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Influence of dietary protein contents on serum biochemical profiles of growing Windsnyer pigs

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Abstract

The response in the concentration of nutritionally-related blood metabolites to decremental dietary protein contents was evaluated in growing Windsnyer pigs. A total of thirty, healthy, Windsnyer indigenous pigs weighing 23 ± 0.62 kg were randomly allocated into individual pens in a complete randomized design. Each of the six dietary treatments was replicated five times. The diets were formulated to contain 193, 174, 154, 135, 116, and 97 g/kg CP contents with a similar energy content of 9.5 MJ/kg. Pigs were allowed ad *libitum* access to the diets and clean water. After 8 w of feeding, Windsnyer pigs were fasted overnight before blood collection. A 10-ml blood samples was collected by jugular venupuncture in non-coagulated vacutainer tubes containing sodium heparin as an anticoagulant. Nutritional-related blood metabolites and liver enzyme activity were determined. There was a linear decrease in total serum protein as dietary protein decreased. There was a quadratic relationship between serum protein and serum albumin. A negative quadratic relationship for serum globulin was observed as dietary protein decreased and in serum aspartate aminotransferase as dietary protein increased. The plateau for albumin, globulin, albumin to globulin ratio, and aspartate aminotransferase occurred at 4.54 g/dl, 5.37 g/dl, 0.8, and 208 U/L and optimum protein was estimated to be 129.2, 125.3, 119.3, and 130.4 g/kg DM, respectively. The results suggest that 135 g/kg crude protein could be appropriate to meet the protein requirements for growing Windsnyer pigs.

Keywords: albumin, cholesterol, glucose, liver enzymes, slow-growing pig, total serum protein
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Introduction

In most developing countries, particularly southern Africa, a large number of slow-growing pigs are found in resource-poor communities (Chimonyo *et al*., 2006; Halimani *et al*., 2012). Kolbroek and Windsnyer pigs, indigenous to South Africa, dominate South African smallholder pig production (Ramsey *et al*., 2000). Windsnyer pigs are the most common indigenous South African breed and are mainly produced in smallholder production systems which are characterised by low to no inputs (Randolph *et al*., 2007). Due to their adaptability to the local South African production environment, Windsnyer pigs are best suited for small-scale farming, backyard production systems, and integrated farming. These pigs contribute to the protein supply for human food security and poverty alleviation at the communal content. Considering that approximately 26% of South Africa's total pork production

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comes from communal areas, Windsnyer pigs, amongst other breeds produced in the communal areas, make a substantial contribution to South Africa's agricultural gross domestic product (AGDP).

The real economic contribution of slow-growing pigs to the AGDP of many countries is constrained by feeding and marketing constraints. In South Africa, communally-produced pork is mainly sold through the informal market and used for dietary purposes. Windsnyer pigs are characterized by low body weights, low growth rate, and poor feed efficiency resulting from low productivity (Kanengoni *et al*., 2014). Regardless of their low productivity, Windsnyer pigs form an important genetic resource base that needs to be sustained. The low body weights and slow growth rates of the pigs have been attributed to poor feeding programs and poor nutrition. Under communal production systems, pigs are mostly fed of kitchen scraps and are allowed to roam freely (Ncobela *et al*., 2017). The potential of these pigs when kept on big commercial farms where there are better management practices for feeds and feeding is not known. If scientifically proven, the high growth performance of these pigs when put on a high nutritional plane coupled with their adaptability to climatic extremes, diseases, and parasite challenges, could be of huge benefit to the South African pork industry with commercialization prospects. Commercialization of the hardy Windsnyer pig might help reduce the high costs of production associated with high technologies such as climate control systems, vaccines, and other preventative measures currently used to manage diseases and parasites amongst current commercial breeds.

Crude protein, an important and expensive nutrient in pig nutrition, is the most limiting factor for pig production in smallholder production systems (Pham *et al*., 2010). Although growth performance is considered to differ between the fast-growing breeds and slow-growing pigs (Kanengoni *et al*., 2014), it is not clear how Windsnyer pigs perform when subjected to optimum protein requirements. The optimum content of dietary protein for Windsnyer pigs has not been established. The protein requirements of Windsnyer pigs are expected to be lower than those of their fast-growing commercial counterparts due to their generational exposure to poor quality feeds (Kanengoni *et al*., 2014). The generational exposure of Windsnyer pigs to poor quality feed sources could have triggered some adaptive mechanisms of these pigs to low protein feeds, however, these postulations still warrant further exploration (Ncobela *et al*., 2017). Optimum crude protein contents of 130–160 g/kg have been reported on hardy pig breeds such as Kadon pigs (Vasupen *et al*., 2004), Cinta Senese pigs (Sirtori *et al*., 2010), Mong Cai pigs (Pham *et al*., 2010), Moo Lath pigs (Phengsavanh and Lindberg, 2013), Indian pigs (Paul *et al*., 2017), and Ghungroo pigs (Hazorika *et al*., 2017). This suggests that, generally, hardy genotypes require less dietary protein than those that are recommended by the NRC (2012).

