Influence of reduced dietary protein level on quality of pork carcasses in Windsnyer pigs

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Abstract

To promote the sustainable production of local pigs, their dietary protein requirements need to be determined. Meat production from these pigs when fed on appropriate diets, coupled with their adaptability to climatic extremes and disease and parasite challenges, could be of huge benefit to the pork industry. The objective of the study was to determine the carcass traits, primary pork cuts, and internal organ weights of pigs fed decreasing dietary protein levels. Thirty, slow-growing, Windsnyer male pigs were randomly allocated to six dietary treatments in a complete randomized design. There were five replications for each of the six treatments. Dietary crude protein levels in the six experimental diets were 193, 174, 154, 135, 116, and 97 g/kg, respectively. The diets were formulated to contain similar net energy levels of ~9.5 MJ/kg. Lysine, methionine, threonine, and tryptophan levels were the same for all diets. A two week adaptation was followed by an 8w feeding phase. At slaughter, pigs had an average weight of ~39.13±0.85 kg. Pigs were humanely slaughtered at the end of feeding period to determine carcass characteristics, primary pork cuts, and internal organ size. A negative linear relationship was observed between protein levels and cooler shrink. There was a positive linear relationship between protein level and dressing percentage, cooler shrink, and shoulder fat. There was a quadratic relationship between dietary protein level and shoulder fat, ham diameter, P2(3) backfat depth, and kidney weight. The thickness of dorsal fat at the last rib, the thickness of back fat, and the width of back fat at P2(2) increased linearly as protein level decreased. The reduction in dietary protein level had an influence on carcass traits, primal pork cuts, and internal organs in slow-growing Windsnyer pigs. A reduction in dietary protein level below 116 g/kg compromised ham diameter, P2(3) width of back fat thickness, shoulder fat, and kidney weight.

Introduction

African countries have a large number of local pigs (Halimani *et al*., 2012). The Kolbroek and Windsnyer pigs are commonly found in South Africa, Swaziland, Lesotho, Malawi, and Zimbabwe. These slow-growing pigs face extinction, and there is a need to conserve them through sustainable use (Hlatini *et al*., 2020). Local pigs have high thermotolerance, low feed requirements, low labour requirement, and are adapted to endemic parasites. These attributes are beneficial to resource-limited farmers (Kanengoni *et al*., 2004; Halimani *et al*., 2012). Slow-growing indigenous pigs are rejected by mainstream economies, but they have the potential to alleviate poverty in rural communities and improve human dietary nutrition (Halimani *et al*., 2012). The human demand for a good source of protein and their responsiveness to the healthy meat consumption are other factors influencing the need to improve the production of local pigs. The intense demand for pork recently has put pressure to increase pork production in South Africa. The strong demand for pork implies a desperate need for protein-rich feedstuffs to meet a potential growth rate of pigs. However, conventional protein-rich feedstuffs are prohibitively expensive and not always available (Pham *et al*., 2010; Ncobela *et al*., 2018). This is due to climate change and competition with human and biofuels. The commercial pig industry in South Africa prioritises fast-growing breeds with high feed

conversion rates such as the Large White. The pork industry only relies on fast-growing imported pigs (Wood *et al.*, 2004). These breeds, however, have high feeding requirements and need high inclusion levels of specific nutrients, particularly protein (NRC, 2012). There is a need to reconsider harnessing local pigs such as Windsnyer pigs, which may require less protein needs than fast-growing pigs.

The Windsnyer is a slow-growing pig indigenous to southern Africa and is adapted to survive under low planes of nutrition; they have the ability to reproduce at low dietary protein levels. Indigenous pigs are avoided in pork production due to their low growth rate and high feed conversion ratio (Ncobela *et al.*, 2018). As a result, South Africa opt to imports pork every year instead of paying attention on the commercial value of Windsnyer pigs. This occurrence could change by including traits from indigenous pigs through crossbreeding. Indigenous pigs have been used to produce pork for fresh consumption (Hoffman *et al.*, 2005). Hence, improving the performance and pork quality of indigenous pigs to meet the market expectations is necessary. Lean meat is in high demand among consumers as an excellent addition to a healthy diet (Peres *et al.*,2014). The challenge in the pig industry is to improve the nutritional value, quality of pork, and meet consumer demand. To improve economic meat production in indigenous pigs, the correct supply of nutrients is critical, and should not exceed or be below the requirement.

