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 × Landrace (LW × 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. Bodyweight, feed intake, average daily gain (ADG) and feed conversion ratio (FCR) were recorded weekly throughout the 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 twelve hours, weighed, slaughtered, and carcass samples were collected for analysis. Feed intake was not affected by dietary treatment, but ADGs were reduced at ANC levels > 15%, resulting in poor FCR. Protein digestibility was reduced at ANC levels > 15%, while ether extract and fibre levels increased. Warm and cold carcass weights were lower at ANC levels > 15, with improved meat redness and lightness. It was concluded that ANC could replace SBM in the diet of growing pigs at less than 15% inclusion level.

Introduction

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). The majority (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. Consequently, researchers in animal nutrition have attempted to identify protein source alternatives to SBM to reduce feed costs. However, performance results of pigs from dietary replacement of SBM with other protein sources are variable. Some researchers (e.g. Choi et al., 2015) reported reduced pig growth performance while others did not report any significance when SBM was substituted in the diet (Florou-Paneri *et al*., 2014). This depends mainly on the balance or imbalances of amino acids in the diets (McDonald et al., 2010) and the presence of anti-nutritional factors (ANFs) in the diet (Choi et al., 2015) when SBM is replaced with other crude protein (CP) sources.

The banning of 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. Due to the shortage in local production and increasing imports of SBM, the possibility of replacing SBM with other plant protein sources from local oil production, can contribute to sustainable pig production. It is thus 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). Research has shown that ANC contains crude protein (CP) that ranges from 325 to 470 g/kg dry matter (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 EE/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).

Previous studies showed that ANC could be a good source of CP in diets for ruminants (Mlambo et al., 2011, Mdziniso et al., 2016) and for poultry (Mthiyane and Mhlanga, 2017, Mazizi *et al*., 2019) without adverse effects on animal performance. According to Mthiyane and Mhlanga (2017) and Mazizi *et al*. (2019) one of the benefits for dietary 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. 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. The ANC nutrient composition was analysed at ARC-Irene Campus, and was used to formulate the five experimental diets (Table 1) that contained ANC at 0 (control), 50, 100, 150 and 200 g/kg DM; 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. The thirty LW × LR pigs with an average body weight of 20 ± 5 kg were randomly allocated to the experimental diets (treatments) in a completely randomized block design. The pigs were housed individually in 1.54 × 0.8 m pens in environmentally controlled houses with temperatures ranging from 22 to 25°C. The pigs were weighed once every week. The feeders were adjusted twice each day to ensure constant access to fresh feed and minimize any possible wastage. The pigs also had free access to water.

Nutrient digestibility

A week after completion of the growth study, digestibility study carried out. In brief, chromic oxide was mixed with the treatments at a concentration of 3 g chromic oxide/kg feed as an indigestible marker as described by Moughan et al. (1991). The 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, crude protein (CP), gross energy (GE), ash, EE and fibre fractions [neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL)] following the standard procedures of AOAC (2003).

Animal processing and carcass measurements

At the end of the nutrient digestibility study, pigs were fasted for 12 hours 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 220V and 1.8A with a current flow for 6s and exsanguinated within 10s 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 (pH_i) 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 hours, after which cold carcass weight (CCW) of each carcass was measured. After determination of cold carcass weight, cold carcass temperature and pH (pH_u) 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 weight to determine the hindquarter weight (HQW). The hid 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 hrs at 2°C. After 24 hours, 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 × 100.

Meat colour determination

Meat colour was measured using a Minolta colour meter (Model CR200, 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 wherein 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 1h 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 SAS version 9.4 statistical software (SAS, 2016).

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

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_j is the effect of the j-th block replicate, and ε_{ij} is the residual error.

Results

Increasing dietary inclusion of ANC increased (P0<05) the ether extract, energy and fibre while reducing the CP contents of the diets (Table 4). Inclusion of ANC reduced compositions of lysine and threonine in the diets (Table 5). The ADFI (average daily feed intake) was not affected by dietary treatments (Table 6). However, the ADG and BWG (body weight gain) were reduced with more than 15 % dietary inclusion of ANC, which further resulted in a poor FCR. The digestibility of DM was not affected by the dietary treatments (Table 7). However, the digestibility of CP was reduced while that of EE and fibre was improved with increased (> 15 %) dietary ANC.

Warm and cold carcass weights were reduced with increased (> 15 %) dietary ANC (Table 8). The warm and cold carcass temperature and pH, DP, back fat thickness, eye muscle area, drip loss 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 9).