Serum biochemistry and haematology can be used to interpret the quality of dietary protein in the diet and the metabolic state of pigs because they are the main indices of physiological, pathological, and nutritional status of animals (Fasuyi *et al*., 2013; Etim *et al*., 2014). Blood metabolites have a direct relationship with nutritional status and health of pigs (Radostis *et al*., 2000; Mutle *et al*., 2006; Hlatini & Chimonyo, 2016; Kumar *et al*., 2017).The concentration of glucose and urea in the blood of pigs is used to assess their nutritional status (Mashatise *et al*., 2005; Kanengoni *et al*., 2014). The serum concentration of total protein, cholesterol, and uric acid reflects health together with the nutritional status of pigs (Etim *et al*., 2014; Hazorika *et al*., 2017); alkaline phosphatases (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) are used to determine the presence of hepatic disease and liver cytolysis (Mutle *et al*., 2006; Hlatini & Chimonyo, 2016). The content of minerals in grower–finisher pigs has been recommended as 0.3–0.56% per kg (Hall *et al*., 1991). Kumar *et al*. (2017) determined health and disease status of indigenous pigs in Tamil Nadu using their serum biochemical profile. Apart from Ncobela *et al*. (2018), information about serum biochemistry profiles of South African Windsnyer pigs is lacking. The objective of the current study was to determine the serum biochemical responses of indigenous, growing Windsnyer pigs to different dietary protein contents. It was hypothesized that the reduction in dietary protein content had no influence in blood metabolites.

Materials and Methods

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 study was then conducted at the Agricultural Research Council (ARC), Animal Production Institute, Irene, South Africa. The average annual temperature during the period of the study was 20.7 °C. 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. Animals were 6 ± 3.5 months of age. The animals were ear tagged and housed in a room with artificial heating, lighting, and proper ventilation systems. The house temperature was maintained at 21.9 ± 2.24 ^{o} C. The temperature was measured using a thermometer hanging from the roof. 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.

Six diets were formulated to supply protein contents of 100, 90, 80, 70, 60, and 50% of the NRC (2012) recommended standard with similar energy contents of ~9.5 MJ in all diets (Table 1). The control diet was 193 g/kg CP; five low protein diets containing 173.7, 154.4, 135.1, 115.8, and 96.5 g/kg CP were designed by reducing protein incrementally by 19.3 units. Diets were randomly allocated to pens with one pig in each pen in a complete randomized design. Each pig was treated as an experimental unit. There were five pigs per treatment. Pigs were fed experimental diets in mash form for 8 w. The pigs were allowed 2 w of environmental and dietary adaptation.

Low protein diets were formulated by reducing CP content through partial substitution of soybean meal with yellow maize and wheat bran. Diets for the current study were formulated using the software program, Bestmix® (Adifo, Belgium). The major ingredients used in the formulations 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). Samples were taken per dietary treatment 3 d after the beginning and 3 d before the end of the experiment. Samples were kept at a room temperature (~25 °C) until they were homogenised, subsampled, and prepared for chemical composition analysis (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) contents were analysed using an ANKOM Fibre Analyser (Ankom Macedon, NY, USA) according to method of van Soest *et al*. (1991). Samples for mineral analyses were ashed at 550 °C for 6 h and the ash was dissolved in 1 M HCl (Abdou *et al*., 2011). Mineral contents were detected using a Varian 720 inductively coupled plasma emission spectrometer (ICP-OES, Frankfurt, Germany).

On the 8th week of the feeding period, samples were collected from five pigs to represent each of the six dietary protein contents. Blood samples were taken from each pig by a veterinarian to determine the serum metabolites indices. The pigs were fasted overnight before collection of blood samples. All the experimental Windsnyer indigenous pigs were sampled between 07:00 and 09:00. A 10-ml blood sample was collected by jugular venipuncture in non-coagulated vacutainer tubes containing sodium heparin as an anticoagulant (Becton Dickinson, Franklin, NJ, USA). After collection, the blood samples were kept in cooler box with ice and transported from the Agricultural Research Council to the University of KwaZulu-Natal laboratory. Collected blood samples were allowed to coagulate at room temperature (25 °C) prior to serum analysis. Blood samples for serum metabolites were centrifuged at 1000 × *g* for 10 min within 2 h of collection. Serum was carefully transferred to 1.5 mL polypropylene tubes and kept at -20 °C for preservation pending analysis.

