
	Roasted Cowpeas	26.6	1.29	0.78	0.20	6.46	9.75^{bcd}	71.0	67.0
	Control	66.4	1.97	0.60	0.63	3.24	10.50^{abc}	76.1	73.7
W × LW × LR	Sprouted Cowpeas	61.0	1.98	0.64	0.43	4.61	11.75^{a}	76.2	74.3
	Roasted Cowpeas	57.5	1.88	0.62	0.41	4.74	9.75^{abcd}	78.3	75.9
SEM		0.549	0.0339	0.0144	0.0234	0.361	0.164	0.511	0.551
	LW × LR	60.2^{a}	1.94^{a}	0.63^{b}	0.50^{a}	5.20	7.41^{b}	77.7^{a}	75.6^{a}
Genotype	Windsnyer	30.1^{b}	1.31^{b}	0.72^{a}	0.29^{b}	4.84	9.75^{a}	72.0^{b}	68.1^{b}
	W × LW × LR	61.7^{a}	1.94^{a}	0.62^{b}	0.49^{a}	4.20	10.66^{a}	76.9^{a}	74.6^{a}
SEM		0.841	0.0479	0.0204	0.0331	0.551	0.321	0.723	0.780
	Control	55.4^{a}	1.87^{a}	0.66	0.58^{a}	3.41^{b}	9.91^{a}	75.9	73.1
Diet	Sprouted Cowpeas	50.0^{b}	1.68^{ab}	0.64	0.38^{b}	4.94^{ab}	8.50^{b}	74.8	72.2
	Roasted Cowpeas	46.4^{c}	1.65^{b}	0.68	0.32^{b}	5.93^{a}	9.41^{ab}	75.8	72.9
SEM		0.841	0.0479	0.0204	0.0331	0.551	0.321	0.723	0.780
P-values	*Genotype*	0.000	0.000	0.012	0.002	0.528	0.000	0.000	0.000
	Diet	0.000	0.025	0.539	0.000	0.024	0.005	0.625	0.784
	Genotype × Diet	0.949	0.348	0.450	0.907	0.896	0.037	0.603	0.696

^{abcde} Within each factor or combination of factors means within a column with different letter superscripts are significantly different at (P<0.05), SEM- Standard error of the mean, W-Windsnyer, Large White (LW), Landrace (LR), FCR = Feed conversion ratio. ; [1]Control –Standard commercial maize-soybean grower diet, [2]Sprouted- 12-hour soaking, 4-day open air sprouting, sun-dried, [3]Roasted- 150^{o} C initial, empty cast-iron drum temperature, 20-minute roasting to 105° C terminal grain temperature

Table 4 Visceral organ sizes of indigenous, exotic and crossbred growing pigs fed a standard, and roasted or sprouted cowpea diets

Treatments		Visceral organs and tissues (% slaughter Weight)					
		Heart	**Liver**	**Kidneys**	**Spleen**	**Stomach**	**Lungs**
Genotype	**Diet**						
LW × LR	Control	0.396	1.55	0.384	0.156	2.26	1.64
	Sprouted cowpeas	0.382	1.62	0.396	0.139	2.20	1.78
	Roasted cowpeas	0.383	1.31	0.419	0.127	1.03	1.79
W	Control	0.403	2.12	0.460	0.248	1.55	1.69
	Sprouted cowpeas	0.339	2.40	0.458	0.178	2.25	1.52
	Roasted cowpeas	0.623	2.99	0.567	0.296	1.97	2.28
W × LW × LR	Control	0.480	1.99	0.412	0.187	3.09	1.28
	Sprouted cowpeas	0.414	1.73	0.410	0.196	1.49	1.47
	Roasted cowpeas	0.418	1.76	0.452	0.232	1.24	1.58
SEM		0.0224	0.0768	0.0105	0.0109	0.147	0.0638
Genotype							
LW × LR		0.387	1.49[b]	0.400[b]	0.141[b]	1.83	1.74[a]
W		0.455	2.50[a]	0.495[a]	0.241[a]	1.93	1.83[a]
W × LW × LR		0.437	1.83[b]	0.425[b]	0.205[ab]	1.94	1.44[b]
SEM		0.0317	0.109	0.0148	0.0155	0.208	0.0902
Diet							
Control		0.426	1.89	0.419[b]	0.197	2.30	1.54
Sprouted cowpeas		0.378	1.92	0.421[b]	0.171	1.98	1.59
Roasted cowpeas		0.474	2.02	0.479[a]	0.218	1.41	1.88
SEM		0.0317	0.109	0.0148	0.0155	0.208	0.0902
P-values	*Genotype*	0.449	0.000	0.003	0.003	0.947	0.050
	Diet	0.236	0.767	0.042	0.227	0.060	0.076
	Genotype × Diet	0.150	0.101	0.642	0.335	0.058	0.348