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 (Table 1) is still advantageous to other conventional feed resources such as maize and 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 (Table 4) is due to the fact that ANC contains higher residual oil and thus energy, but lower CP content compared to SBM (Malebana et al., 2018). Lysine is the first limiting amino acid in pigs and the ANC contains 6.8 g lysine/kg (Table 2), which 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/d (Payne, 1990). Pigs in the present study had ADG that meets this benchmark except for those that were fed diets that contained > 15 % ANC, which were lower than this benchmark. In a study by Smit and Beltranena (2017), dietary addition of 15 % of Camelina cake (a by-product with an oil content of between 10 and 20 % and CP of > 30 %) (Pekel et al., 2015) decreased the ADFI, ADG and body weights (BW) of growing pigs. Results by Smit and Beltranena (2017) concurred with the findings of the current study when more than 15 % of ANC is added to the pig diets. Pig diets containing 15.5 % of moringa oleifera as a replacement to SBM resulted in a lower ADFI due to increased dietary fibre (Ruckli and Bee, 2016). Results in this study show 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 > 15 % 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 gastro-intestinal tract (Len et al., 2008). In contrast, the final body weights of pigs fed increased dietary inclusion levels of ANC was reduced (Table 6), 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/d is recommended for finishing pigs. Pigs in the present study had a daily feed intake (DFI) that ranged from 1500 to 1700 g/d, 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 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 fiber 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. 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 (Table 7) 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 (Table 8). 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 reflects a reduction in lipid deposition brought by decreased energy intake. Smit and Beltranena (2017) reported a decreased back fat thickness in pig carcass when Camelina 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.

Data on the effects of dietary ANC inclusion on meat colour is shown in Table 9. The redness (a*) value 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 could be used as a potential feedstuff in pig diets without causing any detrimental effects on the growth performance, nutrient digestibility and carcass traits of pigs. However, the ANC inclusion levels above 15 % in pig diets increased FCR, thus reduced BWG in the pigs. As with reduced BWG, weights of cold and warm carcasses of pigs fed diets with ANC above 15 % were reduced. It is therefore concluded that ANC can partially replace SBM in the diet of growing pigs at less than 15 % 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 extraction cost can be compensated by the cost of the extracted 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. In addition,

Declarations

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Author contribution

PMM and BDN conceived and designed research. PMM, MMR and BDN conducted experiments and analysed data. PMM wrote the first version of the manuscript. BDN, IMM, MMR and TTN revised and edited the final version of the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

Not applicable

Code availability

Not applicable

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 that they have no conflict of interests.

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Tables

Table 1
Nutrients and mineral composition of amarula nut meal (n = 3)

Parameters	ANC	SD
Nutrients composition		
DM (g/kg DM)	963.5	0.04
Ash (g/kg DM)	48.5	0.11
CP (g/kg DM)	322.9	0.05
EE (g/kg DM)	343.9	0.09
GE (g/kg DM)	25.3	0.01
NDF (g/kg DM)	244.0	0.46
ADF (g/kg DM)	223.2	0.10
ADL (g/kg DM)	98.1	0.69
Mineral composition		
Calcium (g/kg)	1.8	0.06
Magnesium (g/kg)	5.7	0.12
Phosphorus (g/kg)	9.4	0.24
Potassium (g/kg)	8.3	0.21
Sodium (mg/kg)	345.7	223.9
Iron (mg/kg)	95.5	13.3
Copper (mg/kg)	27.9	1.05
Manganese (mg/kg)	10.2	0.43
Zinc (mg/kg)	60.2	1.30
Cobalt (mg/kg)	0.14	0.04
Molybdenum (mg/kg)	0.31	0.01

ANC = Amarula nut cake; SD = standard deviation; ADF = Acid detergent fibre; ADL = Acid detergent lignin; CP = crude protein; DM = dry matter; EE = ether extract; GE = Gross Energy; NDF = Neutral detergent fibre.

Table 2
Amino acid composition of amarula nut cake (n = 3)

	g/kg	SD
Essential amino acids		
Arginine	50.5	0.04
Histidine	6.1	0.01
Isoleucine	12.9	0.01
Leucine	12.3	0.01
Lysine	6.8	0.02
Methionine	4.4	0.01
Phenylalanine	12.8	0.01
Threonine	8.1	0.03
Tryptophan	4.4	0.01
Valine	15.6	0.03
Non-essential amino acids		
Alanine	10.4	0.04
Aspartic acid	27.4	0.01
Glutamic acid	84.0	0.01
Glycine	18.0	0.01
Hydroxy-proline	0.9	0.01
Proline	11.3	0.02
Serine	16.5	2.72
Tyrosine	6.9	0.01

ANC = Amarula nut cake; SD = standard deviation.