The analyses of serum biochemical parameters and blood minerals were performed. Serum metabolites as well as minerals were analysed using the IDEXX Vettest® Chemistry Analyser (IDEXX Laboratories, Inc., Westbrook, ME, USA). The IDEXX Vettest® Chemistry Analyser uses dry-slide technology that uses a potentiometric endpoint. The analyte was used to catalyse a reaction sequence to yield products that absorb light at wavelengths in various regions (340–680 nm) and diffuses into an underlying layer and are monitored by reflectance spectrophotometry. The dry-slide technology minimizes interference from lipemic, icteric, and haemolysed samples.

Serum biochemical parameters were measured: total protein, albumin, urea nitrogen, uric acid, glucose, creatinine, cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglyceride, bicarbonate, total bilirubin, and amylase. Blood minerals were assessed, including calcium, phosphorus, sodium, potassium, magnesium, chloride, and iron. The serum was analysed for total protein (TP) (Doumas and Biggs, 1972); spectrophotometrically for iron (Fe) (Tietz, 1976); and phosphorus, was assayed using the colorimetric method (Tietz *et al*., 1993). Albumin was determined using the bromocresol green (BCG) method. The globulin content was determined by subtracting albumin from the serum total protein. The enzymatic method was used for the determination of cholesterol (Allain *et al*., 1974) and uric acid (Tietz 1995). AST and ALT were analysed using the ultraviolet method (Bergmeyer *et al*., 1986; Horder *et al*., 1991); ALP was assayed using the colorimetric method (Tietz *et al*., 1993).

The PROC GLM (SAS, 2009) procedure was used to determine the effect of dietary protein content on serum biochemistry of the Windsnyer pigs. The fixed effect was the decreasing content of protein. The PROC REG of SAS (2009) was used to determine relationships between serum biochemical parameters and blood minerals with protein inclusion content in the pig diet. A significant difference was considered at $P < 0.05$. The model used was:

$$Y = \beta_0 + \beta_1 P + \beta_2 P^2 + E \quad (1)$$

where: Y is the response variable (serum blood metabolites and minerals), β_0 is the regression component, β_1 and β_2 are the quadratic regression components, P is the protein content, and E is the error.

A piecewise regression (broken-stick) analysis using the PROC NLIN (SAS, 2009) procedure was used to determine the optimum inclusion of dietary protein on biochemical profile. A content of $P < 0.05$ was set at the criterion for statistical significance.

Table 1 Ingredient and chemical composition of diets used for growing Windsnyer pigs

Ingredients (g/kg)	Crude Protein (g/kg DM)					
	193	173.7	154.4	135.1	115.8	96.5
Yellow maize	602	633	688	725	767	720
Soya oil cake	273	208	171	117	750	50
Wheat bran	85	120	100	120	120	155
Limestone	13	12.5	12.1	13	13.5	10
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	3.0
Threonine	1.1	1.1	1.1	1.1	1.1	1.1
Methionine	1.0	1.0	1.0	1.0	1.0	0.9
Synthetic 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	880
Net energy value (MJ)	9.91	9.92	9.92	9.9.1	9.9.1	9.92
Crude protein	192	169	153	137	117	100
Crude fibre	27.3	27.2	27.3	27.2	27.3	27.3
Ash	54.8	52.8	50.3	46.5	45.0	55.2
Fat	38.2	38.0	38.2	38.0	38.1	38.1
Chemical analysis						
Dry matter	882	882	876	875	875	876
Crude protein	202	177	161	145	121	103
Ether extract	27.2	21.7	22.0	21.2	22.4	23.1
Neutral detergent fibre	142	144	148	143	144	147
Acid detergent fibre	44.2	45.5	47.2	47.8	45.3	46.5
Ash	46.7	46.2	40.6	30.6	45.0	39.4

The piecewise regression model used was:

$$Y_i = y_o + y_1 + y_2 (Ix_c) (x_i - x_c) + \varepsilon_i, \quad (2)$$

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

$$Y_j = y_o + y_1 (x_i), \text{ for } x_i \leq x_c; \text{ and } Y_k = y_o + (y_1 + y_2) x_i, \text{ for } x_i \geq x_c, \quad (3, 4)$$

where Y_i is the response variable when crude protein inclusion content affects biochemical profile; Y_j is the response variable before the content of crude protein affects the biochemical profile; and Y_k is the response variable when the content of crude protein in the diet exceeds the optimal content.