Dietary protein is the most important and expensive nutrient in pig diets (Whittemore *et al.*, 2001). The rate of protein and lipid deposition is largely determined by the ability of the pig to grow lean tissue and the protein to energy ratio of the diet (Ruusunen *et al.*, 2007). In improved pig genotypes, reduced dietary protein levels enhance carcass traits, pork quality, and pork cuts (Alonso *et al.*, 2010). Estimated protein levels for slow-growing pigs are valuable in ration formulation because maximizing the dietary content of crude protein is a viable strategy to increase profitability. It has been shown that dietary protein content can be reduced from 193 g/kg to 135 g/kg without affecting the nitrogen balance and growth potential of growing Windsnyer pigs (Hlatini *et al* 2020; 2021). Reports on the relationship between decreasing levels of protein and pork quality traits in slow-growing pigs are sparse. Zhang *et al.* (2008) reported that reduced protein levels were associated with more intramuscular fat and greater meat tenderness. Determining the relationship between dietary protein level and pork characteristics would help to identify the threshold value for protein. To determine the optimal protein level in pigs, there is need to examine the relationship between crude protein and carcass traits. Optimising protein levels for slow-growing pigs is a viable strategy for increasing profitability of pig enterprises. For the slow-growing Windsnyer pig, the protein requirements for growth have not been accurately estimated, as is the case for improved pig genotypes (NRC, 2012). In addition to Windsnyer growth performance (Hlatini *et al.*, 2021) and nitrogen balance (Hlatini *et al.*, 2020), carcass traits and meat quality needs to be understood because incorrect supply of some nutrients such as protein, could increase fat deposition (Monteiro *et al.*, 2017; Nørgaard *et al.*, 2014). Data on the effect of a decreasing protein level on carcass characteristics and primal pork cuts in the slow-growing Windsnyer pigs is not available. Such information can assist in reducing fat deposition while meeting protein requirements for Windsnyer pigs. The objectives of the current study were to determine carcass traits, primary pork cuts, and internal organ weight responses to decreasing dietary protein levels. It was hypothesized that decreasing the dietary protein level would not affect carcass traits, primary pork cuts, and internal organ weight in native Windsnyer pigs until a certain inclusion level of dietary protein where these variables would be negatively affected.

Materials and Methods

The study was conducted at the Agricultural Research Council (ARC), Animal Production Institute, Irene, South Africa. The institute lies at 25°34′0″ S; 28°22′0″ E and is ~1526 m above sea level. The average annual temperature during the period of the study was 20.7 °C. The care and use of experimental animals was according to the approved standards of the Animal Ethics Committee of the Agricultural Research Council–Animal Production Institution (Reference number APIEC 17/12)

The experimental diets contained 193, 174, 154, 135, 116, and 97 g/kg crude protein. The six diets were formulated to contain similar net energy values of ~9.5 MJ/kg. All diets were supplemented with synthetic lysine, methionine, threonine, and tryptophan levels to match the content of the control diets. Diets were allocated randomly to pens and each pig was treated as an experimental unit in a complete, randomized design. There were five pigs per treatment. Pigs were fed experimental diets in mash form for 8 w during the rainy season.

The control diet containing the NRC (2012) recommended CP (193 g/kg) for growing pigs was formulated. Low protein diets were formulated by reducing the CP content through partial substitution of soybean meal by yellow maize and wheat bran. The experimental diets were formulated to meet and exceed NRC requirements for all other nutrients for growing pigs (NRC, 2012). Diets for the current study, were formulated using the software program Bestmix® (Adifo, Belgium). The major ingredients used in the diets included yellow maize, soybean meal, wheat bran, sunflower oil, limestone, monocalcium phosphate,

salt, lysine HCL, methionine, threonine, tryptophan, and a mineral–vitamin premix (Table 1). Diet samples were taken from all the feeders per dietary treatment three days after the beginning and three days before the end of the experiment and kept in a cool dry place until they were homogenised, subsampled, and prepared for chemical composition analysis. The composition and chemical analyses of the different diets are given in Table 1.

