Growth performance, nutrient digestibility and carcass characteristics of pigs fed diets containing amarula (*Sclerocarya birrea* **A. Rich) nut cake as replacement to soybean meal**

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Abstract

This experiment evaluated varying levels of Amarula (*Sclerocarya birrea* A. Rich) nut cake (ANC) on growth performance, nutrient digestibility and carcass characteristics in pigs. Thirty Large White \times Landrace (LW \times LR) pigs were stratified by weight (average live weight of 20 ± 5 kg) and randomly allocated to the five experimental diets that contained 0 (control), 50, 100, 150 and 200 g ANC/kg DM. Each pig served as a replicate unit, housed individually. The pigs were fed the experimental diets *ad-lib* once in the morning, allowing a 10% of feed refusal, and had free access to water. Bodyweight, feed intake, average daily gain (ADG) and feed conversion ratio (FCR) were recorded weekly throughout the 56-day trial period. On completion of the growth trial, following a 3-day adaptation, a nutrient digestibility study was conducted over 5 days. Thereafter, pigs were fasted for 12 h, weighed, slaughtered, and carcass samples were collected for analysis. Data on the effects of treatments on growth performance, nutrient digestibility and carcass characteristics were analysed using a two-way ANOVA in randomised blocks and were compared using Student's *t*-LSD. Feed intake was not affected by dietary treatment, but ADGs were reduced at ANC levels > 150 g/kg, resulting in poor FCR. Protein digestibility was reduced at ANC levels $>$ 150 g/kg, while ether extract and fibre levels increased. Warm and cold carcass weights were lower at ANC levels > 150 g/kg, with improved meat redness and lightness. It was concluded that ANC could replace SBM in the diet of growing pigs at less than 150 g/kg inclusion level.

Keywords: Energy; Fats; Fibre; Meat; Nitrogen; Soybean

Introduction

The ban on the use of protein sources from animal origin in animal feed during the year 2001 increased the demand for locally produced plant protein sources in South Africa. Soybean meal (SBM) is the mostly used protein source in animal nutrition, due to its high quality in amino acid profile (McDonald et al., 2011), and accounts for 85% of the protein supplements fed to pigs (Cortamira et al., 2000). About 72% of SBM supplies in South Africa is imported from non-African countries (Sihlobo and Kapuya, 2016), making it difficult to be afforded by some pig producing farmers, especially those in the emerging sector. The possibility of

partially replacing SBM with other plant protein sources from local oil production contributes to sustainable pig production. Unlike the global trend, where pork is the most consumed of all meat products, the South African pork industry is relatively small compared to chicken and beef markets. Despite this, the pork industry consumed 10% of all feed produced, and almost 75% of producers mix their own feed (Grimbeek et al., 2014). Thus, to reduce the cost of feeding, researchers persistently attempt to identify protein source alternatives to SBM, although with variable results (Choi et al., 2015). This is attributable mainly to the balance or imbalances of amino acids (McDonald et al., 2011) and the presence of anti-nutritional factors (ANFs) (Choi et al., 2015) in the diet when SBM is replaced with other plant protein sources.

Nonetheless, it is important to evaluate the nutritional value of locally produced plant protein sources. Amarula (*Sclerocarya birrea* A. Rich) nut cake (ANC) is a by-product that is derived from oil extraction of the dry seeds of ripe amarula fruit, an indigenous fruit tree that is widely distributed throughout the sub-Saharan Africa (Mthiyane and Mhlanga, 2017). The availability of ANC is seasonal (i.e. July to December) and an estimated amount of 90 t per month is produced in South Africa, which is often dumped without use. Research has shown that ANC contains crude protein (CP) that ranges from 325 to 470 g/kg DM (Mthiyane and Mhlanga, 2017; Malebana et al., 2018; Nkosi et al., 2019), comparable to that of SBM, but higher than that of other conventional protein sources such as canola seed meal (270 g CP/kg DM, NRC, 2001). The ANC contains ether extract (EE) that ranges from 344 to 498 g $E E/kg$ DM (Mthiyane and Mhlanga, 2017; Malebana et al., 2018; Nkosi et al., 2019), which depends on the processing method for oil extraction (Malebana et al., 2018). The high fat content in ANC could provide sufficient energy in diets and allow pigs to improve feed efficiency, daily gains and thus increase muscle accretion (De la Llata et al., 2001; Stephenson et al., 2016). When compared to SBM, Malebana et al. (2018) reported an amount of $21.2 \text{ mg}/100 \text{ g}$ DM and 0.07 mg/100 g DM for saponin and tannins in ANC, respectively, which are lower than those in SBM, giving an advantage for using ANC as dietary feed ingredient for pigs.

Previous studies showed that ANC could be a good source of CP in diets for poultry (Mthiyane and Mhlanga, 2017, Mazizi et al., 2020 without adverse effects on animal performance. According to Mthiyane and Mhlanga (2017) and Mazizi et al. (2020), one of the benefits for addition of ANC in animal diets is the increase in oleic acid content in the meat of birds, which is a potential health benefit to consumers. It has not yet been determined how much SBM can be replaced by non-conventional ANC in diets for growing pigs without affecting growth performance or carcass characteristics. With the high feed cost involved with pig production (especially under intensive system), it is worth to evaluate ANC as a dietary ingredient for pigs. The hypothesis that ANC would be safe as feedstuff for growing pigs, support adequate growth performance, and enrich the carcass with lean meat was tested. The objectives of this study were therefore to evaluate the effects of feeding increasing inclusions levels of ANC in diets on growth performance, nutrient digestibility and carcass characteristics of pigs.