$$Y_o = y_o - y_2 x_c; \text{ when } x_i = 0; \quad (5)$$

y_o 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 content of crude protein in the diet;
x_c is the optimal content beyond which biochemical profile is compromised by a decrease in the content of crude protein in the diets; and
Ix_c is a dummy variable with value 0 when $x_i < x_c$ and 1 when $x_i \geq x_c$

Results

The relationship between dietary protein content and serum protein and liver enzymes is shown in Table 2. As the content of CP in the Windsnyer pig diet decreased, serum total protein concentration also decreased linearly (P <0.05). A quadratic relationship between reduced protein content and serum globulin was observed in Windsnyer pigs (P <0.01). As the dietary protein content decreased, the globulin concentration increased at an increasing rate (P <0.05; Figure 1a). Serum albumin content exhibited a quadratic response to the content of dietary protein (P <0.05). Serum albumin increased when protein content decreased from 193 g/kg to 174 g/kg, and thereafter began to decrease with decreasing content of dietary CP (Figure 1a). There was a quadratic relationship between dietary protein content and albumin to globulin ratio in Windsnyer pigs (P <0.01). When dietary protein content decreased, there was a quadratic response in serum AST (Figure 1d; P <0.05).

The AST concentration increased when protein content decreased from 193 to 154 g/kg before started to decrease at an increasing rate. Serum amylase (AMYL), ALP, and ALT concentrations were not influenced by decreasing contents of protein (P >0.05). Table 3 shows the response in serum metabolites and renal function to decreasing dietary protein content. Decreasing content of dietary protein linearly increased the serum triglycerides and blood urea nitrogen (P <0.05). As the content of CP in the diet decreased, the triglycerides increased linearly (Figure 1c; P <0.05). The blood urea nitrogen decreased in a linear fashion as protein content decreased (P <0.05). The serum cholesterol, high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol, creatinine (LDLC), creatine kinase (CK), creatine, glucose, uric acid, glucose, and total bilirubin were not influenced by decreasing content of dietary protein (P >0.05).

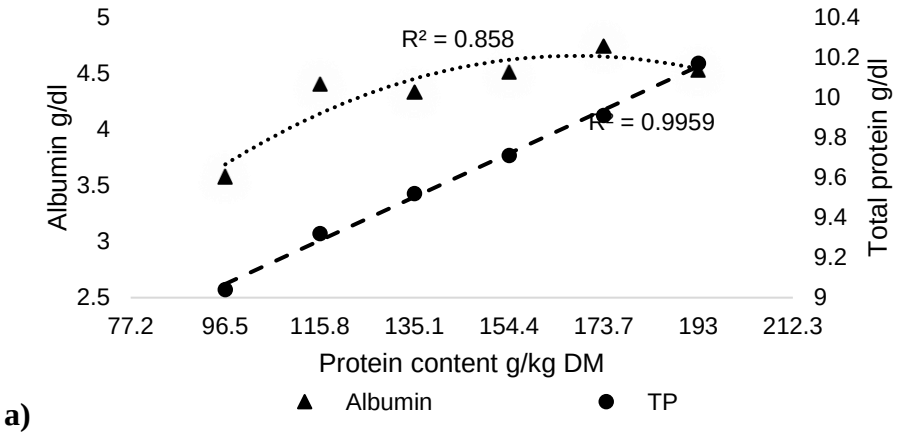
The influence of decreasing dietary protein content on serum mineral concentration is shown in Table 4. Decreasing contents of protein caused a linear decrease in calcium concentration (P <0.05). The concentrations of iron (Fe), phosphorous (P), sodium (Na), potassium (K), magnesium (Mg), chloride (Cl), and bicarbonate were not influenced by the decreasing content of dietary CP (P >0.05).

Table 5 presents the segmented regression indicating the optimum protein content beyond which the maximum blood metabolites were attained. The optimum content of protein for albumin was attained at 129.2 g/kg CP and the plateau was at 4.54 g/dl (P <0.01). The threshold for globulin was estimated to be at 125.3 g/kg CP and the plateau was at 5.37 g/dl (P <0.01). The maximum values for albumin:globulin and AST were obtained at 119.3 and 130.4 g/kg CP, and the plateaus were at 0.80 g/dl and 208 u/l, respectively (P <0.01).

