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Growth performance and nutrition-related serum metabolites in growing pigs fed on *Acacia Tortilis* leaf meal

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ABSTRACT

The objective of the study was to determine the response in metabolites and growth performance in growing pigs fed on *Acacia tortilis* leaf meal-based diets using a dose-response trial. Forty-eight male hybrid pigs (28.5 ± 2.18 kg BW) were individually penned and assigned in a complete randomized design to six experimental diets containing 0, 30, 60, 90, 120, and 150 g/kg DM of *A. tortilis* leaf meal. Pigs were bled once after three weeks for biochemical analyses. An increase in *A. tortilis* resulted in quadratic reductions in ADFI ($P < 0.0001$) and ADG ($P < 0.05$), and linear decreases in G:F ($P < 0.001$). Serum iron, cholesterol and total protein initially increased, and then started decreasing with incremental levels of *A. tortilis*. There was a quadratic increase in alanine aminotransferase (ALT) ($P < 0.001$) and aspartate aminotransferase (AST) ($P < 0.01$) and a linear increase ($P < 0.001$) in alkaline phosphatases (ALP) observed as *A. tortilis* inclusion increased. Using the broken-stick model, the optimum levels of leaf meals marking break points at which threshold values of ADG, serum iron, serum cholesterol and total protein occurred when *A. tortilis* was included at 64.8, 60.0, 87.1 and 63.2 g/kg DM, respectively. In conclusion, growth performance, serum iron and total proteins are reliable indicators of optimum inclusion levels of leaf meals in pigs.

1. Introduction

Increase in price for the common conventional protein supplements calls for the need to come up alternative protein sources to feed livestock. Sustainable cost-effective feedstuffs such as nitrogen-rich leguminous leaf meals can be used as protein sources. The leaves of *Acacia* species namely; *Acacia karroo, Acacia nilotica, Acacia angustissima* and *Acacia tortilis*, have been widely used as feedstuffs in farm animals (Halimani et al., 2005; Mapiye et al., 2011; Ncube et al., 2012). *A. tortilis* dominate arid regions of Africa, where they are causing bush encroachment, which is detrimental to the sustainability of plant diversity and also reduces grazing capacity of rangelands. The leaf meals from these plants are rich in tannins and dietary fibre but if consumed at optimum levels, they induce health-enhancing properties such as anti-helmintic, antioxidant, antidiarrheic and anticancerogenic effects, without compromising growth performance (Funatogawa et al., 2004; Geidarn et al., 2007; Khanyile et al., 2014). Therefore, moderate tannins in the diets can replace the need for banned growth promoters. Accurate inclusion levels are, however, largely unavailable. Dietary inclusion of these leaf meals above optimum levels leads to endogenous losses of minerals and amino acids, hepatotoxicity and toxic nephrosis (Lee et al., 2010).

Growth performance has commonly been used to determine optimum inclusion levels of *A. tortilis* leaves in finishing pigs diets (Khanyile et al., 2014). The presence of anti-nutritional factors compromises nutrient intake, digestibility, absorption and metabolism (Halimani et al., 2005) implying that blood metabolites also have the potential to be used to determine accurate optimum inclusion levels. Dose-response trials, in conjunction with broken-stick analyses, are appropriate and essential in accurately determining the inclusion levels when each measured parameter starts to decrease. It is, therefore, important to assess whether metabolites and growth performance indicators yield similar estimates of optimum inclusion levels in growing pigs. Therefore, the objective of the study was to determine the response of metabolites to increasing *A. tortilis* leaf meal in growing pigs.

2. Materials and methods

Care and use of the pigs were performed according to the ethical needs by Certification of Authorization to Experiment

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on Living Animals provided by University of KwaZulu–Natal (UKZN) Animal Ethics Committee, Pietermaritzburg, South Africa (Reference Number 041/13/Animal).

