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Nutraceutical and preservative potential of *Acacia mearnsii* and *Acacia dealbata* leaves for ruminant production and product quality enhancement

T. Uushona[1,2], O. C. Chikwanha[1], T. Tayengwa[1,3], C. L. F. Katiyatiya[1], P. E. Strydom[1] and C. Mapiye[1]

[1]Department of Animal Sciences, Faculty of AgriSciences, Stellenbosch University, Private BagX1, Matieland 7602, South Africa; [2]Department of Animal Production, Agribusiness and Economics, Faculty of Agriculture, Engineering and Natural Resources, University of Namibia, Private Bag 13188, Windhoek, Namibia and [3]Agriculture and Agri-Food Canada, Lacombe Research and Development Centre, 6000 C & E Trail, Lacombe, AB T4L 1V7, Canada

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Author for correspondence:
C. Mapiye,
E-mail: cmapiye@sun.ac.za

Abstract

Seasonality and species are key aspects of the abiotic and biotic environment, respectively, that influence nutrient, phytochemical and antioxidant profiles of invasive alien plants leaf-meals. The current study investigated the effects of season on nutrient, amino acid, fatty acid, mineral, bioactive polyphenolic composition and antioxidant activity of *Acacia mearnsii* and *A. dealbata* leaf-meals. Overall, *A. mearnsii* leaf-meal harvested in the hot-dry-season had greater crude protein, ether extract, neutral detergent soluble fibre, 24 and 48 h *in vitro* neutral detergent fibre digestibility, macro-mineral contents, individual polyphenols, C18:1*n*9 and C18:2*n*6 compared to other leaf-meals. Regardless of the season, *A. mearnsii* leaf-meals had greater amino acid concentrations than *A. dealbata* leaf-meals. Hot-dry-season leaf-meals had a greater concentration of total flavonoids than the leaf-meals in the cool-wet-season irrespective of species. Antioxidant potency composite of the Acacia leaf-meals was in the order of *A. dealbata* × hot-dry-season > *A. mearnsii* × hot-dry-season > *A. mearnsii* × cool-wet-season > *A. dealbata* × cool-wet-season. The findings suggested that *A. mearnsii* leaf-meal harvested in the hot-dry-season has superior nutritional and polyphenolic profiles, which could support its use as a nutraceutical and preservative for the enhancement of ruminant production and product quality compared to other leaf meals.

Introduction

Invasive alien plants (IAPs) reduce biodiversity and the capacity of rangelands to support livestock production (Jones and McDermott, 2018). Amongst IAPs, the genus *Acacia sensu stricto* (formerly subgenus *Phyllodineae)* native to Australia is the most widespread with 24 out of 1000 species classified as invasive globally (Richardson *et al*., 2015). In South Africa, black wattle (*Acacia mearnsii*) and silver wattle (*A. dealbata*) are the dominant IAPs covering about 10 million hectares of mesic areas land (Gwate *et al*., 2016; Gouws and Shackleton, 2019). These species were introduced in South Africa from Australia for the commercial production of tannins and woodchips for export but became invasive (Chan *et al*., 2015). Control of *A. mearnsii* and *A. dealbata* species has, therefore, become a policy priority for the South African government (Richardson *et al*., 2015; Shackleton *et al*., 2018). Mechanical and chemical methods, which involve cutting the Acacias with chainsaws and applying herbicides on the cut stump are currently being used to prevent new establishments and reducing existing ones (Richardson *et al*., 2015; Shackleton *et al*., 2018). In the smallholder farming areas, the cut branches are used for fencing, firewood or charcoal (De Neergaard *et al*., 2005; Gouws and Shackleton, 2019) but leaves are left to decompose naturally.