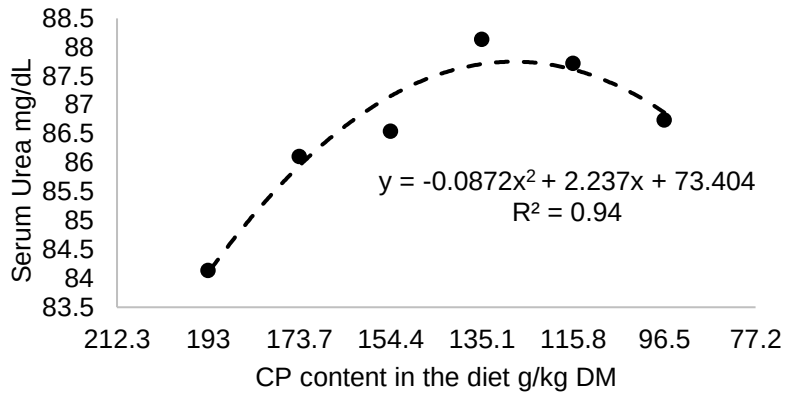
Table 2 Serum protein and enzymatic activity profile of Windsnyer growing pigs fed diets with different contents of protein

Parameter	Protein content in the diet, g/kg DM							Regression P-value	
	193.0	173.7	154.4	135.1	115.8	96.5	MSE	Linear	Quadratic
Proteins Total Protein g/dL	10.17	9.91	9.71	9.52	9.32	9.04	0.28	0.048	0.837
Albumin g/dL	4.53	4.74	4.51	4.33	4.40	3.58	0.14	<0.001	0.007
Globulin g/dL	5.64	5.4	5.2	5.19	4.92	5.46	0.21	0.219	0.028
A:G ratio	0.80	0.88	0.87	0.83	0.89	0.66	0.03	<0.001	0.001
Enzymes ALP U/L	102.0	118.2	95.67	85.48	117.19	84.09	12.6	0.328	0.862
AST U/L	206.1	205.3	214.3	206.3	202.8	178.5	7.2	0.011	0.011
ALT U/L	84.1	85.68	87.07	81.04	87,5	86.3	1.7	0.495	0.684
AMYL U/L	601.3	612.0	610.3	615.2	605.8	603.3	16.6	0.283	0.628

Abbreviations: ALP; alkaline phosphatase, AST; aspartate aminotransferase, ALT; alanine transaminase, AMYL; amylase



a)



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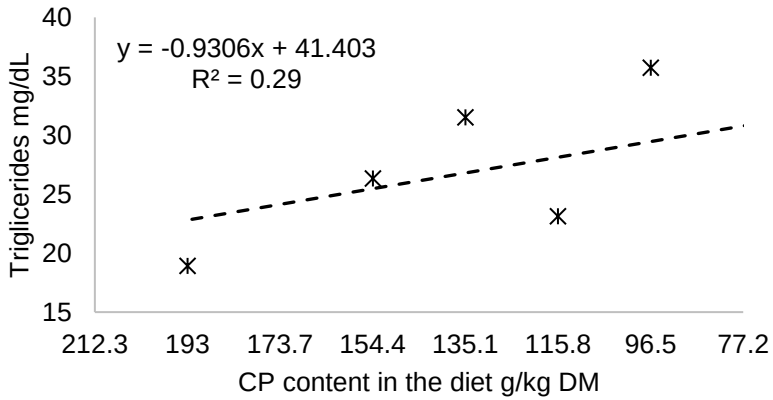

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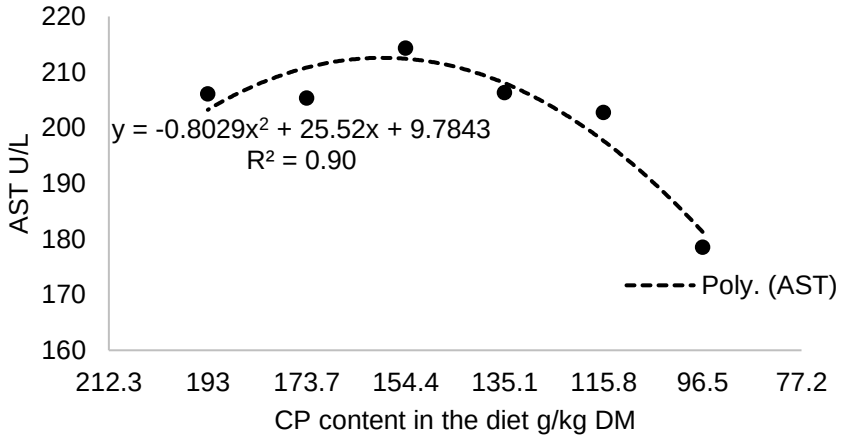

d)

Figure 1: Response of (a) serum total protein, albumin, b) urea, c) triglycerides and d) aspartate aminotransferase (AST) as affected by dietary protein content in growing Windsnyer pigs

Discussion

The study was conducted to determine the extent to which the dietary protein content can be reduced without influencing blood-related metabolites of Windsnyer pigs. Experimental diets were formulated to match or exceed the ideal crude protein content for fast growing pigs. Hlatini *et al*. (2021) reported that to perform optimally, Windsnyer pigs require a diet containing ~135.1 g/kg of crude protein. Understanding the response in serum biochemical profile to dietary protein content informs the correct dietary protein content for Windsnyer pigs.