2.1. Acacia tortilis leaf-meal preparations and diets formulation

A. tortilis leaves were harvested during the hot-dry season from a single grazing camp. The leaf meals were air-dried under well ventilated shade (with no direct sunlight exposure), for five days. During the drying period, the leaf meals were often turned to prevent accumulation of microbial growth. After drying, the leaf meals were passed through a 1 cm sieve-wire to get rid of thorns and twigs, packed into polythene plastic bags, sealed and stored under room temperature prior to chemical analysis and feed formulation.

Six experimental diets were used in this trial: a basal diet and five diets based on varying incremental levels of *A. tortilis* were formulated according to meet the nutrient requirements for growing pigs with 25–50 kg BW (NRC, 2012). The basal diet had a low dietary fibre and no leaf meal. The other five diets were formulated to contain 30, 60, 90, 120 and 150 g/kg (as-is basis) inclusion levels of *A. tortilis* leaf meal. Diets were formulated using Winfeed diet formulation software programme (WinFeed Limited, Cambridge, UK). The maximum inclusion level of *A. tortilis* was adapted by not to exceeding optimum inclusion level proposed for finishing pigs in our previous study (Khanyile et al., 2014). The ingredient compositions of the experimental diets are given in Table 1.

Table 1
Ingredient and analyzed composition (g/kg DM) experimental diets.

Item	*Acacia tortilis* inclusion level (g/kg DM)					
	0	30	60	90	120	150
Ingredient						
Maize	527	490	454	418	382	346
Wheat bran	191	192	192	193	194	194
Soybean meal	206	209	212	215	217	220
AT	–	30.0	60.0	90.0	120	150
$_{L}$-Lysine HCL	2.90	2.89	2.91	2.86	2.84	2.81
$_{DL}$-Methionine	0.48	0.56	0.56	0.58	0.58	0.58
$_{L}$-Threonine	1.08	1.07	1.03	1.00	0.98	0.95
Vitamin-mineral premix[a]	1.50	1.50	1.50	1.50	1.50	1.50
Limestone	19.2	18.9	18.6	18.1	17.9	17.6
Sodium chloride	3.25	3.26	3.26	3.27	3.29	3.28
Monocalcium phosphate	12.8	12.9	13.0	13.1	13.2	13.3
Oil-sunflower	34.8	37.4	40.5	43.7	46.9	50.0
[†]Analyzed composition						
DM	867	871	873	877	882	889
Crude protein	180	182	186	186	186	190
Gross energy (MJ/kg)	16.7	17.0	17.1	17.5	17.8	18.1
NDF	161	176	181	208	237	241
ADF	52.4	75.8	87.2	93.4	88.5	104.2
Condensed tannins (mg/kg DM)	–	1.55	3.09	4.64	6.18	7.73
Lysine	11.2	11.4	11.4	11.5	11.6	11.9
Threonine	6.7	7.1	6.8	6.6	6.7	7.6
Methionine	4.2	4.3	4.2	4.3	4.4	4.7
Calcium	11.7	12.3	12.6	13.1	13.4	14.1
Phosphorus	8.1	8.4	8.6	8.8	8.8	9.1
Iron (mg/kg DM)	189	198	218	231	251	258
WHC (g_{water}/g_{feed} DM)	3.21	3.30	3.77	3.82	3.87	4.09
Swelling capacity (ml/g DM)	2.69	2.73	3.02	2.97	3.16	3.26

AT=*Acacia tortilis* leaf meal; DM=Dry matter; WHC=water holding capacity.