[abc] Within each factor or combination of factors, means within a column with different letter superscripts are significantly different at (P<0.05), SEM- Standard error of the mean, W- Windsnyer, Large White (LW), Landrace (LR), FCR = Feed conversion ratio; [1]Control –Standard commercial maize-soybean grower diet, [2]Sprouted- 12-hour soaking, 4-day open air sprouting, sun-dried, [3]Roasted- 150[o] C initial, empty cast-iron drum temperature, 20-minute roasting to 105° C terminal grain temperature

Table 5 Plasma metabolite and enzyme biomarkers of indigenous, exotic and crossbred growing pigs fed a standard, and roasted or sprouted cowpea diets

Parameter		Glucose	Creatinine	Urea	Total Protein	Albumin	Globulin	ALT	AST	ALP	Bilirubin - Total	Cholesterol	Triglycerides
Normal Range		**4.72 - 8.88 mmol/L**	**44.2 - 185.64 µmol/L**	**2.14 - 10.71 mmol/L**	**60 - 80 g/L**	**18 - 33 g/L**	**9 - 43 U/L**	**16 - 65 U/L**	**92 - 294 U/L**	**92 - 294 U/L**	**1.71 - 5.13 µmol/L**	**0.47 - 2.04 mmol/L**	**0.46 - 0.94 mmol/L**
Treatment		**Glucose mmol/L**	**Creatinine µmol/L**	**Urea mmol/L**	**Total Protein g/L**	**Albumin g/L**	**Globulin U/L**	**ALT U/L**	**AST U/L**	**ALP U/L**	**Bilirubin µmol/L**	**cholesterol mmol/L**	**Triglycerides mol/L**
Genotype	**Diet**												
	[1]Control	6.61	75.14	3.21	71.75	37.00	34.75	51.00	62.00	100.50	8.12	1.77	0.63
	[3]Sprouted	6.02	72.93	4.46	67.25	32.75	34.50	42.50	74.00	136.00	8.55	2.15	0.43
LW×LR	**[2]Roasted**	5.89	66.30	4.46	68.50	33.00	35.50	42.00	50.25	140.50	5.99	2.13	0.71
	Control	7.27	46.41	3.84	73.50	39.75	33.75	56.75	62.75	118.75	5.13	0.85	0.39
W	**Sprouted**	6.16	41.99	5.71	68.25	35.25	33.00	56.25	62.00	102.75	5.13	1.28	0.40
	Roasted	6.62	46.41	5.54	67.75	35.25	32.50	66.25	69.25	136.50	5.56	1.42	0.37
	Control	6.56	64.09	3.66	75.00	41.50	33.50	53.50	62.50	91.25	8.12	1.53	0.40
W×LW×LR	**Sprouted**	6.30	59.67	5.62	73.50	39.00	34.50	49.75	56.25	94.00	5.56	2.02	0.50
	Roasted	6.36	63.30	3.57	72.25	38.50	33.75	53.25	70.75	113.25	10.64	1.48	0.47
SEM		0.197	1.64	0.180	0.647	0.637	0.446	2.420	3.901	4.310	0.618	0.0589	0.0387
	LW×LR	6.17	71.46^{a}	4.05	69.20^{b}	34.25^{b}	34.92	45.20	62.13	125.70^{a}	7.55	2.02^{a}	0.62
Genotype (G)	**W**	6.68	44.94^{b}	5.03	69.83^{ab}	36.75^{ab}	33.13	59.75	64.70	119.33^{ab}	5.27	1.23^{b}	0.40
	W×LW×LR	6.23	63.35^{a}	4.28	73.63^{a}	39.67^{a}	33.92	59.20	63.20	99.50^{b}	8.11	1.77^{a}	0.43
SEM		0.279	2.32	0.255	0.915	0.902	0.631	3.420	5.510	6.090	0.874	0.0833	0.0547
	Control	6.81	61.88	3.57^{b}	73.42	39.42^{a}	34.00	53.75	62.42	103.50^{b}	7.13	1.41^{b}	0.53
Diet (D)	**Sprouted**	6.22	58.20	5.30^{a}	69.67	35.58^{b}	34.00	49.50	64.13	110.92^{a}	6.41	1.82^{a}	0.44
	Roasted	6.29	59.67	4.52^{ab}	69.50	35.60^{b}	33.92	53.83	63.42	130.13^{a}	7.43	1.72^{ab}	0.52
SEM		0.279	2.32	0.255	0.915	0.902	0.631	3.420	5.510	6.090	0.874	0.0833	0.0547
P-values	**Genotype**	0.579	0.000	0.085	0.020	0.007	0.261	0.064	0.964	0.050	0.160	0.000	0.112
	Diet	0.373	0.658	0.003	0.320	0.032	0.996	0.707	0.985	0.049	0.801	0.016	0.737
	Genotype × Diet	0.953	0.670	0.288	0.986	0.986	0.901	0.798	0.561	0.410	0.373	0.326	0.589