Materials and methods

Study site and management of animals

The study was conducted at the Pig Nutrition Section of the Agricultural Research Council (ARC)-Irene Campus, South Africa (longitude 25° 55′ S; 28° 12′ E). Amarula nut cake (ANC) was sourced from African Exotic Oils, Phalaborwa, Limpopo Province, South Africa. Prior to oil extraction, the amarula nuts were air-dried to 3% moisture and the dried nuts were cold-pressed at $6.1-9.5$ kg/h and $35-40$ min rotations of the screw at $55-60$ °C. The ANC was brought to the ARC-Irene Campus for the analyses of nutrient composition (Table 1) and was used to formulate the five experimental diets (Table 2) that contained ANC at 0 (control), 50, 100, 150 and 200 g/kg, respectively. The diets were formulated to be isoenergetic and isonitrogenous to provide 14 MJ/kg digestible energy (DE), 180 g/kg crude protein (CP) g/kg and 11.6 g/kg lysine for growing pigs. Thirty Large White \times Landrace (LW \times LR) pigs with an average body weight of 20 ± 5 kg were randomly allocated to the experimental diets (6 animals/treatments). Experimental diets were allocated randomly to pens and each pig was treated as an experimental unit, in a complete randomized design. The pigs were housed individually in 1.54×0.8 m pens in a pig barn, which is an enclosed house with windows and fans (Termotecnica Pericoli®) for ventilation, that can be operated in response to the external weather to make the internal housing environment conducive for the pigs. The temperature fluctuated between 22 and 25 \degree C, which was recorded daily in the morning and afternoon at pig-shoulder level using a thermometer. Prior to data collection, the pigs were adapted to the experimental environment and diets for 14 days and the feeding trial lasted for 56 days. The pigs were fed the experimental diets *ad-lib* once in the morning, allowing a 10% of feed refusal, and had free access to water. The feeders were adjusted twice each day to ensure constant access to fresh feed and minimize any possible wastage. Pigs were individually weighed at the start and weekly until end of the trial and data on daily feed intake, average daily gains and feed conversion rates were recorded.

Nutrient composition		Essential amino acids, g/kg			
DM (g/kg DM)	963.5	Arginine	50.5		
Ash (g/kg DM)	48.5	Histidine	6.1		
CP (g/kg DM)	322.9	Isoleucine	12.9		
EE (g/kg DM)	343.9	Leucine	12.3		
GE (MJ /kg DM)	25.3	Lysine	6.8		
NDF (g/kg DM)	244.0	Methionine	4.4		
ADF (g/kg DM)	223.2	Phenylalanine	12.8		
ADL (g/kg DM)	98.1	Threonine	8.1		
Mineral composition		Tryptophan	4.4		
Calcium (g/kg)	1.8	Valine	15.6		
Magnesium (g/kg)	5.7	Non-essential amino acids, g/kg			
Phosphorus (g/kg)	9.4	Alanine	10.4		
Potassium (g/kg)	8.3	Aspartic acid	27.4		
Sodium (mg/kg)	345.7	Glutamic acid	84.0		
Iron (mg/kg)	95.5	Glycine	18.0		
Copper (mg/kg)	27.9	Hydroxy-proline	0.9		
Manganese (mg/kg)	10.2	Proline	11.3		
Zinc (mg/kg)	60.2	Serine	16.5		
Cobalt (mg/kg)	0.14	Tyrosine	6.9		
Molybdenum (mg/kg)	0.31				

Table 1 Nutrient, mineral and amino acids composition of amarula nut meal $(n=3)$

ANC-amarula nut cake, SD-standard deviation, ADF-acid detergent fibre, ADL-acid detergent lignin, CPcrude protein, DM-dry matter, EE-ether extract, GE-gross energy, NDF-neutral detergent fibre

Ingredient (g/kg)	Treatment								
				Control 50 g/kg 100 g/kg 150 g/kg 200 g/kg					
Maize	545.1	694.7	697.5	636.0	609.8				
Sunflower oil cake	150								
Wheat bran	150	182.4	155.7	168.4	147.9				
Sunflower oil	26.4	\equiv	$\qquad \qquad =$	۰	$\qquad \qquad =$				
Monocalcium phosphate	8.9	14.5	12.9	9.9	8.3				
Feed lime	12.4	9.6	10.9	12.9	19.7				
Lysine	17.0	46.5	21.0	20.6	12.3				
ANC	-	50	100	150	200				
Soybean oil cake	88.2	-	-	-	-				
Premix ^a	2.0	2.0	2.0	2.0	2.0				