Acacia trees have high rates of leaf-litter accumulation that is deposited in large quantities, which become waste and proliferate pests and diseases over time (Eyles *et al*., 2008). These leaves could be beneficial for improving the production and quality of animal-sourced foods as they contain moderate contents of crude protein (CP, 130–175 g/kg DM; Salem, 2005), high contents of neutral detergent fibre (NDF, 180–457 g/kg; De Neergaard *et al*., 2005; Giridhar *et al*., 2018) and proanthocyanidins (up to 150 g/kg DM; Degen *et al*., 2000; Xiong *et al*., 2016) that have nutritional, helminth suppressant, anti-bloat, antioxidant and antimicrobial properties (Sottie *et al*., 2014; Yoshihara *et al*., 2014; Koenig *et al*., 2018; Lima *et al*., 2019). In this regard, the valorisation of Acacia leaves presents an economic incentive for the sustainable management of IAPs and opportunities to unlock new value chains for the Acacia and animal feed industries to progress towards a circular bioeconomy.

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The utilisation of Acacia leaves as livestock feed is dependent upon their nutritional composition and bioactive phytochemical profile, which are largely influenced by biotic and abiotic environmental factors, particularity plant species and seasons, respectively (Mueller-Harvey *et al.*, 2019). Intra- and inter-species chemical variability and diversity is attributable to the interactive effects of plant genes, ontogeny, phenology and phenotypic plasticity (Moore *et al.*, 2014; Hasan *et al.*, 2020). The phenotypic plasticity of plants is largely influenced by seasonality (Chuine and Régnière, 2017). Seasonal changes result in annual variations in day length, temperature, light, radiation, humidity, precipitation and nutrient supply, which subsequently influence plant chemical profiles (Williams *et al.*, 2017; Choudhary *et al.*, 2019). However, there is a dearth of information regarding the effects of season on nutritional, phytochemical and antioxidant profiles of *A. mearnsii* and *A. dealbata* leaves. The information is crucial in unmasking the potential of Acacia leaves as nutraceuticals and preservatives for enhancing ruminant production and product quality, respectively. The objective of the current study was, therefore, to evaluate the effects of season on the nutrient, phytochemical and antioxidant profiles of *A. mearnsii* and *A. dealbata* leaves.

Materials and methods

Study site, collection and preparation of Acacia leaf-meals

Acacia mearnsii and *A. dealbata* leaves were harvested during the hot-dry (i.e. February) and cool-wet (i.e. June) seasons in two locations (33°52′27″ 18°58′33″E; 33°55′31.8″S 18°52′45.3″E, respectively) in the Mediterranean region of Pniel, South Africa. These two locations are in the same agroecological zone with an annual temperature ranging from 6 to 30°C and receive rainfall between 250 and 350 mm per annum. A systematic sampling technique was used to sample Acacia trees for leaf harvesting. Two 100 m transect lines were set diagonally across each other in a one-hectare plot and held in place by metal pins. Along each transect line, four 5×5 m^2 quadrants were placed at 20 m intervals using a measuring tape. In each quadrant, leaves were harvested from four trees with a breast height diameter between 6 and 8 cm by pruning six lower branches from each tree. The harvested leaves from each quadrant ($n = 8$/species) were placed in brown khaki bags and transported to the Department of Animal Sciences at Stellenbosch University. Exactly, 2 kg of fresh leaves per sample were oven-dried at 50°C for 72 h according to Makkar (2003) and ground using a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA) with a 1 mm sieve and stored in pre-labelled airtight containers at 4°C pending analyses.

Proximate and fibre analysis

The AOAC (2002) procedures were used to determine the dry matter (DM), ash and ether extract (EE) contents of the samples. The Dumas method with a macro-Nitrogen analyser (LECO® FP528, LECO Corporation, Miami, USA) was used to determine total nitrogen content and multiplied by a 6.25 factor to calculate the crude protein (CP) content. Starch was analysed using a commercial assay (Total Starch Megazyme kit KTSTA, Megazyme International Ireland Ltd., Wicklow, Ireland), following methodology by Hall (2009). Neutral detergent fibre (aNDFom) was analysed according to Mertens (2002), using sodium sulphite and alpha-amylase. Acid detergent fibre (ADFom) was determined according to AOAC (2002). Lignin (sa) was determined by solubilization of cellulose with sulphuric acid according to Raffrenato and Van Amburgh (2011). Neutral detergent fibre, ADFom and lignin (sa) were expressed exclusive of ash content. All analyses were performed in triplicate. The non-fibrous carbohydrates (NFC) were determined using the following formula:

$$1000\ \text{g/kg} - ([Ash + aNDFom + CP + EE]\ \text{g/kg}).$$

Neutral detergent soluble fibre (NDSF) was computed by subtracting aNDFom (g/kg) from 1000 g/kg. Pectin and sugars were calculated according to López *et al.* (2014) by subtracting the content of starch (g/kg DM) from NFC (g/kg) content.

In vitro *digestibility analysis*

Two rumen-cannulated Holstein dairy cows were used as rumen content donors for the *in vitro* aNDFom digestibility (*iv*NDFd). The animals were grazing on Kikiyu pasture, and the rumen fluid was collected at 08:00 before the animals were let out for grazing. The rumen fluid was immediately transported to the laboratory in a pre-warmed insulated thermos flask. The rumen fluid was filtered through four layers of cheesecloth, 100 µm mesh and glass wool prior to inoculation. About ~0.5 g ground sample of *A. mearnsii* and *A. dealbata* leaf-meals in duplicates were weighed into 125 ml Erlenmeyer flask, with the addition of 40 ml of Van Soest buffer according to Raffrenato *et al.* (2018). The flasks were placed in a heated (39.5°C) shaking water bath under carbon dioxide positive pressure to ensure an anaerobic environment, thereafter, 10 ml of rumen fluid was added.

In vitro aNDFom digestibility was measured at 24 and 48 h. After incubation, the undigested matter was analysed for aNDFom content by filtering the boiled samples through Gooch sintered glass crucible (40–60 µm) crucibles with a Whatman glass microfiber filter (934-AH®, GE Healthcare, Pittsburgh, PA, USA), which minimises particle loss and increase recovery. The difference between the undigested aNDFom after boiling and the aNDFom content of the leaves was used to calculate the digestibility coefficient of *iv*NDFd on a DM basis. Each sample within treatment was analysed in duplicate, and the analysis was performed in three runs.

Amino acid analysis

Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) fitted with a photo-diode array detector (UPLC-PDA) was used for separation and detection of amino acids (AA) using AccQ Fluor reagent Kit, Waters (En Yvelines Cedex, France). Briefly, 0.1 g grounded dried leaf-meals of *A. mearnsii* and *A. dealbata* was used to determine AA composition using 0.5 ml of 6 M hydrochloric acid for extraction and L-Norvaline as an internal standard. In the vials, 10 µl of the standard and sample was added, with 70 µl buffer solution (0.2 M borate buffer) and 20 µl of derivatisation reagent (2 mg/ml 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). The vials were incubated at 55°C for 10 min to build stable derivatives. A volume of 1 µl of standard/sample solution was injected into the mobile phase onto the Waters UltraTag C18 column (2.1 × 50 mm × 1.7 µm) at 60°C. The amino acids were quantified against a 16 amino acid standard (AAS18-5ml, Sigma-Aldrich, USA). During the development of the chromatographic method,

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individual amino acids standards were prepared to identify the unique retention time of each amino acid. After derivatization of the calibration standards, they were analysed using the Waters AQC UPLC chromatographic method. The gradient of the method was adjusted until baseline separation of all 16 amino acids, the AMQ peak from the derivatizing agent and the L-Norvaline internal standard peak was achieved. Analytes eluting off the column were detected by the PDA detector, with each AA coming off the column at a unique retention time. Calibration curve was plotted, and AA concentration quantified in MassLynx V4.1 2011 software (Waters, Milford, USA), using peak areas and retention times. The analyses were performed in triplicate per sample.