Table 3
Experimental diets with varying inclusion levels of amarula nut cake

Ingredient (%)	**Treatment**				
	Control	5%	10%	15%	20%
Maize	54.51	69.47	69.75	63.60	60.98
Sunflower oil cake	15	-	-	-	-
Wheat bran	15	18.24	15.57	16.84	14.79
Sunflower Oil	2.64	-	-	-	-
Monocalcium	0.89	1.45	1.29	0.99	0.83
Feed lime	1.24	0.96	1.09	1.29	1.97
Lysine	1.70	4.65	2.10	2.06	1.23
ANC	-	5	10	15	20
Soybean oil cake	8.82	-	-	-	-
[a]Premix	0.20	0.20	0.20	0.20	0.20

ANC = Amarula nut cake; DM = dry matter; CP = crude protein; CF = crude fibre; DE = digestible energy.

[a]Provided the following per kilogramme of diet: 6500 IU vitamin A, 1200 IU vitamin, D_3, 40 IU vitamin E, 2mg vitamin K_3, 1–5 mg vitamin B_1, 4.5 mg vitamin B_2, 0.03 mg vitamin B_{12}, 2.5 mg vitamin B6, 25mg niacin, 12mg 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.

Table 4
Nutrient and mineral profile of experimental diets containing amarula nut cake at varying levels (n = 3)

Parameters	Treatment						*P*-value		
	Control	5 %	10 %	15 %	20 %	SEM	Treatment	Linear	Quadratic
Nutrient composition									
DM (g/kg)	956.8^{b}	958.9^{a}	954.4^{c}	953.8^{c}	956.3^{b}	0.19	< .0001	-0.039	0.001
Ash (g/kg)	51.2	50.1	47.7	47.0	48.8	0.20	0.0687	-0.058**	0.002*
CP (g/kg)	171.0^{a}	164.6^{b}	162.8^{b}	149.8^{c}	145.2^{d}	1.01	< .0001	-0.088***	-0.00
EE (g/kg)	44.4^{e}	56.0^{d}	72.4^{c}	78.1^{b}	96.8^{a}	1.88	< .0001	0.235***	0.001
GE (MJ/kg DM)	17.6^{c}	17.7^{c}	17.8^{bc}	18.1^{b}	18.5^{a}	0.37	0.0006	-0.015***	0.003**
NDF (g/kg)	313.4^{b}	291.8^{c}	295.1^{c}	337.5^{a}	337.1^{a}	2.05	< .0001	-0.280***	0.02**
ADF (g/kg)	88.7^{bc}	83.6^{c}	85.8^{c}	95.4^{b}	103.8^{a}	0.82	0.0003	-0.114***	0.010***
ADL (g/kg)	14.1^{c}	19.1^{b}	23.3^{a}	26.4^{a}	24.9^{a}	0.50	0.0003	0.138***	-0.004**
Mineral profile									
Calcium (g/kg)	7.1^{b}	7.9^{a}	8.3^{a}	8.3^{a}	8.2^{a}	0.55	< .0001	0.19***	-0.01***
Magnesium (g/kg)	2.4^{a}	2.2^{b}	2.1^{bc}	2.1^{bc}	2.0^{c}	0.13	< .0001	-0.03***	0.001*
Phosphorus (g/kg)	6.3^{a}	5.5^{b}	5.5^{b}	5.5^{b}	5.1^{c}	0.42	< .0001	-0.09***	0.002
Potassium (g/kg)	7.90^{a}	6.6^{b}	6.2^{c}	5.8^{d}	5.1^{e}	0.95	< .0001	-0.21***	0.004***
Sodium (mg/kg)	243.2	255.4	239.8	233.8	227.3	24.4	0.6023	0.52	-0.08
Iron (mg/kg)	161.5	169.8	149.5	143.3	135.5	23.8	0.2566	-0.54*	-0.05
Copper (mg/kg)	73.3	58.4	58.2	60.2	59.8	8.6	0.0416	-2.29*	0.09*
Manganese (mg/kg)	84.9^{a}	73.8^{b}	70.2^{b}	84.6^{a}	63.0^{c}	9.4	< .0001	-0.49*	-0.01
Zinc (mg/kg)	48.5^{a}	44.8^{b}	45.0^{b}	42.2^{b}	38.7^{c}	3.7	< .0001	-0.3***	-0.01
Cobalt (mg/kg)	0.30^{a}	0.26^{ab}	0.28^{ab}	0.29^{a}	0.23^{b}	0.03	0.0230	0.0003*	-0.0001

$^{a-d}$ Values with different superscripts within a row are different; SEM = standard error mean; Level of significance (*P < 0.05; **P < 0.01; ***= P < 0.001); control = no amarula nut meal; ADF = acid detergent fibre; ADL = acid detergent lignin; CP = crude protein; DM = dry matter; EE = ether extract; GE = gross energy; NDF − neutral detergent fibre.