Table 2 Experimental diets with varying inclusion levels of amarula nut cake

Treatments: control = no ANC inclusion; 50 g/kg = 50 g/kg inclusion of ANC; 100 g/kg = 100 g/kg inclusion of ANC; 150 g/kg = 150 g/kg inclusion of ANC; 200 g/kg = 200 g/kg inclusion of ANC

ANC amarula nut cake, *DM* dry matter, *CP* crude protein, *CF* crude fibre, *DE* digestible energy

^aProvided the following per kilogramme of diet: 6500 IU vitamin A, 1200 IU vitamin, D₃, 40 IU vitamin E, 2 mg vitamin K₃, 1–5 mg vitamin B₁, 4.5 mg vitamin B₂, 0.03 mg vitamin B₁₂, 2.5 mg vitamin B6, 25 mg niacin, 12 mg calcium pantothenate, 190.5 mg choline, 0.6 mg folic acid, 0.05 mg biotin, 40 mg manganese, 100 mg zinc, 125 mg copper, 1 mg iodine, 100 mg ferrous, and 0.3 mg selenium

Nutrient digestibility

A week after completion of the growth study, the digestibility study carried out. Chromic oxide was added to the treatment diets at a concentration of 3 g chromic oxide/kg feed as an indigestible marker as described by Moughan et al. (1991). Pigs were allowed an adaptation period of 3 days to the chromic oxide mixed diets before collection of faecal samples for 5 days using the grab sampling method was carried out according to Moughan et al. (1991). The faecal samples were collected once per day, weighed and DM determined. The samples were then freeze-dried, ground, and stored at -20 °C in 250 ml plastic containers until further analysis. Both diets and faecal samples were analysed for DM (ID 945.15), crude protein (CP) (ID 945.18), ash (ID 923.03) and ether extract (EE) (ID 920.85) following the standard procedures of AOAC (2005). The gross energy (GE) was determined using bomb calorimeter (Parr Instruments Co., Moline, IL, USA) while the fibre fractions [neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL)] were analysed following the procedures of Van Soest et al. (1991).

Preparation for slaughter and carcass measurements

At the end of the nutrient digestibility study, all pigs were fasted for 12 h and weighed to determine the final weight. The pigs were then transported to the Irene Campus's abattoir for slaughtering. Prior to being slaughtered, the pigs were handled according to the routine abattoir procedures, which included rest for the pigs before slaughter and ante-mortem inspection. The pigs were then stunned with an electrical stunner set at 220 V and 1.8 A with a current flow for 6 s and exsanguinated within 10 s of stunning. The head from each carcass was then removed at the *atlanto-occipital* joint and the tail at the junction of the third and fourth sacral vertebrae. Carcass dressing and evisceration were done according to the abattoir`s standard operating procedures.

Following carcass dressing and evisceration, carcass length was measured from the first rib to the pubic bone along the median plane using a measuring tape, followed by measurement of warm carcass weight (WCW) using an overhead scale. Warm carcass temperature and pH (pHi) were measured on each of the carcasses *Longissimus thoracis* muscle (eye muscle) with a portable pH meter between the third and the fourth rib, 60 mm from the midline. Dressing percentage (DP) of each carcass was calculated as described by Bonvillani et al. (2010).

The carcasses were then stored in a cold room at a temperature of 0° C for 24 h, after which cold carcass weight (CCW) of each carcass was measured. After determination of cold carcass weight, cold carcass temperature and pH (pHu) as measured on the *Longissimus thoracis* muscle. Back fat thickness was measured between the 2nd and 3rd ribs about 60 mm from the midline on the left carcass using a pair of Vernier Calipers (Bruwer et al., 1991). The rib was cut between the 4th and 12th thoracic vertebrae dorsally and along a parallel line (16 cm) from the spinal cord midline ventrally. The rib was then weighed using a digital scale to obtain the rib weight. The hind leg was removed between the 2nd and 3rd sacral vertebrae perpendicular to the stretched leg and at the hock joint distally and weighed to determine the hindquarter weight (HQW). The hind leg was also measured from the ischiopubic symphysis to the hock joint to determine the hindquarter length (HQL) using a measuring tape.

Drip loss determination

An 80 g chop was cut from the *Longissimus dorsi* muscle between the 4th and 8th ribs of each carcass and used to determine drip loss (DL) of the muscles. Each chop sample was placed in a nylon mesh and then sealed in a pre-weight plastic bag for a period of 24 h at 2 °C. After 24 h, each sample was taken out of the plastic bag and re-weighed. Drip loss was calculated according to the method described by Choi and Oh (2016), using the equation: Drip loss = Final weight of sample/Initial weight of sample \times 100.

Meat colour determination

Meat colour was measured using a Minolta colour meter (Model CR 200, Osaka, Japan) according to Krzywicki (1979). Chops samples (40 g) from the *Longissimus dorsi* muscle were used to measure the meat colour. The three fundamental colour constituents $(L^*, a^*$ and b*) were evaluated where L* indicates lightness on a scale of 0 to 100; a* indicates red to green colour, and b* indicates yellow to blue colour. The chop samples were placed on a tray to bloom for 1 h at 4° C. After 1 h of blooming, meat colour was measured at three different locations of each chop.

