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Short communication

Nutritionally-related blood metabolites and liver enzymes in growing pigs fed on *Acacia tortilis* treated with polyethylene glycol

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ABSTRACT

Presence of polyphenolic compounds limits the utilization of leaf meals. Incorporation of polyethylene glycol (PEG) can prevent the formation of tannin-protein complex. The study aimed to determine the nutritionally-related metabolites in pigs fed on *Acacia tortilis* leaf meal treated with polyethylene glycol (PEG). Forty-eight clinically healthy male growing pigs were randomly allotted to individual pens, in a completely randomized design. The diets included 150 g/kg *A. tortilis* which had been treated with six increasing levels (0, 5, 10, 15, 20 and 25 g/kg) of PEG. There were eight pigs per treatment. The pigs were allowed *ad libitum* access to the diets and clean water throughout. Inclusion of PEG showed a linear response on total protein (TP) and globulin, but quadratic to albumin ($P < 0.01$). There was a linear relationship between PEG inclusion and cholesterol, creatinine and uric acid ($P > 0.05$). The concentration of aspartate aminotransferase (AST) ($P < 0.01$) and alanine aminotransferase (ALT) ($P < 0.05$) decreased linearly as PEG inclusion increased. There was a quadratic increase in alkaline phosphatase (ALP) as the PEG inclusion level increased ($P > 0.05$). Inclusion of PEG in *Acacia tortilis* leaf meal based-diet, therefore, had no adverse effects on the health and nutritional status of growing male pigs.

1. Introduction

There is a need to identify and characterize non-conventional feed resources to feed pigs. Although leaf meals are abundant in the tropics, polyphenolic compounds, high fibre content and presence of thorns, reduce their nutritive value (Halimani et al., 2005; Martens et al., 2013). If present at high concentrations, polyphenolic compounds depress feed intake, nutrient digestibility and compromise functions of the liver, kidneys and intestines (Ndou et al., 2015). Concentrations of cholesterol, uric acid and total protein reflect the health and nutritional status of pigs (Etim et al., 2014). Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are markers of hepatic disease and liver cytolysis in pigs. There is need to counter the nutritional and toxic effect of polyphenolic compounds. Polyethylene glycol (PEG), a tannin-binding agent, prevents formation of tannin-protein complexes which, in turn, is expected to improve the health and nutritional status of pigs. The response in the metabolites and activity of liver enzymes can indicate the levels of PEG to be added to pig diets containing 150 g/kg of *Acacia tortilis* leaf meal. Khanyile et al. (2014) reported that growing pigs are able to effectively utilise diets containing less than 150 g/kg *A. tortilis* leaves, if no tannin-binding agent is incorporated. Optimum inclusion levels of PEG are, therefore not known. The objective of the study was to establish the influence of PEG inclusion in *A. tortilis* leaf meal diet on nutritionally-related metabolites and liver enzymes. It was hypothesised that PEG inclusion in leaf meals has a linear relationship with blood metabolites.

2. Materials and methods

2.1. Pigs and housing

The study was conducted at Ukulinga Research farm at the University of KwaZulu-Natal in Pietermaritzburg, South Africa. Forty-eight clinically healthy male PIC pigs were randomly allotted into individual pens. The pigs were housed in a room with artificial heating, lighting, and proper ventilation systems. The house conditions were kept at a temperature of 21.9 ± 2.24 °C, and 45.2 ± 6.85% relative humidity. The pigs were not given any antibiotics or growth promoters.

Ethical approval for the experiment was granted by the UKZN Animal Ethics Committee (Reference no: 076/14/Animal).

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2.2. Experimental design, diets and feeding

Male pigs weighing 15.8 (s.d.=0.032) kg were randomly allocated to each of the six diets in a completely randomized design (n=8). Each pen was provided with water through low-pressure nipple drinkers. Feed was supplied *ad libitum* in a plastic self-feeder trough (Big Dutchman Lean Machine®, Postfach). Preparation of leaf meal has been fully detailed (Hlatini et al., 2016). Feed containing 150 g/kg DM basis *A. tortilis* was treated with 0, 5, 10, 15, 20 or 25 g/kg of PEG. The diet contained 346, 194, 220, 50, 17.6, 13.3, 3.3, 2.0, 1.4, 0.9 and 1.5 g/kg maize, wheat bran, soybean meal, oil sunflower, limestone, monocalcium phosphate, salt, L-lysine HCL, DL-methionine, L-threonine and premix, respectively. The diet was prepared to provide 220 g/kg crude protein. The trial lasted 31 days, excluding the seven day treatment adaptation period. Data on pig performance parameters were reported earlier (Hlatini et al., 2016).