The dry matter, ash, crude protein, and ether extract were determined using AOAC (1990) Official Methods 945.15, 942.05, 979.09, and 920.39, respectively. Nitrogen content was determined using the Dumas Combustion method in a Leco Truspec Nitrogen Analyser (St Joseph MI, USA). Crude protein was then calculated as N × 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using ANKOM Fibre Analyser (Ankom Macedon, NY, USA), according to Van Soest (1973) and Van Soest *et al.* (1991). Samples for mineral analyses were ashed at 450 °C overnight (Abdou *et al.*, 2011). The bulk density was determined according to the method described by Kyriazakis and Emmans (1995).

Table 1: Ingredient and chemical composition of diets used for growers

Ingredient (g/kg)	Levels of crude protein (g/kg DM)					
	193	174	154	135	116	97
Yellow maize	602	633	688	725	767	728
Soya oil cake	273	208	171	117	75.0	50.0
Wheat bran	85.0	120	100	120	120	185
Limestone	13.0	12.5	12.1	13.0	13.5	11.0
Monocalcium phosphate	6.0	6.0	4.0	4.0	4.0	5.0
Oil-Sunflower	5.0	5.0	5.0	5.0	5.0	5.0
Salt	5.0	5.0	5.0	5.0	5.0	5.0
Lysine HCL	3.8	3.8	3.8	3.8	3.8	3.8
Threonine	1.1	1.1	1.1	1.1	1.1	1.1
Methionine	1.1	1.0	1.0	1.0	1.0	1.0
Tryptophan	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin-mineral premix	4.7	4.7	4.7	4.8	4.7	4.9
Calculated nutrient analysis						
Dry matter	884	883	882	880	879	881
Net energy value (MJ/kg)	9.76	9.78	9.92	10.1	10.1	9.99
Crude protein	192	169	153	137	117	100
Crude fibre	27.3	28.6	25.9	26.4	25.3	27.3
Ash	54.8	52.8	50.3	46.5	45.0	55.2
Fat	37.2	38.0	38.2	39.0	39.4	44.1
Laboratory chemical analysis						
Dry matter	882	882	876	875	875	876
Crude protein	202	177	161	145	121	103
Crude fat	27.2	21.7	22.0	31.2	27.2	24.1
Neutral detergent fibre	142	174	158	173	199	197
Acid detergent fibre	44.2	50.5	47.2	47.8	45.3	46.5
Ash	46.7	46.2	40.6	30.6	44.9	39.4

Thirty, clinically healthy, slow-growing Windsnyer pigs, with an initial body weight of 23 ± 0.622 kg were randomly allocated into individual pens measuring 1.5 × 1 m^2. The animals were ear tagged and housed in a room with artificial heating, lighting, and proper ventilation systems. The house temperature and humidity were maintained at 21.9 ± 2.24 °C and 45.2 ± 6.85%, respectively. A 12 h dark–12 h artificial light cycle was applied. Each pen was provided with water through a low-pressure nipple drinker and feed was supplied in a self-feeder trough. Feed and water were supplied *ad libitum*. The pigs received no antibiotics or growth promoters.

At the end of the experiment, Windsnyer pigs were transported to the processing plant, located 1.5 km from the pig trial facility. Each pig was weighed to determine slaughter weight. Windsnyer pigs were weighed 8 h before transportation after being feed fasted for 8 h, but with *ab libitum* access to clean water. The final live body weight at slaughter was 39.13 ± 0.85 kg. Pigs were gently driven from the pens to slaughter. At the entrance, pigs were electrically stunned with electrodes at the base of each ear and

exsanguinated using a thoracic puncture. Afterwards Windsnyer pigs were bled and processed following the standard procedures of the slaughterhouse. After dehairing and evisceration, warm carcass weight was measured after dressing using an overhead scale. The dressing percentage was calculated by taking the warm carcass weight as a percentage of slaughter weight. The carcasses were then driven to a chilling room set at 2–4 °C for 24 h, after which cold carcass weights were measured. Cooler shrink, which is the amount of water lost from a carcass in the first 24–48 h after harvest (Schweihofer, 2011), was calculated using the following formula:

Cooler shrink (%) = (1 − (cold carcass weight / warm carcass weight) x100).

The pH of the homogenate of 3.0 g muscle was measured in 27 ml of deionized water, using a pH meter (MP230, Mettler Toledo, Switzerland).