Statistical analysis

The experiment was carried out as a randomized blocked design (RBD) with five treatments randomly allocated in 6 blocks. ANOVA (analysis of variance) was used to test the differences between the five treatments effects in order to determine the growth performance, nutrient digestibility and carcass characteristics in pigs (Snedecor and Cochran, 1980). To test for deviations from normality, Shapiro–Wilk's test was performed on the standardized

residuals (Shapiro and Wilk, 1965). In cases where significant deviation from normality was evident and the deviation was due to skewness, the outliers were removed until the data was normally or symmetrically distributed (Glass et al. 1972). Student's t-LSD (least significant difference) were calculated at a 5% confidence level to compare treatment means (Snedecor and Cochran, 1980). All the above data analysis was performed with GenStat (2019).

Data on the effects of treatments on growth performance, nutrient digestibility and carcass characteristics were fitted with the following model: $Y_{ij} = \mu + t_i + \beta_j + \varepsilon_{ij}$.

where Y_{ij} is the individual observations of the *i*th treatment and the *j*th replicate, μ is the overall mean, t_i is the effect of the *i*th treatment, β_i is the effect of the *j*th block replicate and *εij* is the residual error.

Data on of growth performance, nutrient digestibility and carcass characteristics were also fitted using a polynomial equation of the second degree (i.e. linear and quadratic term) as:

$$
\gamma_{ij} = \mu + t_i + \omega_1 + \omega_2 + \beta_j + \varepsilon_{ij}
$$

where Y_{ij} is the individual observations of the *i*th treatment and the *j*th block, μ is the overall mean, t_i is the effect of the *i*th treatment, ω_1 is a linear component, ω_2 is a quadratic component, *βj* is the effect of the *j*th block and *εij* is the residual error.

Results

Increasing dietary inclusion of ANC increased $(P < 0.05)$ the ether extract, energy and fibre while reducing the CP contents of the diets (Table 3). Adding ANC in the diet reduced (*P* < 0.05) dietary magnesium, zinc and molybdenum levels while increasing calcium and manganese levels compared to the control. Inclusion of ANC reduced compositions of lysine and threonine in the diets (Table 4). Further, most essential amino acids (i.e. histidine, leucine, phenylalanine, threonine and valine) and some non-essential amino acids (i.e. alanine, aspartic acid, glycine, etc.) were reduced $(P < 0.05)$ with the inclusion of ANC in the diet. The ADFI (average daily feed intake) was not affected by dietary treatments (Table 5). However, the ADG and BWG (body weight gain) were reduced with more than 150 g/kg dietary inclusion of ANC, which further resulted in a poor FCR. The digestibility of DM was not affected by the dietary treatments (Table 6). However, the digestibility of CP was reduced while that of EE and fibre was improved with increased $(>150 \text{ g/kg})$ dietary ANC.

Parameters	Treatment				P value				
	Control	50 g/kg	$100 \frac{\text{g}}{\text{kg}}$	150 g/kg	200 g/kg	SEM	Treatment	Linear	Quadratic
Nutrient composition									
DM(g/kg)	956.8b	958.9a	954.4c	953.8c	956.3b	0.19	0.001	0.039	0.001
Ash (g/kg)	51.2	50.1	47.7	47.0	48.8	0.20	0.069	0.058	0.002
CP (g/kg)	171.0a	164.6b	162.8b	149.8c	145.2d	1.01	0.001	0.088	0.000
EE(g/kg)	44.4e	56.0d	72.4c	78.1b	96.8a	1.88	0.001	0.235	0.001
GE (MJ/kg DM)	17.6c	17.7c	17.8bc	18.1 _b	18.5a	0.37	0.0006	0.015	0.003
NDF(g/kg)	313.4b	291.8c	295.1c	337.5a	337.1a	2.05	0.0001	0.280	0.021
$\text{ADF}(\text{g/kg})$	88.7bc	83.6c	85.8c	95.4b	103.8a	0.82	0.0003	0.114	0.010
ADL(g/kg)	14.1c	19.1 _b	23.3a	26.4a	24.9a	0.50	0.0003	0.138	0.004
Mineral profile									
Calcium (g/kg)	7.1 _b	7.9a	8.3a	8.3a	8.2a	0.55	$-.0001$	0.191	0.011
Magnesium (g/kg)	2.4a	2.2 _b	2.1 _{bc}	2.1 _{bc}	2.0 _c	0.13	$-.0001$	0.030	0.001
Phosphorus (g/kg)	6.3a	5.5 _b	5.5 _b	5.5 _b	5.1 _c	0.42	$-.0001$	0.098	0.002
Potassium (g/kg)	7.90a	6.6 _b	6.2c	5.8d	5.1e	0.95	$-.0001$	0.212	0.004
Sodium (mg/kg)	243.2	255.4	239.8	233.8	227.3	24.4	0.6023	0.521	0.082
$\mathop{\text{Iron}}\nolimits$ (mg/kg)	161.5	169.8	149.5	143.3	135.5	23.8	0.2566	0.543	0.051
Copper(mg/kg)	73.3	58.4	58.2	60.2	59.8	8.6	0.0416	2.290	0.090
Manganese (mg/kg)	84.9a	73.8b	70.2 _b	84.6a	63.0c	9.4	$-.0001$	0.492	0.013
Zinc (mg/kg)	48.5a	44.8b	45.0 _b	42.2 _b	38.7c	3.7	$-.0001$	0.323	0.011
Cobalt (mg/kg)	0.30a	0.26ab	0.28ab	0.29a	0.23 _b	0.03	0.0230	0.0003	0.0001
Molybdenum (mg/kg)	0.45a	0.35 _b	0.31c	0.26d	0.21e	0.08	$-.0001$	0.022	0.0003