2.3. Chemical analyses

The dry matter, ash, crude protein and ether extract of the diets were determined using the standard AOAC (1990) methods. Nitrogen content were analysed following Dumas Combustion method in a Leco Truspec Nitrogen Analyser, St Joseph MI, USA. A factor of 6.25 was used to calculate CP content. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using Ankom Fibre Analyser (ANkom Macedon, NY, USA) following methods by Van Soest et al. (1991). Acid hydrolyses were used for amino acid preparation before analyses using an amino acid analyser (SY-KAM, Erising, Germany). Condensed tannins were estimated calorimetrically by the butanol-HCL method (Reed, 1986). Water holding and swelling capacity were determined following methods described by Whittemore et al. (2003). Samples for minerals were ashed at 450 °C and dissolved in 1 M HCl. Mineral contents were detected using Varian 720 Inductively Coupled Plasma Emission Spectrometer (Frankfurt, Germany) using atomic absorption. Table 1 shows the chemical analysis of *A. tortilis* leaves and diet.

Table 1
Chemical analyses of *Acacia tortilis* leaves and diet.

Component	Leaf meal	Diet
Dry matter (g/kg)	992	894
Ash (g/kg DM)	65.1	72.2
Organic matter	934.9	927.8
Crude protein (g/kg DM)	218.4	215.2
Ether extract (g/kg DM	40.2	86.4
Neutral detergent fibre (g/k DM)	495	241.2
Acid detergent fibre (g/kg DM)	298.6	104.2
Neutral detergent insoluble nitrogen (g/kg DM)	27.5	ND
Acid detergent insoluble nitrogen (g/kg DM)	18.3	ND
Condensed tannins (mg/kg DM)	51.6	7.7
Water holding capacity (g_{water}/g_{feed} DM)	6.1	4.09
Swelling capacity (ml/g DM)	4.8	3.26
Lysine (g/kg DM)	ND	13.5
Threonine (g/kg DM)	ND	8.5
Methionine (g/kg DM)	ND	5.3
Calcium (g/kg DM)	ND	12.1
Phosphorus (g/kg DM)	ND	7.9
Iron (mg/kg)	ND	250

ND: not determined.

2.4. Collection and analyses of blood samples

A 10 ml blood was collected through jugular venipuncture in vacutainer tubes containing sodium heparin as the anticoagulant (Becton Dickinson, Franklin, NJ), from each pig. This was done on day 31 of the trial. Blood samples, collected between 0700 and 0900 h, were kept on ice. They were allowed to coagulate prior to centrifugation ($1000 \times g$ 10 min at 25 °C) within two hours of collection, and kept at −20 °C, until analysis. The serum was analysed spectrophotometrically for total protein (TP) concentration (Doumas and Biggs, 1972). Alkaline phosphatases (ALP) were assayed using the colometric method (Tietz, 1995). The enzymatic method was used for determination of both cholesterol and uric acid, as described by Tietz (1995). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analysed using the ultraviolet method (Bergmeyer et al., 1986).

2.5. Statistical analyses

The data were analysed by the response surface regression (RSREG) procedure to determine relationships between TP, albumin, globulin, creatinine, and cholesterol, uric acid, AST, ALT and ALP with PEG inclusion level. Differences in metabolites and liver enzyme values were analysed using the PROC GLM procedure of SAS (2008).

3. Results

The response of selected nutritionally-related blood metabolites to PEG treated *Acacia tortilis* leaf meal diet in growing pigs is shown in Table 2. Inclusion of PEG influenced TP, globulin and albumin concentrations ($P<0.01$). There was no relationship between PEG inclusion and cholesterol, creatinine and uric acid concentrations. As shown in Fig. 1, TP concentration increased linearly as the PEG inclusion increased (b=0.21; $R^2=0.92$). A linear relationship between PEG inclusion level and globulin ($P<0.01$) was observed. Globulin concentration also increased linearly with PEG inclusion increased (b=0.20; $P<0.01$; $R^2=0.78$).

Table 2
Response of selected nutritionally-related blood metabolites to PEG treated *Acacia tortilis* leaf meal diet.

Parameter	PEG inclusion level (g/kg DM)						SEM	Regression coefficient		
	0	5	10	15	20	25		Linear	Quadratic	Sig.
Total protein (g/dl)	6.47	6.77	7.00	7.24	7.16	7.65	0.12	0.10 (0.05)	−0.01 (0.01)	*
Globulin (g/dl)	3.26	3.62	3.80	3.99	3.76	4.51	0.15	0.23 (0.06)	−0.02 (0.01)	**
Albumin (g/dl)	3.21	3.14	3.20	3.24	3.40	3.14	0.08	−0.12 (0.03)	0.01 (0.03)	**
Cholesterol (mg/dl)	74.0	73.4	77.1	69.1	71.8	72.1	2.43	0.73 (0.94)	−0.10 (0.09)	NS
Creatinine (mg/dl)	2.56	1.50	1.47	2.10	0.70	1.24	0.40	−0.12 (0.15)	0.01 (0.02)	NS
Uric acid (mg/dl)	0.23	0.15	0.35	0.25	0.19	0.25	0.05	0.01 (0.02)	−0.001 (0.00)	NS

SEM: Standard error of Mean; Sig.: Significance level; NS: Not significant.
* $P<0.05$;
** $P<0.01$.