Mineral content analysis

Minerals were quantified using the inductively coupled plasma-mass spectroscopy (ICPMS) and –atomic emission spectrometry (ICPAES) for micro, and macro minerals, respectively, according to Sah and Miller (1992). The samples were digested using CEM MARS microwave digester. For ICPMS digested solid samples were introduced into the instrument via autosampler by a peristaltic pump. Small droplets pass through to the plasma, which produces ions that are directed into the MS and extracted from the interface region. An ion detector converts the ions into an electrical signal. This signal is expressed as counts using Masshunter software for calculating results using a calibration acceptance criterion of $R^2 > 0.9995$. For ICPAES, the ions excite in the plasma and emit characteristic light, measured by the Echelle optical design and charge injection device solid-state detector to provide elemental analysis. The instrument was controlled, and data were processed by iTEVA software using a calibration acceptance criterion of $R^2 > 0.9995$. The analyses were performed in triplicate.

Fatty acids analysis

Following the procedures of Sukhija and Palmquist (1988), individual and total lipids for *A. mearnsii* and *A. dealbata* leaf-meals were determined in triplicate. Briefly, 2.5 ml of n-hexane was used to extract 0.5 g dried samples with 100 µl of 0.1 ml/l of heptadecanoic acid as an internal standard. Thereafter, 1 ml of 20% (v/v) sulphuric acid in methanol was added for the derivatisation step. The samples were then vortexed and incubated at 70°C for 60 min in a water bath. After cooling at room temperature, 2 ml of 20% (w/v) sodium chloride was added and samples vortexed. The top layer was transferred using a glass Pasteur pipette into fatty acid methyl esters (FAMEs) vials. The FAMEs were analysed using a gas chromatograph (6890 N, Agilent Technologies) coupled to a flame ionisation detector. Separation of the FAMEs was performed on a polar RT-2560 (100 m length × 0.25 mm internal diameter × 0.20 µm film thickness) (Restek, USA) capillary column. Helium was used as the carrier gas at a flow rate of 1 ml/min. The injector temperature was maintained at 250°C and 1 µl of the sample was injected in a 5:1 split ratio. The oven temperature was programmed at 50°C for 2 min and ramped up to 180°C at a rate of 25°C/min and held for 5 min and finally ramped up to 250°C at a rate of 3°C for 2 min. The peak areas and retention times relative to the Supelco™ 37 FAME mix, (Supelco, USA) were used to identify the FAME. The content of fatty acids (FA) was expressed as g/100 g of total FAME.

Polyphenol extraction

About 500 g of dried *A. mearnsii* and *A. dealbata* leaves were ground to pass a 2 mm sieve on a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA), thereafter, 100 g of the ground sample was further ground to pass a 0.5 mm sieve. For extraction, 20 ml of 80% aqueous methanol (v/v) was added to 2 g of the ground sample and placed in an ultrasonic water bath for 20 min and then centrifuged for 20 min set at 10 000 × g at 4°C. The supernatant was collected, and the sample was re-extracted using 20 ml of 80% aqueous methanol (v/v). The supernatants were mixed and stored at −80°C pending for analysis. Before analysis, the supernatants were diluted by 63.7% for all analyses besides simple phenolics analysis which was diluted at 21.2%. All polyphenols were analysed in triplicate.

Polyphenol analysis

Individual phenolic compounds were identified based on methods described by Tsugawa *et al.* (2015) and Lai *et al.* (2018). A waters synapt G2 quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a UPLC (Waters, Milford, MA, USA) was used for greater-resolution UPLC-MS analysis. Column eluate first passed through a photodiode array (PDA) detector before going to the MS, allowing simultaneous collection of UV and MS spectra. Electrospray ionisation was used in negative mode with a cone voltage of 15 V, desolvation temperature of 275°C, desolvation gas at 650 l/h and the rest of the MS settings optimised for best resolution and sensitivity. Data were acquired by scanning from 150 to 1500 m/z in resolution mode as well as in MSE mode. In MSE mode two channels of MS data were acquired, one at a low collision energy (4 V) and the second using a collision energy ramp (40–100 V) to obtain fragmentation data. Leucine enkaphalin was used as lock mass (reference mass) for accurate mass determination and the instrument was calibrated with sodium formate. Separation was achieved on a waters HSS T3, 2.1 × 100 mm, 1.7 µm column. An injection volume of 2 µl was used and the mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid as solvent B. Data were processed using MSDIAL and MSFINDER (RIKEN Center for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan).