Table 3 Nutrient and mineral profile of experimental diets containing amarula nut cake at varying levels $(n = 3)$

Values with different letters (a-d) within a row are different. Treatments: control=no ANC inclusion; 50 $g/kg = 50 g/kg$ inclusion of ANC; 100 g/kg = 100 g/kg inclusion of ANC; 150 g/kg = 150 g/kg inclusion of ANC; 200 g/kg = 200 g/kg inclusion of ANC

SEM standard error mean, control no amarula nut cake, ADF acid detergent fibre, ADL acid detergent lignin, CP crude protein, DM dry matter, EE ether extract, GE gross energy, NDF neutral detergent fibre

Amino acid	Treatment						P value			
	Control	50 g/kg	100 g/kg	150 g/kg	200 g/kg	SEM	Treatment	Linear	Quadratic	
Essential amino acids (g/kg)										
Arginine	13.4 _b	13.4 _b	14.0ab	13.7 _b	14.3a	0.17	0.0004	0.002	0.182	
Histidine	4.9b	5.2a	5.0ab	4.4c	3.4d	0.05	$-.0001$	0.010	0.001	
Isoleucine	6.4	6.2	4.7	5.3	5.1	0.54	0.284	0.134	0.168	
Leucine	12.3a	12.4a	12.0a	11.6 _b	10.7 _b	0.19	0.0011	0.003	0.001	
Lysine	22.3a	20.6 _b	18.6c	15.1e	17.8d	0.15	$-.0001$	0.071	0.002	
Methionine	2.5	2.6	2.5	2.5	2.2	0.11	0.174	0.003	0.089	
Phenylalanine	7.0a	7.1a	6.8a	6.1 _b	6.0 _b	0.024	0.0272	0.001	0.066	
Threonine	6.2a	5.6b	5.3 _c	4.9d	4.3 _e	0.007	$-.0001$	0.010	0.254	
Tryptophan	2.3	$2.2\,$	$2.2\,$	8.5	4.9	0.190	0.2143	0.167	0.153	
Valine	7.9a	7.4a	7.4a	6.7 _b	6.5 _b	0.015	0.0004	0.011	0.074	
Non-essential amino acids (g/kg)										
Alanine	0.75a	0.73 _b	0.71c	0.68d	0.63 _e	0.010	$-.0001$	0.002	0.0002	
Aspartic acid	1.43a	1.29 _b	1.19c	1.08d	0.98e	0.004	$-.0001$	0.032	0.0002	
Glutamic acid	3.04a	2.92c	2.95 _b	2.93c	2.80d	0.004	$-.0001$	0.014	0.251	
Glycine	0.86a	0.77 _b	0.77 _b	0.71c	0.67d	0.003	$-.0001$	0.012	0.092	
Ho-proline	0.05c	0.06 _b	0.07a	0.04d	0.04d	0.002	$-.0001$	0.002	0.0002	
Proline	0.94a	0.90 _b	0.88 _b	0.88 _b	0.83c	0.008	$-.0001$	0.004	0.145	
Serine	0.83a	0.77 _b	0.75c	0.70d	0.66e	0.003	$-.0001$	0.013	0.162	
Tyrosine	0.50a	0.62 _b	0.67a	0.51a	0.44d	0.006	$-.0001$	0.030	0.002	

Table 4 Amino acid composition of the experimental diets containing varying levels of amarula nut cake (*n***= 3)**

Means with different letters (a-d) within a row differ significantly ($P < 0.05$). Treatments: control=no ANC inclusion; 50 g/kg=50 g/kg inclusion of ANC; 100 g/kg = 100 g/kg inclusion of ANC; 150 g/kg = 150 g/kg inclusion of ANC; 200 g/kg = 200 g/kg inclusion of ANC SEM standard error mean, control no amarula nut cake

Parameters	Treatment						P value		
							Control 50 g/kg 100 g/kg 150 g/kg 200 g/kg SEM Treatment Linear Quadratic		
IBW (kg)	21.93	21.40	21.98	21.63	21.83	0.46	0.89	0.132	0.741
FBW (kg)	62.30a	65.28a	61.40a	50.98b	54.77b	1.74	$-.0001$	0.312	0.088
BWG (kg)	40.37a	43.88a	39.42a	29.35b	32.93 _b	1.67	$-.0001$	0.283	0.072
\overline{ADG} (kg)	0.64a	0.70a	0.63a	0.47 _b	0.52 _b		$0.026 \le 0.001$	0.004	0.067
$ADFI$ (kg)	1.71	1.67	1.54	1.50	1.58	0.073	0.23	0.082	0.105
FCR (kg/kg)		2.70abc 2.41c	2.50 _{bc}	3.25a	3.04ab	0.16	0.004	0.017	0.069