[abc] For each factor, and interactions, [means] within a column with different letter superscripts are significantly different at (P<0.05). SEM- Standard error of the mean, ALT - Alanine aminotransferase, AST - Aspartate aminotransferase, ALP - Alkaline phosphatase, LW- Large White LR- Landrace, W- Windsnyer, ; [1]Control –Standard maize-soybean grower diet, [2]Sprouted- 12-hour soaking, 4-day open air sprouting, sun-dried, [3]Roasted- 150º C initial, empty cast-iron drum temperature, 20-minroasting to 105° C terminal grain temperature. [4]Extracted from Milinković-Tur *et al*. (2005) and Ozer *et al*., 2008).

The higher intake of the control diet explained the higher back fat thickness, for which the genotype × diet interaction suggested unique treatment effects on energy or lipid metabolism. Similarly, the enlarged liver and kidney in W pigs, and the enlarged kidney in pigs on the roasted cowpea diet could be unique genotype adaptive traits. The W × LW × LR pigs had higher plasma total protein compared to the commercial type, LW × LR pigs, with an intermediate level for the W pigs, which suggested the same order in protein demand or the reverse order in efficiency of protein utilization.

In the present study, across the treatments, the plasma creatinine, glucose, urea, cholesterol, total protein, and the ALP and AST activities were considered within the reference normal pig ranges (Milinković-Tur *et al.,* 2005; Ozer *et al.,* 2008). The elevated plasma albumin, bilirubin, and ALT activity suggested infection or non-infectious hepatocellular disease (Nathwani *et al.,* 2005). However, these were not treatment-related, and were attributed to an inflammatory response triggered by the transient high ammonia levels to which the pigs had been exposed. The observed genotypic influences on plasma parameters such as the total protein, ALP activity, and the variable lipid components were consistent with indications from more robust metabolomic exploration, which similarly differentiated both protein and lipid metabolism in fast growing, lean versus unimproved indigenous pigs (Yang *et al.,* 2011; Bovo *et al.,* 2016). The intensity of metabolic change, mainly of protein, is also reflected in the concentration of other biochemical indicators of blood, such as total protein, urea, aspartate aminotransferase or alanine aminotransferase (Kapelański *et al.,* 2000; Więcek and Skomiał 2000). In the current study, plasma total protein was 68.5–75 g/L across the treatments. Most of the biochemical parameters of blood related to protein metabolism were higher (73.63 g/L) in W × LW × LR pigs characterized by a high rate of growth compared to the commercial type LW × LR pigs (69.20 g/L), with an intermediate level (69.83) for the slow-growing W pigs. The relative imbalance in dietary amino acids in relation to the animal requirement was likely to be sufficient to compromise the liver or whole-body protein synthesis (Hassan *et al.,* 2020). Friendship *et al.* (1984) previously reported such variations in the range of plasma total protein of 52–83 g/L in Ontario pigs on maize diets. Harapin *et al.* (2003) reported higher values of 76–88 g/L in wild boar pigs of 3–5 years old scavenging in protected camps. Albumin constitutes up to 60% of the total plasma proteins and is involved in the transport of plasma lipids (Shen et al., 2004). Protein nutrition influences albumin synthesis, which decreases with protein malnutrition (Juliene *et al.,* 2004). In the present study, a range of 32.5–39.5 g/L plasma albumin was observed across the treatments. The sprouted and roasted cowpea diets similarly (35.58–35.60 g/L) decreased pig plasma albumin compared to the control (39.42 g/L). The LW × LR pigs had lower (34.25 g/L) plasma albumin than the W × LW × LR pigs (39.67 g/L), with an intermediate level (36.75 g/L) for the W pigs. Low plasma albumin suggests less plasma transport of lipid compounds (Juliene *et al.,* 2004). In the present study, pigs on the sprouted diet had higher plasma urea compared to those on the control diet, which suggested either an excess or imbalance in dietary amino acids. Surprisingly, despite dietary influences on urea N, plasma urea N was not differentiated by genotype to reflect effects of a higher demand for amino acids expected of the lean-type LW × LR genotype (Liu *et al.,* 2015).