After the heads and tails of each carcass were removed, the kidneys, kidney fat, glands, and remaining parts of the diaphragm were removed, as well as flare fat, kidneys, kidney fat, and remaining diaphragm. Using a carcass splitting bandsaw, carcasses were then divided along the median plan from the remaining sacral vertebra to the first cervical vertebra. A measuring tape was used to measure the length of the carcass from the first rib to the pubic bone in the median plane. Back fat measurements were taken at the first rib (dorsal fat thickness at first rib [DFT1]), last rib (dorsal fat thickness at last rib [DFT2]), and last lumbar vertebra (dorsal fat thickness at last lumbar vertebra [DFT3]) off the median plane cut surface. For all other carcass measurements, the left side was used. There was a cut between the 10th and 11th rib followed by a cut through the spinal column. Over the eye muscle, 60 mm from the carcass midline, Vernier callipers were used to measure the P2, P2(1) length, P2(1) width, P2(2) width and P2(3) width fat on each carcass. The cut interface yielded three measurements of the eye muscle length (EML) and three measures of eye muscle width (EMW). The following formula from Bruwer (1992) was used to compute the lean meat percentage:

Lean % = 72.511 − 0.418V + 0.0547S,

where V is the fat thickness (mm), and S is the muscle depth (mm).
The eye muscle area (EMA) was calculated using the equation of Zhang *et al.* (2007):
EMA (mm^2)= EML × EMW × 0.7,

where EML is measured in millimetres and EMW is the average of the three width measurements (mm) of the eye muscle.

A sample joint (measuring 2.5 cm thick and 16 cm long) along the surface of the rear of the eye muscle was cut off and weighed from the same cut where P2 values were taken. This sample joint was placed in a netlon bag and inserted into a small plastic bag, which was then secured in such a way that the sample joint did not come into contact with the bottom of the bag or air. They were then stored in a refrigerator between 0–5 °C for 24 h, after which the mass of the water lost was calculated from the weight of the water in the bag and was used to calculate drip loss. Following that, the primary cuts (shoulder, ham, and rib) were made. The shoulder was removed by cutting between the third and fourth ribs caudally and the junction of the caudal edge of the second rib with the sternum cranially, and the front trotter was removed by cutting through metacarpal region (at the joint of the carpal bones and the radius and ulna) and weighed to determined shoulder weight (SW). The rib was sliced along a parallel line 16 cm from the spinal cord midline ventrally, between the fourth and twelfth thoracic vertebrae dorsally. The rib weight (RW) was determined by weighing this.

The hind leg was removed between the second and third sacral vertebrae perpendicular to the stretched leg and at the hock joint distally and weighed to get the hindquarter weight (HQW). It was also measured to get the hindquarter length (HQL) from the ischiopubic symphysis to the hock joint and the hindquarter circumference (HQC), in maximum amplitude near the base of the tail. The RW, SW, and HQW were then each presented as a proportion of CCW to give RW proportion (RWP), SW proportion (SWP), and HQW proportion (HQWP), respectively. The selected primal pork cuts are important commercial pork cuts in South Africa. The weight of the heart, lungs, kidneys, liver, spleen, small intestine, large intestine, and stomach was recorded. The weights of the internal organs were expressed relative to the weight of each pig at slaughter.

A PROC GLM (SAS, 2008) procedure was used to examine the response of carcass traits, pork cuts, and internal organs to reduced dietary protein level. A polynomial regression (PROC REG) procedure (SAS, 2008) was used to determine the relationships between protein levels in the diet and carcass traits, pork

cuts, and internal organs. Least square means were compared using the probability difference (PDIFF) option in the LS Means statement of SAS (2008). The model that was used was:

$$Y = Bo + B_1A + B_2A^2 + E,$$

where Y = is the response variable (pork quality parameters), Bo= is the intercept, B_1A = is the linear regression component, B_2A^2 = is the quadratic regression component, and E = is the error.