Table 5 Effects of varying dietary inclusion levels of amarula nut cake on growth performance of pigs $(n = 6)$

Means with different letters (a-c) within a row differ significantly ($P < 0.05$). Treatments: control = no ANC inclusion; 50 g/kg = 50 g/kg inclusion of ANC; 100 g/kg = 100 g/kg inclusion of ANC; 150 g/kg = 150 g/kg inclusion of ANC; 200 $g/kg = 200 g/kg$ inclusion of ANC

SEM standard error mean, control no amarula nut cake; ANC amarula nut cake; IBW initial body weight, FBW final body weight, BWG body weight gain, ADG average daily gain, ADFI average daily feed intake, **FCR** feed conversion rate

Table 6 Effects of varying dietary inclusion levels of amarula nut cake on nutrient digestibility by pigs $(n = 6)$

Parameters	Treatment			P value					
	control	50 g/kg	$100 \frac{\text{g}}{\text{kg}}$	150 g/kg	200 g/kg	SEM	Treatment Linear		Ouadratic
DM%	92.4	86.4	87.0	88.3	91.9	2.61	0.3765	1.07	0.051
$CP\%$	86.4a	85.9a	81.6b	80.2bc	79.4c	0.54	$-.0001$	0.334	0.031
EE %	67.5b	67.8b	69.7b	76.8ab	79.7a	2.51	0.005	0.011	0.032
aNDF $%$	58.9c	61.6c	64.2bc	68.6ab	71.5a	1.81	0.001	0.503	0.010
ADF%	49.3c	54.8b	57.3ab	62.4a	60.1ab	1.50	0.0002	1.272	0.043
ADL%	46.6d	53.6c	58.4b	62.1a	63.1a	1.15	$-.0001$	1.561	0.044
Hemi %	68.1	72.2	73.4	74.7	75.3	2.41	0.387	0.721	0.024

Means with different letters (a-d) within a row differ significantly ($P < 0.05$). Treatments: control=no ANC inclusion; 50 g/kg=50 g/kg inclusion of ANC; 100 g/kg=100 g/kg inclusion of ANC; 150 g/ $kg = 150$ g/kg inclusion of ANC; 200 g/kg = 200 g/kg inclusion of ANC

SEM standard error mean, *control* no amarula nut cake, ANC amarula nut cake, ADF acid detergent fibre, ADL acid detergent lignin, CP crude protein, DM dry matter, EE ether extract, Hemi hemicellulose, NDF neutral detergent fibre

Warm and cold carcass weights, hind quarter length and hind quarter weight were reduced with increased (>150 g/kg) dietary ANC (Table 7). The warm and cold carcass temperature and pH, DP, back fat thickness, eye muscle area, drip loss, carcass length, rib weight and the water holding capacity were not affected by the dietary treatments. The redness (a*) and lightness (L^*) of the meat sample was improved (P < 0.05) with increased dietary inclusion of ANC, while the yellowness (b^*) was not affected by the dietary treatments (Table 8).

Parameters	Treatment					P value			
	Control	50 g/kg	100 g/kg	150 g/kg	200 g/kg	SD	Treatment	Linear	Quadratic
WCW (kg)	50.37a	50.32a	47.67a	42.45b	42.23b	2.24	0.028	0.322	0.008
CCW (kg)	48.97a	48.97a	46.42a	41.23b	40.97b	2.20	0.027	0.291	0.009
BF (mm)	16.50	14.17	14.67	15.17	15.50	1.43	0.820	0.323	0.015
Dressing %	68.17	69.17	69.17	68.17	68.50	0.42	0.254	0.134	0.007
CL (cm)	46.00	43.00	45.00	44.33	44.83	0.66	0.052	0.27	0.012
HOL (cm)	14.00a	12.33 _b	13.67ab	14.17a	13.67ab	0.39	0.029	0.061	0.004
HQW (kg)	23.33a	19.50b	22.83a	20.83ab	23.00a	0.80	0.009	0.372	0.019
RW (kg)	2.64	1.97	2.10	2.25	2.20	0.185	0.154	0.084	0.004
Warm muscle pH _i	6.48	5.95	6.16	5.93	5.99	0.15	0.092	0.060	0.021
Cold muscle pH _u	4.75	4.81	4.88	4.62	4.72	0.10	0.488	0.009	0.0007
Temp 45 min	32.73	32.80	32.28	33.15	33.70	1.43	0.996	0.014	0.001
Temp 24 h	8.53	8.80	8.45	9.82	9.28	0.58	0.449	0.04	0.0003
Drip loss $(\%)$	6.06	4.31	4.75	4.73	4.85	0.61	0.348	0.22	0.009
WHC ratio	0.41	0.31	0.33	0.29	0.28	0.05	0.502	0.01	0.0003
Eye muscle area	2548.8	2529.0	2677.3	2375.3	2276.2	166.2	0.483	20.82	1.74

Table 7 Effects of varying dietary inclusion levels of amarula nut cake on carcass characteristics of pigs $(n = 6)$