Parameters	Treatment						P-value		
	Control	5 %	10 %	15 %	20 %	SEM	Treatment	Linear	Quadratic
Molybdenum (mg/kg)	0.45[a]	0.35[b]	0.31[c]	0.26[d]	0.21[e]	0.08	< .0001	-0.02***	0.0003**

[a–d] Values with different superscripts within a row are different; SEM = standard error mean; Level of significance (*P < 0.05; **P < 0.01; ***= P < 0.001); control = no amarula nut meal; ADF = acid detergent fibre; ADL = acid detergent lignin; CP = crude protein; DM = dry matter; EE = ether extract; GE = gross energy; NDF − neutral detergent fibre.

Table 5

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

Amino acid (g/kg)	**Treatment**						***P*-value**		
	Control	**5%**	**10%**	**15%**	**20%**	**SEM**	**Treatment**	**Linear**	**Quadratic**
Essential amino acids									
Arginine	13.4^{b}	13.4^{b}	14.0^{ab}	13.7^{b}	14.3^{a}	0.17	0.0004	0.002**	NS
Histidine	4.9^{b}	5.2^{a}	5.0^{ab}	4.4^{c}	3.4^{d}	0.05	< .0001	0.01***	-0.001***
Isoleucine	6.4	6.2	4.7	5.3	5.1	0.54	0.284	NS	NS
Leucine	12.3^{a}	12.4^{a}	12.0^{a}	11.6^{b}	10.7^{b}	0.19	0.0011	0.003***	-0.001**
Lysine	22.3^{a}	20.6^{b}	18.6^{c}	15.1^{e}	17.8^{d}	0.15	< .0001	-0.07***	0.002**
Methionine	2.5	2.6	2.5	2.5	2.2	0.11	0.174	0.003*	NS
Phenylalanine	7.0^{a}	7.1^{a}	6.8^{a}	6.1^{b}	6.0^{b}	0.024	0.0272	-0.001**	NS
Threonine	6.2^{a}	5.6^{b}	5.3^{c}	4.9^{d}	4.3^{e}	0.007	< .0001	-0.01***	NS
Tryptophan	2.3	2.2	2.2	8.5	4.9	0.190	0.2143	NS	NS
Valine	7.9^{a}	7.4^{a}	7.4^{a}	6.7^{b}	6.5^{b}	0.015	0.0004	-0.01***	NS
Non-essential amino acids									
Alanine	0.75^{a}	0.73^{b}	0.71^{c}	0.68^{d}	0.63^{e}	0.010	< .0001	-0.002***	-0.0002***
Aspartic acid	1.43^{a}	1.29^{b}	1.19^{c}	1.08^{d}	0.98^{e}	0.004	< .0001	-0.03***	0.0002**
Glutamic acid	3.04^{a}	2.92^{c}	2.95^{b}	2.93^{c}	2.80^{d}	0.004	< .0001	-0.01***	NS
Glycine	0.86^{a}	0.77^{b}	0.77^{b}	0.71^{c}	0.67^{d}	0.003	< .0001	-0.01***	NS
Ho-proline	0.05^{c}	0.06^{b}	0.07^{a}	0.04^{d}	0.04^{d}	0.002	< .0001	0.002**	-0.0002**
Proline	0.94^{a}	0.90^{b}	0.88^{b}	0.88^{b}	0.83^{c}	0.008	< .0001	-0.004***	NS
Serine	0.83^{a}	0.77^{b}	0.75^{c}	0.70^{d}	0.66^{e}	0.003	< .0001	-0.01***	NS
Tyrosine	0.50^{a}	0.62^{b}	0.67^{a}	0.51^{a}	0.44^{d}	0.006	< .0001	0.03**	-0.002***

$^{a-d}$ Values with different superscripts within a row are different; SEM = standard error mean; Level of significance (*P < 0.05; **P < 0.01; ***= P < 0.001); control = no amarula nut cake; NS = not significant.