Total serum protein results indicate the total amount of protein in the blood. The serum protein and albumin concentrations of pigs fed different dietary protein were above the normal reference range (6–8.9 g/L; Radostis *et al*., 2000). This could not be linked to liver disease or malnutrition because protein synthesis was not limited, causing high concentration of total serum protein. Serum total protein in the blood may decrease in conditions that interfere with the production of albumin or globulin. The high total serum protein value in Windsnyer pigs could be associated with an improved utilization of amino acids for growth and protein accretion of pigs. Kumar *et al*. (2017) reported the mean value of total protein for growth and protein accretion in indigenous pigs as 6.5 and 7.7 g/dl, respectively. Serum total protein and albumin values above the standard physiological range are an indication that the pigs suffered from hyperproteinaemia (which is fuelled by increased production of proteins) (Toledo *et al*., 2014; Hlatini & Chimonyo, 2016; Hazorika *et al*., 2017).

The quadratic response of albumin as dietary protein decreases could be associated with protein absorption at each content of protein. The optimum content of protein was attained at 129.2 g/kg for albumin in slow-growing Windsnyer pigs and the plateau was 4.54 g/dl. The response of albumin in the study suggests normal function of the organs because kidney and liver diseases are usually associated with decreased albumin and total serum protein. The strong albumin synthesis might be associated with physiological and genetic mechanisms of Windsnyer pigs. Mutle *et al*. (2006) reported that rations high in protein led to hyperalbuminaemia in pigs. Therefore, the high concentration of serum albumin at an optimum content of 129.2 g/kg protein is an indicator of adequate quantity and quality of dietary protein in the experimental diet. The quality of dietary protein can be evaluated with albumin, because it mainly transports the organic protein reserve and amino acids (Babatunde *et al*., 1990; Radostis *et al*., 2000).

The quadratic relationship between globulin and dietary protein was expected. The globulin concentration with a plateau of 5.37 g/dl was attained at 125.3 g/kg crude protein in the diet. The optimum protein content led to a globulin concentration that eliminates the link with infectious and immune-mediated diseases, which are associated with increased contents of globulin. The serum globulin concentration in the present study was within the normal reference range for pigs (85–160 g/dL; Radostis *et al*., 2000). Kanengoni *et al.* (2014) also reported a globulin concentration within the normal range in Windsnyer pigs fed maize–soya-based diets and fibre-rich diets.

The albumin: globulin ratio showed a quadratic response; the results indicate a decrease in albumin and increase in globulin as dietary protein content decreases. An albumin:globulin that is close to one indicated that dehydration increased the concentration of albumin, as observed in the study. Infections causes high globulin concentrations and it could have depressed the albumin concentration, thus altering the albumin:globulin (Hascheck and Walling, 2010). The plateau for albumin: globulin in the current study occurred at an optimum protein content of 119.3 g/kg DM. The albumin:globulin reflects whether changes in the protein concentration include either changes in albumin or globulin (Haschek and Walling, 2010). Pigs suffering from a disease would have low albumin concentration and that will cause an albumin:globulin not close to 1.

Table 3 Metabolites and renal function of Windsnyer growing pigs fed diet with different contents of dietary protein

	Protein content in the diet, g/kg DM							Regression P-value	
Parameter	193.0	173.7	154.4	135.1	115.8	96.5	MSE	Linear	Quadratic
Metabolites									
Cholesterol mg/dL	102	77.5	99.1	71.9	101	96.6	10.8	0.882	0.219
HDLC mg/dL	47.56	35.58	46.40	35.19	46.79	46.02	4.64	0.419	0.167
LDLC mg/dL	92.80	65.35	90.87	63.80	92.03	90.10	9.28	0.418	0.175
Glucose mg/dL	114.85	88.88	92.3	100.4	116.24	102.0	7.8	0.719	0.135
Uric acid mg/dL	0.37	1.08	0.8	0.5	0.54	0.63	0.13	0.646	0.188
Triglycerides mg/dL	18.93	31.92	32.35	34.53	33.13	35.74	3.28	0.045	0.699
CK U/L	3077	4376	3249	4332	6203	3498	633	0.134	0.255
T.bili mg/dL	0.54	0.51	0.49	0.48	0.52	0.41	0.14	0.253	0.781
Renal function BUN mg/dL	88.14	87.72	86.74	86.55	86.11	84.14	1.18	0.035	0.074
Creatinine mg/dL	4.62	4.57	4.6	4.45	464	4.49	0.1	0.425	0.885