A piecewise regression (broken stick) analysis using the PROC NLIN procedure in SAS was used to determine the optimal inclusion levels of dietary protein on carcass traits and pork cuts. A level of $P <0.05$ was set at the criterion for statistical significance. The piecewise regression model used was:

$$Y_i = y_{0 + } y_{1 + } y_2 (Ix_c) (x_i - x_c) + \varepsilon_i.$$

Using parameters (y_0, y_1, y_2) and the x_c, the two segmented simple regression functions were:

$$Y_j = y_0 + y_1 (x_i), \text{ for } x_i \le x_c; \text{ and}$$
$$Y_k = y_0 + (y_{1 + } y_2) x_i, \text{ for } x_i \ge x_c,$$

where Y_i is the response variable when crude protein inclusion is inhibiting carcass traits and pork cuts; Y_j is the response variable before crude protein in the diet inhibits carcass traits and pork cuts; Y_k is the response variable when crude protein in the diet exceeds the optimal level;

$Y_0 = y_0 - y_2 x_c$; when $x_i = 0$; y_0 is the intercept or minimum Y_i when $x_c < 0$; y_1 is the rate of change of Y_i when $x_i < x_c$; y_2 is the rate of increase in Y_i when $x_i > x_c$; x_i is the level of crude protein in the diet; x_c is the optimal level below which carcass traits and pork cuts are compromised by the decrease in the crude protein content in the diet; and Ix_c is a dummy variable with a value = 0 when $x_i < x_c$ and 1 when $x_i \ge x_c$.

Results

Table 2 shows the relationship between dietary protein level and pork traits. The relationship between protein content and warm carcass weight and cold carcass weight was not significant. A negative linear relationship was observed between protein content and cooler shrink in pigs ($P <0.01$). Cooler shrink decreased with dietary protein content ($P <0.01$). Protein content and dressing percentage in growing pigs were linearly related ($P <0.05$). As the protein content in the diet decreased, dressing percentage increased ($P <0.05$). A quadratic relationship between dietary protein content and shoulder fat was observed ($P <0.05$). As the protein content dropped, shoulder fat increased at a decreasing rate ($P <0.05$). There was no relationship between protein content and carcass length in pigs ($P >0.05$). Reduced protein had no significant influence on drip loss, lean percentage, eye muscle area, and ultimate pH.

The relationship between dietary protein content and primary pork cuts in Windsnyer pigs is shown in Table 3. There was a positive quadratic relationship between protein content and ham diameter ($P <0.05$). The ham diameter decreased at an increasing rate as the dietary protein content decreased ($P <0.05$). The reduction in dietary protein content had no effect on ham length, ham circumference, hind quarter mass, shoulder mass, and rib mass ($P >0.05$). There was a linear relationship between protein content and dorsal fat thickness at the last rib (P2) in pigs ($P <0.05$). As the dietary protein content decreased, dorsal fat thickness at the last rib (P2) declined. A negative linear relationship between protein content and P2 back fat thickness was observed ($P <0.01$). P2 back fat thickness decreased with protein content ($P <0.01$). A decreasing linear relationship was observed between protein content and P2(2) back fat depth width in pigs ($P <0.05$). The relationship between protein content and dorsal fat thickness at rib (f1), and dorsal fat thickness at the last lumbar vertebra (fat) was not significant in pigs ($P >0.05$). Dietary protein content did not influence P2(1) back fat depth length, P2(1) back fat depth width, and P2(2) back fat depth width ($P >0.05$). There was a quadratic relationship between dietary protein content and P2(3) back fat width ($P <0.05$). As the protein content in the pig diet decreased, P2(3) back fat depth width decreased at an increasing rate ($P <0.05$). Reducing the protein content in the diets had no influence on shoulder weight proportions, rib weight proportions, and hindquarter weight proportions ($P >0.05$).

Table 4 shows the relationship between dietary protein content and internal organ weight in slow-growing Windsnyer pigs. There was a quadratic relationship between protein content and kidney weight ($P <0.05$). As the protein content in the pig diet decreased, the kidney weight increased at a decreasing rate. Protein content was not related to heart weight, lung weight, spleen, liver weight, small intestine, large intestine, and stomach weight in pigs ($P >0.05$).

Table 5 shows the optimal levels below which the shoulder fat, ham diameter, and P2(3) width (back fat depth), together with kidney weight of growing Windsnyer pigs was compromised by low dietary protein content. The optimum protein content for shoulder fat of Windsnyer pigs was estimated to be 136.8 g/kg and the plateau was estimated at 24.7 mm ($P >0.05$). For ham diameter and P2(3) backfat depth width, the threshold levels of protein were estimated at 111.3 and 115.3 g/kg, respectively. Their plateau was

estimated at 44.6 cm and 24.8 mm (P <0.05), respectively. The optimum dietary protein content for kidney weight was estimated at 114.7 g/kg and the plateau was at 0.19 kg in Windsnyer pigs (P <0.05).