Although the diet did not influence plasma total protein, an opposite trend to plasma total protein was observed for plasma ALP activity among the pig genotypes. The W is a smaller, fatter breed, with slower growth potential compared to the genetically-improved commercial pig breeds (Qin *et al.,* 2002). Both cowpea diets increased plasma ALP, regardless of the cowpea processing method. Plasma ALP activity is positively correlated to pig weight gain (Pond *et al.,* 1997; Yang *et al.,* 2011; Liu *et al.,* 2015), and is a signal for lipid metabolism (Liu *et al.,* 2015), likely the effects of the pig growth rate, given that, compared to the LW × LR, creatinine was lower in W pigs, consistent with a lower muscle mass or less turnover for the slow-growing breed. (Fisher, 1954) indicated that most incoming nitrogen is condensed to form tissue proteins and that catabolic processes are secondary to anabolic ones. Some nitrogen in unutilized forms (e.g., creatinine) may pass immediately out of the body. A little may be used directly for endogenous processes.

The primary mammalian energy repository is lipid. Plasma TG, total, low, and high density lipoprotein cholesterol levels reflect patterns of both lipid absorption and utilization (Ma *et al.,* 2020) The principal storage lipid classes are the TG, phospholipids, and steroids, with the TG quantitatively the most important (Godsland, 2004). Mammals revert to breaking down stored lipids to meet energy requirements only when in dietary energy deficit, or to preserve glucogenic fuel substrates for the dependent organs (Lomb *et al.,* 2010). The TG are considered indices of total body fat (Griffin *et al.,* 1982; Whitehead and Griffin 1984). Yang *et al.* (2011) stated that, in animal species, the blood levels of molecules related to lipid, glucose and protein metabolism, such as non-esterified fatty acids, triglyceride, glucose, and alanine aminotransferase (ALT), reflect nutritional and disease status. Though

not statistically significant, the genetically-improved LW × LR genotype had higher plasma TG, which was consistent with a higher feed intake. Apart from dietary supply, plasma TG include those mobilized from adipocytes to increase the plasma levels during energy deficit (Jensen *et al.,* 2000). In the fed state, unsaturated fatty acids, saturated fatty acids, and glycerol are used to synthesize triglycerides (Miyazaki *et al.,* 2001). During fasting, fatty acids are released from triacylglycerol stored in adipocytes of growing pigs, resulting in increased levels in plasma (Jansen *et al.,* 2001). Therefore, the higher plasma TG in the LW × LR pigs could also partially indicate marginal energy deficit. Thyroid hormones and their receptors are reported to stimulate reverse cholesterol transport in animal models (Pedrelli *et al.,* 2010) and such increase was observed when cowpea diets increased plasma cholesterol, particularly the sprouted cowpea diet, likely an effect of low dietary intake. Adequate cholesterol in blood is therefore crucial for achieving maximum weight gain in growing animals. The blood levels of lipids and cholesterol depend on the breed of pigs, their genotype in relation to lipoproteins, sex, and the type of feed given (Migdał *et al.,* 1999; Migdał *et al.,* 2003). In the present study, differences were shown in the concentration of blood serum cholesterol between the pig genotypes studied; plasma cholesterol was low in W pigs. Similarly, in chickens, slow-growing genotypes had low plasma cholesterol (Tudorache *et al.,* 2022). Therefore, it is possible that, for the W pigs, low plasma cholesterol levels suggested either low intake, or greater or more efficient transport and/or clearance of dietary cholesterol through catabolism in the liver, or via deposition in adipose tissues. In the present study, the treatments did not affect plasma glucose, which suggests the insulin–glucagon axis control of glucose or energy metabolism was not active.

In addition to the chemical modification of feed nutrients, their absorption in the gut, and the subsequent pig intermediary metabolism, the efficacy of processing legume grains is also a function of potentially residual toxic compounds. The liver is a primary nutrient processing organ, including digestion, metabolism for assimilation or detoxification, and excretion, which exposes it to dietary toxins, effects which are indirectly indicated by serum biomarkers or by biopsy (Chen *et al.,* 2017). In the present study, although genotype differences were observed in liver and kidney sizes, with dietary effects on the kidney size, the profile of the clinical biomarkers did not suggest dietary or genotype discrimination.

Conclusion

Overall, pig performance was consistent with the productive efficiency expected of the genetically improved, compared to the native pig genotypes, and reflected beneficial non-additive gene effects expected of their crossed genotype. The findings suggest that, though less efficient than the standard diet, cowpeas either roasted or sprouted can be substituted for soybean oil cake in growing pig diets with minimal compromise to pig productivity, more so if cowpeas are sprouted, compared to roasted. Pig genotype influences on the utilization of differently-processed cowpeas imply pigs either naturally (indigenous pigs) or artificially (improved pigs) acquired metabolic or physiological functional traits to differently digest and metabolize energy substrates from the experimental diets. The findings provide valuable insights for optimizing pig breeding programs, and efficient processing of alternative legumes to enhance growth and performance in different pig genotypes.

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Authors' contributions

M.W., J.J., and F.F. collaborated in the planning, design, conduct of the experiment, the interpretation of results, and drafting of the manuscript.

Conflict of interest declaration

The authors declare no conflict of interest associated with the research, including any influential financial support.

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