Means with different letters (a, b) within a row differ significantly ($P < 0.05$). Treatments: control=no ANC inclusion; 50 g/kg=50 g/kg inclusion of ANC; 100 g/kg = 100 g/kg inclusion of ANC; 150 g/kg = 150 g/kg inclusion of ANC; 200 g/kg = 200 g/kg inclusion of ANC

WCW warm carcass weight, CCW cold carcass weight, BF back fat thickness, CL carcass length, HQL hindquarter length, HQW hindquarter weight, RW rib weight, WHC water holding capacity, pH_i initial pH at 45 min, pH_u ultimate pH at 24 h

Table 8 Effect of varying dietary inclusion levels of amarula nut cake on meat colour of pig carcasses $(n = 6)$

Parameters Treatments							P value		
							Control 50 g/kg 100 g/kg 150 g/kg 200 g/kg SEM Treatment Linear Quadratic		
L*	49.9b	49.7b	49.9b	52.9a	51.9a		0.650 0.004	0.001	0.647
a^*	3.13	2.69	2.37	3.18	2.77		0.285 0.270	0.812 0.271	
$b*$		12.44bc 12.11c 11.83c		13.13a	13.06ab	0.219 0.001		0.003	0.017

Values with different letters (a-c) within a row are different. Treatments: control = no ANC inclusion; 50 $g/$ kg = 50 g/kg inclusion of ANC; 100 g/kg = 100 g/kg inclusion of ANC; 150 g/kg = 150 g/kg inclusion of ANC; 200 $g/kg = 200 g/kg$ inclusion of ANC

 L^* lightness, a^* redness, b^* yellowness

Discussions

It should be noted that the SBM used in the present study comes from the same batch with that used by Malebana et al. (2018). Although ANC has lower CP compared to SBM (Malebana et al., 2018), the CP of ANC is still advantageous to other conventional feed resources such sunflower meal that contain almost half or even lower CP content than the ANC. The increased fat and energy content with ANC inclusion in the diet is due to the fact that ANC contains higher residual oil and thus energy, but lower CP content compared to SBM (Mthiyane and Mhlanga, 2017; Malebana et al., 2018). Lysine is the first limiting amino acid in pigs and the ANC contains 6.8 g lysine/kg, which is comparable to the 8.8 g lysine/kg reported by Mthiyane and Mhlanga (2017) in ANC. However, the lysine content in ANC is lower than 31 g lysine/kg reported in SBM by Malebana et al. (2018).

Under adequate feeding and management systems, growing pigs are expected to have an average daily gain of 640 g/day (Payne, 1990). Pigs in the present study had ADG that meets this benchmark except for those that were fed diets that contained ≥ 150 g ANC/kg, which were lower than this benchmark. Results by Smit and Beltranena (2017) concurred with the findings of the current study when more than 150 g/kg of ANC was added to the pig diets. These researchers reported that dietary addition of 150 g/kg of Camelina oil cake (a byproduct with an oil content of between 100 and 200 g/kg and CP of $>$ 300 g/kg) (Pekel et al., 2015) decreased the ADFI, ADG and body weights (BWs) of growing pigs. Pig diets that contained 155 g/kg of moringa oleifera leaf meal as a replacement to SBM resulted in a lower ADFI due to increased dietary fibre (Ruckli and Bee, 2016). However, the present study shows that feed intake was not affected by the dietary treatments although the inclusion of ANC increased the dietary fibre in the treatments.

The lower ADG in pigs fed diets containing >150 g/kg of ANC might be attributed to the increased dietary fibre in the treatments, which increased energy requirements for body maintenance at the expense of growth (Agyekum et al., 2015). Mthiyane and Mhlanga (2017) reported the negative effects of ANC on growth performance of broiler chickens to be related to extensive lipid peroxidation of the ANC. Feeding of oxidised lipids has been reported to reduce daily gains, which leads to poor feed efficiency. Unfortunately, lipid peroxidation of ANC was not determined in the present study. It is well documented that pigs fed high fibre diets are heavier in relation to those fed low fibre diets (e.g. Thacker and Campbell, 1999). This could be because of the increased weight of the pigs' visceral organs and the gastrointestinal tract (Len et al., 2008). In contrast, the final body weights of pigs fed increased dietary inclusion levels of ANC was reduced, which might be related to the reduced CP digestibility and ADG by the pigs. According to the National Research Council (NRC, 1998), an intake of 2320 g/day is recommended for finishing pigs. Pigs in the present study had a daily feed intake (DFI) that ranged from 1500 to 1700 g/day, which is lower than the reported value. The low DFI by pigs in the present study could be attributed to the pigs' lower initial body weights and the different breed type than to those reported by the NRC (1998) for finishing pigs. Reduced ADFI by pigs fed the ANC diets was expected due to increased dietary fat compared to the control. This is because pigs often reduce intake as the dietary energy concentration increases (Liu et al., 2019). Surprisingly, the ADFI was not affected by the dietary treatments.