Table 6
Effects of varying dietary inclusion levels of amarula nut cake on growth performance of pigs (n = 8)

Parameters	Treatment						*P*-value		
	Control	5%	10%	15%	20%	SEM	Treatment	Linear	Quadratic
IBW (kg)	21.93	21.40	21.98	21.63	21.83	0.46	0.89	NS	NS
FBW (kg)	62.30^{a}	65.28^{a}	61.40^{a}	50.98^{b}	54.77^{b}	1.74	< .0001	-0.31**	NS
BWG (kg)	40.37^{a}	43.88^{a}	39.42^{a}	29.35^{b}	32.93^{b}	1.67	< .0001	-0.28**	NS
ADG (kg)	0.64^{a}	0.70^{a}	0.63^{a}	0.47^{b}	0.52^{b}	0.026	< .0001	-0.004**	NS
ADFI (kg)	1.71	1.67	1.54	1.50	1.58	0.073	0.23	NS	NS
FCR	2.70^{abc}	2.41^{c}	2.50^{bc}	3.25^{a}	3.04^{ab}	0.16	0.004	-0.017**	NS

$^{a-c}$ Values with different superscripts within a row are different, *P < 0.05; **P < 0.01; ***P < 0.001; NS = not significant; 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 7
Effects of varying dietary inclusion levels of amarula nut cake on nutrient digestibility by pigs (n = 8)

Parameters (%)	Treatment						*P*-value		
	control	5%	10%	15%	20%	SEM	Treatment	Linear	Quadratic
DM	92.4	86.4	87.0	88.3	91.9	2.61	0.3765	-1.07	0.05*
CP	86.4^{a}	85.9^{a}	81.6^{b}	80.2^{bc}	79.4^{c}	0.54	< .0001	-0.33***	0.03**
EE	67.5^{b}	67.8^{b}	69.7^{b}	76.8^{ab}	79.7^{a}	2.51	0.005	-0.01***	0.03
aNDF	58.9^{c}	61.6^{c}	64.2^{bc}	68.6^{ab}	71.5^{a}	1.81	0.001	0.50***	0.01
ADF	49.3^{c}	54.8^{b}	57.3^{ab}	62.4^{a}	60.1^{ab}	1.50	0.0002	1.27***	-0.04*
ADL	46.6^{d}	53.6^{c}	58.4^{b}	62.1^{a}	63.1^{a}	1.15	< .0001	1.56***	-0.04**
Hemi	68.1	72.2	73.4	74.7	75.3	2.41	0.3872	0.72*	-0.02

$^{a-d}$ Means with different superscripts within a row are different; SEM = standard error of means; Level of significance (*P < 0.05; **P < 0.01; ***= P < 0.001); 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.

Table 8
Effects of varying dietary inclusion levels of amarula nut cake on carcass characteristics of pigs (n = 8)

Parameters	Treatment						P value		
	Control	5%	10%	15%	20%	SD	Treatment	Linear	Quadratic
WCW (kg)	50.37^{a}	50.32^{a}	47.67^{a}	42.45^{b}	42.23^{b}	2.24	0.028	-0.32**	-0.008
CCW (kg)	48.97^{a}	48.97^{a}	46.42^{a}	41.23^{b}	40.97^{b}	2.20	0.027	-0.29**	-0.009
BF (mm)	16.50	14.17	14.67	15.17	15.50	1.43	0.820	-0.32	0.015
Dressing %	68.17	69.17	69.17	68.17	68.50	0.42	0.254	0.13	-0.007
CL (cm)	46.00	43.00	45.00	44.33	44.83	0.66	0.052	-0.27	0.012
HQL (cm)	14.00^{a}	12.33^{b}	13.67^{ab}	14.17^{a}	13.67^{ab}	0.39	0.029	-0.06	0.004
HQW (kg)	23.33^{a}	19.50^{b}	22.83^{a}	20.83^{ab}	23.00^{a}	0.80	0.009	-0.37	0.019
RW (kg)	2.64	1.97	2.10	2.25	2.20	0.185	0.154	-0.08	0.004
Warm muscle pH_i	6.48	5.95	6.16	5.93	5.99	0.15	0.092	-0.06*	0.02
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	-0.01	0.001
Temp 24hr	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

$^{a-b}$Values with different superscripts within a row are different; Level of significance (*P < 0.05; **P < 0.01; ***= P < 0.001); WCW = Warm carcass, CCW = Cold carcass, 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 45min, pH_u = ultimate pH at 24 h.

Table 9

Effect of varying dietary inclusion levels of amarula nut cake on meat colour of pig carcasses (n = 3)

Parameters	Treatments						*P*-value		
	Control	5%	10%	15%	20%	SEM	Treatment	Linear	Quadratic
L*	49.9^{b}	49.7^{b}	49.9^{b}	52.9^{a}	51.9^{a}	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*	12.44^{bc}	12.11^{c}	11.83^{c}	13.13^{a}	13.06^{ab}	0.219	0.001	0.003	0.017

$^{a-c}$ Values with different superscripts within a row are different, L* = lightness; a* = redness; b*= yellowness.