Abbreviations: HDLC; high-density lipoprotein cholesterol, LDLC; low-density lipoprotein cholesterol, CK; creatine kinase; T.Bili; total bilirubin, BUN; blood urea nitrogen

Table 4 Serum mineral content of Windsnyer growing pigs fed diets with different contents of dietary protein

	Protein content in the diet, g/kg DM							Regression P-value	
Parameter	193.0	173.7	154.4	135.1	115.8	96.5	MSE	Linear	Quadratic
Minerals									
Ca mg/dL	16.9	16.42	16.34	15.16	15.11	12.83	0.96	0.006	0.276
Na mmol/L	132.7	131.6	133.2	129.0	132.3	128.4	2.0	0.382	0.775
K mmol/L	3.49	3.19	3.25	2.95	3.35	2.92	0.14	0.415	0.858
Mg mmol/L	0.82	0.78	0.80	0.77	0.81	0.79	0.02	0.177	0.565
Cl mmol/L	90.13	89.4	90.8	88.3	90.4	89.8	0.3	0.891	0.218
P mg/dL	13.21	12.92	13.66	12.46	12.83	12.41	0.63	0.285	0.795
Fe ug/dL	292.4	320.3	302.4	289.3	295.9	294.7	15.13	0.519	0.869
Bicarb mmol/L	15.01	15.28	16.12	15.63	16.07	16.82	0.73	0.485	0.863

Abbreviations: Ca; calcium, Na; sodium, K; potassium, Fe; iron, Mg; magnesium, Cl; chloride, P; phosphorus, Bicarb; bicarbonate

Table 5 Decrease in the content of protein beyond the optimal protein content compromises nutritionally-related metabolites

Parameter	Y0		Y1		Y2		Xc	Plateau	P-value
	Estimate	SE	Estimate	SE	Estimate	SE			
Albumin	-2.74	3.38	0.306	0.18	-0.003	0.002	129.2	4.54	<0.001
Globulin	10.99	6.42	-0.246	0.34	0.003	0.004	125.3	5.37	0.035
A:G ratio	-0.062	0.86	-0.055	0.04	0.0008	0.0005	119.3	0.801	<0.001
AST	107.4	157.8	-6.206	0.01	-0.096	0.098	130.4	208.1	0.0019

A:G ratio; albumin:globulin ratio, AST: aspartate amino-transferase

Yi is the response variable when crude protein inclusion affects the biochemical profile; yo is the intercept or minimum Yi when xc < 0; y1 is the rate of change of Yi when xi < xc; y2 is the rate of increase in Yi when xi >xc; xi is the content of crude protein in the diet; and xc is the optimal content beyond which biochemical profile is compromised by a decrease in the content of crude protein in the diet

The linear decrease in blood urea as crude protein content decreased was expected. Blood urea nitrogen content is an indicator of protein utilization, and it is affected by amino acid balance and nitrogen intake, as reported in our earlier study (Hlatini *et al*, 2021). This explains why, in the current study, blood urea nitrogen concentration decreased when dietary crude protein was reduced. Reduction in dietary protein content in the diet led to a decrease in blood urea due to less nitrogen intake. Fang *et al*. (2015) reported similar results; urea nitrogen concentration decreased with dietary protein content in growing pigs. The dietary protein content did not influence the concentration of serum creatinine, glucose, cholesterol, and phosphorus. The similar concentration in creatinine may suggest that the dietary protein in all treatments was of good quality. The concentrations of phosphorus and calcium were outside the normal range, and it is not clear why. This may be due to the increased availability of minerals due to the specialized ability of Windsnyer pigs to utilize nutrients.

The glucose and cholesterol concentration as dietary protein decreased reflects similar energy across the pig diets. Glucose and cholesterol indicate the energy status of the pigs (Kanengoni *et al*., 2014). All six diets were formulated to contain a net energy value of ~9.5 MJ/kg. A similar mean of serum glucose across diets indicates the same amount of net energy provided in all pigs (Kanengoni *et al*., 2014).