The digestibility of nutrients in a diet is mostly affected by the composition of the nutrients as well as ANFs in the nutrients. In most cases, dietary fibre has been reported to reduce nutrient digestibility in monogastric animals (e.g. pigs). Galassi et al. (2010) reported a trend towards a reduction in nutrient digestibility by pigs with increasing dietary fibre content. In addition, Landero et al. (2011) reported a linear reduction in diet nutrient digestibility values with increasing inclusion of canola meal, which was likely attributed to increased fibre content. Reduction of nutrient digestibility in diets with increased dietary fibre in this study was only apparent with the digestibility of CP, whereby diets containing ANC had reduced CP digestibility compared to the control. Woyengo et al. (2017) reported that plant-derived dietary protein sources contain anti-nutritional factors (ANFs) such as tannins, saponins, chelating agents, protease inhibitors, and phytohaemagglutintins. These ANFs interfere with nutrient digestion, absorption, and utilization (Akande et al., 2010). Since Amarula nut cake is a plant-derived protein source, it is likely to contain ANFs, which might have interfered with the digestibility of CP by the pigs in the present study. Our previous study (Malebana et al., 2018) reported 218 mg/100 g DM of phytate-phosphate in ANC, which is higher than 72 mg/100 g DM found in SBM. Phytate has been reported to depresses protein/amino acid

(AA) utilization in pigs due to the formation of complexes, which alter protein structure and in turn decrease protein solubility, enzymatic activity and proteolytic digestibility (Humer et al., 2015). Poor CP digestibility could affect the efficiency with which feed are converted to weight gain. Thus, the poor feed conversion ratio (FCR) in pigs fed diets with increased levels of ANC could be related to ANFs that might be present in the ANC. Wang et al. (2016) demonstrated that high residual oil in diets negatively impacts nutrient digestion and absorption, which was apparent in this study with pigs fed diets that contained high levels of ANC. The higher fibre digestibility observed for pigs fed diets containing higher levels of ANC could have resulted from fermentation of the fibre in the hind gut with the volatile fatty acids (VFAs) produced, contributing to the net energy requirements of the pigs (Mwesigwa et al. 2013).

High fibre diets have been reported to increase total empty weight of the gastrointestinal tract and the volume of digesta in the gut, resulting in lower carcass DP (Smit and Beltranena, 2017). Since the ANC diets contained high fibre compared with the control, it was expected that pigs fed these diets would have carcasses with a low DP. However, the DP was not affected by the dietary treatments. Feeding diets that contain high fibre might result in reduced carcass yield in pigs compared to those fed diets containing SBM, due to reduced dietary fibre (Ruckli and Bee, 2016). Consistently, the warm and cold carcasses of the pigs in the present study were reduced with diets that contained increased inclusion levels of ANC.

Back-fat thickness is usually affected by composition of the diet (Hernandez-Lopez et al., 2016). Apple et al. (2004) reported that higher dietary energy leads to increased back-fat thickness and reduced back fat depth reflecting a reduction in lipid deposition and brought about by decreased energy intake. Smit and Beltranena (2017) reported a decreased back fat thickness in pig carcass when Camelina oil cake was increased in the diet of growing pigs, likely because of feed aversion that caused reduced fat deposition. In contrast, the back-fat thickness of the carcass in the present study was not affected by the dietary treatments.

According to Kanengoni et al. (2014) drip loss in pork is affected by various and complex factors, which include, among other factors, the rate of pH decline and the ultimate pH. The ultimate pH of the pig carcasses in the present study was similar across treatments; hence, the drip loss percentage was not affected.

The redness (a^*) value of meat is related to the concentration of pigments and to the pH value, while the lightness (L^*) value is related to the moisture and fat contents of the carcass, and is also affected by the pH of the carcass. Increasing ANC in the diet improved both the redness and lightness of the meat, while the yellowness of the meat was not affected. The average values of the colour of the meat are consistent with the report of Temperan et al. (2014) who reported colour components $(L^*, a^*$ and $b^*)$ in meat from pigs of different breeds to be in the ranges of 44–58, 5–10 and 4–9, respectively.

Conclusions

This study showed that ANC inclusion levels below 150 g/kg can be used as a potential feedstuff in pig diets to partially replace SBM without causing any detrimental effects on the growth performance, nutrient digestibility and carcass traits. The inclusion levels of ANC above 150 g/kg in pig diets is accompanied by a reduction in feed intake and body weight gain. As with reduced BWG, weights of cold and warm carcasses of pigs fed diets with ANC above 150 g/kg were reduced. It is therefore concluded that ANC can partially replace SBM

in the diet of growing pigs at less than 150 g/kg inclusion level. Although the ANFs (in ANC) that could have negatively affected the pig weights were not assayed, further defatting of the cake in a form of chemical, solvent and or mechanical extraction could reduce not only the ANFs, but the residual oil of the cake for improved nutritional value prior to the cake being supplemented in pig diets. Expenditure amount of the cost of extraction could be compensated by the value of oil, which might also yield profit margins. Thus, further work to evaluate performance of pigs fed diets that contain defatted ANC should be done. Importantly, effects of the ANC oil fatty acids on the pig carcasses should be evaluated.

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Ethics approval

This study was approved by the ARC-AP Animal Ethics Committee on Animal Use of the Institute under protocol No: APAEC (2019/17).

Conflict of interest

The authors declare no competing interests.

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