Discussion

Decreasing the dietary crude protein content led to an increase in the dressing percentage of slow-growing Windsnyer pigs. The observed linear relationship between protein content and dressing percentage may suggest well-muscled meat type breeds, because the heavier the pig, the better the dressing percentage. Castell *et al.* (1994) reported that increasing the protein content in the diet led to a decline in the dressing percentage. Reducing lysine and nutrient content for pigs at ~60 kg body weight causes a decrease in dressing percentage (Zhang *et al.*, 2008). Such findings suggest that low protein diets have adequate nutrient density. The linear relationship between protein content and cooler shrink suggests that at high protein contents, there is a larger water loss from the carcass than at a lower dietary protein content. The decrease in dietary protein content with cooler shrink could be associated with the increase in the full subcutaneous fat cover. Carcass with excess fat lose less water at chilling than trimmer carcasses (Apple *et al.*, 2017).

The carcass length depends more upon genetic selection than dietary specifications. Hence, it was expected that carcass length would not be affected by the dietary protein content. The carcass lengths are associated with genes because slow-growing Windsnyer pigs have a small body frame. Kanengoni *et al.* (2004) reported that the primary pork cuts are affected by the carcass length. The quadratic relationship between protein content and shoulder fat is difficult to explain. The decrease in protein content up to 136.8 g/kg caused changes in shoulder fat, after that, a further improvement in shoulder fat was not observed. Slow-growing Windsnyer pigs fed a fibre-rich diet responded linearly (Ncobela *et al.*, 2018). Reducing dietary protein caused changes resulted in a linear response on the right full side; the observed relationship can be explained by the dressing percentage and cooler shrink outcomes.

A quadratic relationship with protein content caused changes; ham diameter was attributed to shoulder yields. At 111.3 g/kg of protein, ham diameter reached a plateau of 44.6 cm, i.e., for further decreases in the protein content, the ham diameter stayed the same. Our results suggest that a higher protein content in the diet results in carcass leanness and more intramuscular fat. Ruusunen *et al.* (2007) reported that high dietary protein contents lead to higher carcass lean meat and lower fat content than low protein diets. The protein level supplied to pigs can largely influence muscle growth. The quadratic influence of protein content in the exotic pigs on the total weight of the ham has been reported (Young *et al.*, 1967). An increase in total ham as the dietary protein caused changes decreased has also been reported in exotic pigs (Apple *et al.*, 2017). This indicates that the ham diameter can be influenced by several factors including dietary composition, slaughter weight, and breed type.

The linear increase in dorsal fat thickness at the last rib (f2) as the protein content decreased suggests that the rate of fat deposition is inversely proportional to dietary protein content. A lower protein content in the diet increases fat deposition because it provides more net energy due to a reduction in amino acid deamination, urea excretion, and a lower heat production (Noblet *et al.*, 2001). Reducing the protein content in the diet leads to an increase in intramuscular fat content (Teye *et al.*, 2006). Zhang *et al.* (2008) reported that dietary nutrient levels have an influence on the last rib back fat depth. The available information suggests that the regulation of fat deposition is depot-dependent and is regulated by protein inclusion.

Table 2: Relationship between dietary protein content and pork traits in slow-growing Windsnyer pigs

Measurement	Dietary protein levels						SEM	Regression		*P* value
	193	173.7	154.4	135.1	115.8	96.5		Linear	Quadratic	
Warm carcass weight (kg)	28.4	34.1	27.7	32.6	36.8	27.1	1.5	-	-	0.752
Cold carcass weight (kg)	27.03	32.7	26.4	31.3	35.4	26.2	1.4	-	-	0.631
Cooler shrink (kg)	4.87	4.27	4.57	3.66	3.89	3.42	0.35	0.01 (0.09)		0.001
Dressing percentage %	72.1	78.9	72.2	86.7	79.3	81.5	3.1	0.51 (0.86)		0.041
Drip loss %	2.41	2.26	2.65	1.98	1.92	2.60	0.45		-	0.512
Lean %	61.50	63.8	60.08	64.50	66.67	60.01	1.3		-	0.381
Eye muscle area (cm^2)	15.82	15.99	15.12	16.33	16.95	15.01	1.74		-	0.461
Shoulder fat (mm)	19.8	26	26.2	27.2	24	23.6	2.2	-	-0.008(0.003)	0.003
Full side right (cm)	14.2	15.3	14.3	10.9	15.5	10.8	0.67	0.23 (0.24)		0.012
Carcass length (cm)	71.5	74.0	70.0	68.2	73.8	68.2	1.71	-		0.702
Ultimate pH	5.38	5.31	5.40	5.20	5.22	5.42	0.19	-	-	0.265