The Folin-Ciocalteu colorimetric method as described by Makkar (2003) was used for the determination of total phenolics and tannin contents using ultraviolet (UV)-visible spectrophotometer (Thermo Scientific Technologies, Madison, WI, USA) and absorbance was read at 725 nm. The tannin content was calculated as follows; total phenolics without polyvinylpyrrolidone minus total phenolics with polyvinylpyrrolidone addition. A calibration curve was obtained using gallic acid and samples were analysed in triplicate. The results were expressed on a gram gallic acid equivalent (GAE)/kg DM. Proanthocyanidins were determined following the procedure of Porter *et al.* (1986) and the results were expressed as g/kg DM leucocyanidin equivalent.

The total contents of flavonoids were determined following the procedure by Yang *et al.* (2009). Briefly, a 0.25 ml aliquot of extracts (63.7% dilution) was mixed with 1.25 ml of deionised water and added to 0.075 ml of 5% sodium nitrite solution. The solution was incubated for 5 min then 0.15 ml of 10% aluminium chloride was added and incubated for 5 min. Thereafter, 0.5 ml of 1 M sodium hydroxide was added to stop the reaction and 0.775 ml of deionised water was added and read at 510 nm using a

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UV-visible spectrophotometer. The total flavonoids content was calculated using a catechin standard curve and expressed as g of catechin equivalents (CE)/kg extract DM.

Antioxidant activity analysis

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was performed in triplicate following methodology by Tolic *et al.* (2015). A 20 µl aliquot of sample extracts was diluted in 1 ml of 80% methanol and mixed with an equal volume of 0.1 mM DPPH reagent and incubated in the dark for 30 min and read at 517 nm absorbance using a UV-visible spectrophotometer. Methanol was used as a negative control and L-ascorbic acid as a positive control. The results were expressed as % radical scavenging activity.

The ferric reducing antioxidant power (FRAP) assay was conducted in triplicate as outlined by Benzie and Strain (1996). Aliquots of freshly prepared reagent containing 25 ml of 300 mM acetate buffer (pH 3.6), 2.5 ml of 20 mM of iron (III) heptahydrate and 2.5 ml of 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution was mixed with plant extracts before incubation for 30 min in the dark. The method uses low pH to reduce the Fe^{3+}-2,4,6-tris (pyridyl)-s-triazine (TPTZ) complex to the ferrous form. This reduction is monitored by measuring the absorbance change at 595 nm. The standard curve was prepared using Trolox and the FRAP values were reported as µmol Trolox equivalent per g DM.

A 15-Lipoxygenase (15-LOX) inhibition assay was conducted according to Waslidge and Hayes (1995). Dimethyl sulfoxide (DMSO) was used as a negative control and 15-LOX as a positive control. The 100% initial activity (IA) wells were prepared by adding 50 µl of LOX and 20 µl of 10% DMSO. Sample wells (inhibitor) contained 50 µl of LOX and 20 µl of extract. The reaction was initiated by adding 50 µl of linoleic acid (140 µM) to all the wells and incubated for 10 min and 100 µl of 15-LOX as added to terminate the reaction. The Fe^{3+}-dye complex was allowed to develop for 30 min at 25°C and the absorbance was read at 570 nm on a UV-visible spectrophotometer and the result expressed % inhibition of lipoxygenase activity. The per cent lipoxygenase inhibition for each extract was determined as follows:

Inhibition = [1–IA − Inhibitor)/IA] × 100. To quantitatively evaluate the overall phytochemical quality, the antioxidant potency composite (APC) index was calculated based on antioxidant index score = [(sample score/best score) × 100], averaged for all four tests for each extract.