The positive linear relationship in serum triglycerides as dietary protein decreased could be linked to the diet. The observed relationship in the present study for protein content against triglycerides in Windsnyer pigs was expected; increased concentrations of triglycerides and cholesterol are evident in the plasma of obese pigs (Kumar *et al*., 2017). The triglyceride and cholesterol concentration above the range in the study could be linked with heart disease and liver and pancreas problems, but further evidence from meat quality assessments is necessary to reach such a conclusion. Total cholesterol is composed of HDL-C and LDC-C, which is found in the bloodstream in the form of lipoproteins. Hence, determining the content of HDL-C and LDL-C is necessary, because LDL-C contributes to the formation of plaques in the inner walls of the arteries, whereas high contents of HDL-C prevent the building up of plaques (Burke *et al*., 2018). Hence, above-normal range contents of LDL-C and triglycerides are linked with heart disease. High concentrations of triglycerides, cholesterol, and protein in obese pigs have been reported (Etherton & Kris-Etherton, 1980).

Obese and lean breed have different physiological and biochemical characteristics (He *et al*., 2012). ALP, AST, and ALT serum concentrations are used as indicators of hepatic disease and liver cytolysis in pigs (Hlatini & Chimonyo, 2016). These enzymes do reflect the physiological, health, and nutritional status of animals but not the degree of hepatic dysfunction and disease. A decrease in ALP activity for Windsnyer pigs may be associated with the quality of dietary protein, age, and the summer season. Serum ALP concentration across all diets was not influenced by dietary protein content and was below the normal reference range. The older pigs tend to have low demand of the enzyme for skeletal muscle growth (Milinkovic-Tur *et al*., 2005). A lower ALP concentration in pigs is correlated with a lower value of phosphorus in other research on young animals compared to older pigs, but surprisingly not with phosphorus results of the current study. Friendship *et al*. (1984) reported that low dietary content of phosphorus leads to a reduced content of ALP. Mayengbam & Tolenkhom (2015) reported a decrease in ALP activity with an increase in age of pigs, together with a summer season (Sarma *et al*., 2011; Pourouchottamane *et al*., 2013). Decrease in ALP activity corresponds with a high content of TSP, because a high value of ALP is an indication of poor-quality protein in the diet (Awosanya *et al*., 1999). There was no relationship observed between ALT activity and dietary protein content in Windsnyer pigs. The negative quadratic relationship observed between AST and dietary protein content suggests less liver damage in the diet with low protein offered. The optimum protein content attained at 130.4 g/kg led to an AST plateau of 208 g/dl. AST is found in many tissues and organs with high activity in the liver (Milinkovic-Tur *et al.,* 2005). Increased AST activity in the present study could also be due to muscle activity and muscle damage because the indigenous Windsnyer pigs kept on attempting to jump out the cage during feeding and when collecting blood.

The major protein fractions in the blood serum indicated the nutritional adequacy of the six experimental diets. Reduction in dietary protein content in the diet led to a positive response in blood metabolites in Windsnyer pigs. There was a lack of signs of incomplete protein in the diet and severe kidney and liver disease. The responses of pigs to dietary protein vary between breeds, species, and population and part of this variability may have a genetic basis (Kanengoni *et al*., 2014). For the indigenous Windsnyer, serum biochemistry reference intervals are lacking, therefore, relating the results to disease is difficult. For assessment of the physiology, nutritional status, and disease,

references from other breeds and species were used. There is a crucial need for more information on slow-growing pig physiology and metabolism.

Conclusion

The blood metabolite results indicated that the experimental diet provided good quality protein. Albumin, globulin, and albumin:globulin, as well as AST were compromised when protein content declined beyond the optimal content (129.2, 125.3, 119.3, 130.4 g/kg CP, respectively). According to the results of nutritionally-related blood metabolites, a protein content of 70% of the NRC recommended protein in the diet of Windsnyer pigs is appropriate. Hence, a reduction in dietary protein content from 193.0 g/kg to 135.1 g/kg with balanced amino acids is possible with Windsnyer pigs. The present information on protein requirements of indigenous Windsnyer pigs is useful to breeders and farmers considering the breed as an option or complement to pure line breeding. Further evidence from nitrogen balance, pork quality, and meat characteristics studies is necessary to accurately predict protein requirements for slow-growing Windsnyer pigs.

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Contributions made by authors.

VAH & MC conceived project. VAH & CNN conducted the experiment. VAH & CNN analysed data and wrote the manuscript. FT& MC edited and improved the manuscript. All authors read and approved the manuscript.

Data and material availability

The data are available from the corresponding author on request.

Conflict of interest

The authors declare that they have no conflict of interest.

Consent for publication

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