[a] Supplemented per kilogram of diet: vitamin A, 8.250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; vitamin K_3, 4.4 mg; vitamin B_1, 5.6 mg; vitamin B_2, 20.6 mg; niacin (99.5%), 35.6 mg; vitamin B_{12}, 0.08 mg; vitamin B_6 98%, 2.0 mg; choline (chloride 60%), 121 mg; folic acid (96% pure), 0.48 mg; biotin, 0.18 mg; calcium pantothenate (98%), 5.2 mg; Mn, 120.0 mg (manganese sulphate); zinc, 100 mg (zinc bacitracin); Cu, 8 mg (copper sulphate); potassium iodide (Iodine 76.45%), 0.4 mg; cobalt sulphate, 0.2 mg; Fe, 100.0 mg (ferrous sulphate) and selenium, 0.32 mg.

2.2. Pigs and housing

Forty-eight male F_1 hybrid pigs (Landrace female × Large White male, PIC Group) aged four weeks, with an initial mean BW of 28.5 (s.d.=2.18) kg were assigned to individual pens that had slatted floors. Each pen measured 2.1 × 1.1 m^2 and was equipped with a low-pressure nipple drinker which provided water *ad libitum*. An automated plastic feeder (Big Dutchman Lean Machine®, Postfach, Vechta, Germany) was fitted on the opposite side of the drinker.

Pigs were blocked, based on body weight, into eight groups of six. Within each block, pigs were assigned in a complete randomized design to one of the treatment diets. Each of the six diets was offered *ad libitum* to eight individually-penned pigs for 21 days. Individual pig BW and feed intake were measured weekly to calculate average daily feed intake (ADFI), average daily gain (ADG) and gain: feed ratio (G:F). At the end of the 3rd week of feeding, blood from each pig was collected through jugular venipuncture into purple top 5 mL ethylene diamine tetra-acetic acid (EDTA)-coated vacutainer collection tubes. Blood samples were kept on ice, allowed to coagulate and taken to the Genetic and Microbiology Laboratory (School of Biochemistry, UKZN, Westville Campus, Durban, South Africa) for biochemical analyses. Blood serum was obtained by centrifuging blood at 3500*g* for 15 min and aliquots were stored at −20 °C, pending analyses. The housing conditions were automatically maintained at a temperature of 21.9 ± 2.24 °C, 45.2 ± 6.85% relative humidity and a 12 h dark-12 h artificial light cycle, by use of a single heating, lighting and forced-air-ventilation system.

2.3. Laboratory analyses

The experimental diets were milled through a 2 mm sieve and analysed for their physical and chemical properties. The dry matter (method 945.15), ash (method 942.05), crude protein (method 979.09) and ether extract (method 920.39) were determined according to the Association of Official Analytical Chemists methods (AOAC, 1990). A Parr adiabatic oxygen bomb calorimeter (Parr Instrument, Moline, IL, USA) was used to determine gross energy. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using ANKOM Fibre Analyser (Ankom Macedon, NY, USA) according to Van Soest et al. (1991) and Van Soest (1973), respectively. Samples for amino acids analysis were prepared by acid hydrolysis according to AOAC (1984; method 982.30) following modifications by Mills et al. (1989) and analysed using an amino acid analyser (SY-KAM, Erising, Germany). In brief, approximately 100 mg of each of the feed samples was digested in 4 mL of 25% (wt/vol) NaOH and allowed to cool to room temperature. Sodium citrate buffer (pH 2.2) was added so that the mixture reaches 50 mL. Prior to analysis, samples for methionine analysis were initially oxidized with performic acid. Water holding capacity (WHC) was determined according to Whittemore et al. (2003). The swelling capacity was determined according to Canibe and Bach Knudsen (2002). Condensed tannins were estimated calorimetrically by the butanol-HCL method (Reed, 1986). Samples for mineral analyses were ashed at 450 °C and the ash was dissolved in 1 M HCl (Abdou et al., 2011). Mineral contents were detected using Varian 720 Inductively Coupled Plasma Emission Spectrometer (ICP-OES, Frankfurt, Germany) using atomic absorption. The serum metabolites analysed were total protein, cholesterol, iron, uric acid, and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatases (ALP). Blood metabolites were measured spectrophotometrically using an automated chemistry analyzer using kits