*** P <0.001; ** P <0.01; * p <0.05; NS not significant, - denotes regression coefficients which were non-significant

Table 3: Relationship between dietary protein content and primary pork cuts in Windsnyer pigs

| Measurement | Dietary protein levels | | | | | | SEM | Regression | | P value |
	193	173.7	154.4	135.1	115.8	96.5		Linear	Quadratic	
Ham length, cm	30.2	30	29.6	29.2	30.2	28.8	0.77	-	-	0.209
Ham circumference, cm	68.87	68.41	67.78	67.71	68.91	66.95	0.52	-	-	0.084
Ham diameter, cm	48.8	43.8	40.6	43.8	46	42.6	1.25	-	0.005(0.002)	0.004
Hindquarter mass, kg	6.6	5	7.1	3.6	7.3	5.8	0.61	-	-	0.091
Shoulder mass, kg	3.1	3.2	3.3	2.9	3.9	2.74	0.3	-	-	0.715
Rib mass, kg	3.09	3.03	2.98	3.32	3.61	2.48	0.23	-	-	0.441
Dorsal fat thickness at rib (f1), mm	22.8	27	26	26.4	22.6	25.6	1.59	-	-	0.328
Dorsal fat thickness at last rib (f2), mm	18.8	20.2	19.6	17.8	17.2	17.6	0.92	0.10 (0.23)	-	0.018
Dorsal fat thickness at last lumbar vertebra (fat), mm	22.2	23.2	16.4	21	24.2	15.8	2.23	-	-	0.415
P2 fat (back fat thickness), mm	21.8	28	23.6	17.8	15.2	14.6	1.99	0.72 (0.58)		0.010
P2 (1) length (back fat depth), mm	56	50.6	57	53	62.6	57.2	2.72	-	-	0.722
P2 (1) width (back fat depth), mm	20.6	18.8	18.6	20.4	21.6	17.2	1.73	-	-	0.631
P2 (2) width (back fat depth), mm	30.6	30	29.6	29	31.6	24	1.64	0.77 (0.43)		0.032
P2 (3) width (back fat depth), mm	22.8	23	25.2	24.4	29	17.6	2.08	-	-0.007(0.004)	0.008
Shoulder weight proportions %	11.8	11.6	11.9	10.7	12.05	10.4	0.92	-	-	0.521
Rib weight proportions %	9.86	9.71	8.89	9.94	10.5	8.95	0.91	-	-	0.492
Hindquarter weight proportions %	18.8	18.9	18.1	18.4	19.8	17.8	1.40	-	-	0.344

***p <0.001; **p <0.01; * p<0.05; ns: not significant, - denotes regression coefficients which were not significant

Table 4: Relationship between dietary protein content and internal organ weight of Windsnyer pigs

Measurement	Dietary protein levels						SEM	Regression		P value
	193	173.7	154.4	135.1	115.8	96.5		Linear	Quadratic	
Heart weight, g	170	180	140	140	180	160	10.0	-	-	0.295
Lung weight, g	400	450	420	370	480	320	3.0	-	-	0.304
Spleen, g	38.5	38.0	37.9	39.0	39.4	38.2	4.0	-	-	0.453
Kidney weight, g	170	180	200	170	220	160	10.0	-	-0.00004(0.00002)	0.007
Liver weight, g	720	880	810	780	830	760	40.0	-	-	0.469
Small intestine, g	649	669	660	655	661	650	52.0	-	-	0.356
Large intestine, g	702	728	720	710	719	708	81	-	-	0.178
Stomach, g	329	345	340	331	340	329	25	-	-	0.524

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns not significant, - denotes non-significant value

Table 5: Optimum dietary protein content for pork characteristics when further reduction in the crude protein content in the diet caused constraints

Measurement	Y_0		Y_1		Y_2		X_c	Plateau	P value
	Estimate	SE	Estimate	SE	Estimate	SE			
Shoulder fat	22.33	36.02	-0.085	1.01	-0.0076	0.0067	136.8	24.7	0.095
Ham diameter	-16.39	4.87	-1.49	0.0001	-0.0092	0.00068	111.3	44.6	0.027
P 2 (3) widths (back fat depth)	-99.6	4.81	-3.2	0.0007	-0.021	0.00072	115.3	24.8	0.004
Kidney weight	-0.236	0.028	-0.011	0.0004	-0.00007	0.00041	114.7	0.187	0.035

SE-Standard error; y_0 is the intercept or minimum Y_i when $x_c < 0$; y_1 is the rate of change of Y_i when $x_i < x_c$; y_2 is the rate of increase in Y_i when $x_i > x_c$; x_i is the crude protein content in the diet; x_c is the optimal level beyond which carcass traits and pork cuts are compromised by a decrease in the dietary crude protein content

The decreasing linear relationship of P2 back fat and P2(2) width of backfat depth against protein could be due to the reduced dietary protein content. Needham and Hoffman (2015) reported the effect of dietary protein content on back fat thickness. The back fat thickness tends to decrease as dietary protein content is lowered (Hong *et al.*, 2016). The quadratic relationship between dietary protein content and P 2(3) width of backfat depth is not clear. At 115.3 g/kg dietary crude protein, the P2(3) width of backfat depth reach a plateau of 24.8 mm, i.e., a further decrease in protein led to no improvement. Back fat has been reported to be influenced by several factors such as dietary specifications, genotype, sex, and body weight (Needham and Hoffman, 2015; Hong *et al.*, 2016; Apple *et al.*, 2017). The response in back fat in slow-growing Windsnyer pigs may be related to the genotype and dietary composition.

The quadratic relationship between dietary protein content and kidney weight is poorly understood. Organ weights are usually affected by the protein content, so the present results indicate that that the response in kidney weight to dietary protein is not uniform. A decrease in protein level up to 114.7 g/kg had an influence on kidney weight with a plateau at 190 g; beyond that limit, no positive change in kidney weight was observed. Dietary protein content and live body weight have been reported to contribute to the organ weights in slaughter pigs (Ruusunen *et al.*, 2007). The type of diet fed to pigs affects the genetic potential to grow, as well as overall pork quality. It is a common practice in nutritional experiments to use the weights of internal organs as indicators of the influence of diet in animals (Ahamefule *et al.*, 2006). The production level of slow-growing Windsnyer pigs is influenced by the diets that are insufficient or excessive. Our study is the first to report on the effects of a reduced dietary protein content (designed to achieve optimal dietary protein content) on carcass traits, primary pork cuts, and internal organ weights of Windsnyer pigs. There were very few quadratic results in the carcass parameters measured. Due to this, it is difficult to place a strong recommendation on optimum protein levels for Windsnyer pigs without compromising their carcass yield. There are very few studies that focus on the Windsnyer pig carcass and pork quality because of the need to sacrifice numerous pigs for research, despite the fact that their number is declining. The sample size of the study was further limited due to the scarcity of purebred Windsnyer pigs.

Conclusions
The reduction in dietary protein level resulted in linear relationships with cooler shrink, dressing percentage, shoulder fat, dorsal fat thickness at last rib (f2), P2 back fat thickness, and P2(2) width of backfat depth. Decreasing dietary protein content led to a quadratic relationship with few parameters such as shoulder fat, P2(3) width of backfat depth, ham diameter, and kidney weight. Some responses in carcass traits, primal pork cuts, and internal organs are assumed to be related to genetics of the Windsnyer pigs rather than to diet. To optimise carcass performance of the slow-growing Windsnyer pigs, the protein content in the diet should not be reduced below 116 g/kg. The obtained protein content value is needed for use in formulating swine rations for indigenous Windsnyer breed. Knowledge of the protein requirement for indigenous pigs obtained from the present study would enable feed manufacturers and producers to avoid protein deficits and strategically offer supplementary sources of protein. However, further studies should be done to validate the present findings.

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Authors' Contributions
VAH designed the study, collected, and analysed the data, drafted, and revised the manuscript. NCN collected the data and revised the manuscript. MC was responsible for supervising and editing the written manuscript.

Conflict of Interest Declaration
In this study, the authors declare that they have no conflict of interest.

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