Statistical analysis

The data were analysed as a completely randomized design with a 2 (seasons) × 2 (Acacia species) factorial arrangement of treatments using the generalized linear model procedure of SAS v.9.4 (SAS Institute Inc., Cary, NC, USA). The total number of observations for proximate, AA, FA, DPPH, FRAP, LOX, total flavonoids, total phenols, total tannins and proanthocyanidins analyses were 96 (2 Acacia species × 2 seasons × 8 samples × 3 replicates) and for minerals and phenolic compounds were 48 (2 Acacia species × 2 seasons × 4 samples × 3 replicates). For *iv*NDFd the total number of observations was 192 (2 Acacia species × 2 seasons × 8 samples × 2 replicates × 3 runs). After averaging the replications and/or runs, 32 (proximate, *iv*NDFd, AA, FA, DPPH, FRAP, 15-LOX, total flavonoids, total phenols, total tannins and proanthocyanidins), and 16 (minerals and phenolic compounds) observations were subjected to analysis of variance using the following statistical model: $Y_{ijk} = \mu + A_i + B_j + A \times B_{ij} + \varepsilon_{ijk}$ where: Y_{ijk} = variable response of the kth observation in ith species and jth season, μ = overall mean, A_i = effect of ith species (i = *A. mearnsii* and *A. dealbata*), B_j = effect of the jth season (j = hot-dry and cool-wet), $A \times B_{ij}$ = interaction of ith species and jth season, ε_{ijk} = residual error. The treatment means were generated using the least-square means option. Tukey's test was applied for LSMEANS separation. Results were considered significantly different at $P < 0.05$.

Results

Chemical composition

Acacia species and season interaction had a significant effect on all the analysed proximate components (Table 1). Contents of DM and lignin (sa) in the leaf-meals were in the following order: *A. dealbata* × hot-dry-season > *A. mearnsii* × hot-dry-season > *A. mearnsii* × cool-wet-season > *A. dealbata* × cool-wet-season ($P < 0.05$). The ash content was greatest in *A. dealbata* leaf-meal in the hot-dry-season, lowest in *A. dealbata* × cool-wet-season leaf-meal and intermediate in *A. mearnsii* leaf-meals irrespective of the season ($P < 0.05$). The CP and NDSF contents of the leaf meals were in the order of *A. mearnsii* × hot-dry-season > *A. dealbata* × hot-dry-season > *A. dealbata* × cool-wet-season > *A. mearnsii* × cool-wet-season ($P < 0.05$). Starch content was greatest in *A. dealbata* × cool-wet-season followed by *A. dealbata* × hot-dry-season, *A. mearnsii* × cool-wet-season and *A. mearnsii* × hot-dry-season ($P < 0.05$). Ether extract was lowest in leaf-meal of *A. dealbata* in the cool-wet-season, and highest in *A. mearnsii* × hot-dry-season ($P < 0.05$). The aNDFom content in Acacia leaf-meals was in the order of *A. mearnsii* × cool-wet-season > *A. dealbata* × cool-wet-season > *A. dealbata* × hot-dry-season > *A. mearnsii* × hot-dry-season ($P < 0.05$). The ADFom and NFC contents of Acacia leaf-meals were lowest in *A. mearnsii* × hot-dry-season, greatest in *A. dealbata* × cool-wet-season and intermediate in both *A. dealbata* × hot-dry-season and *A. mearnsii* × cool-wet-season leaf-meals ($P < 0.05$). Pectin and sugars were greatest in the *A. mearnsii* × hot-dry-season leaf-meal, intermediate in the *A. mearnsii* × cool-wet-season and lowest in the *A. dealbata* leaf-meals regardless of season ($P < 0.05$).

In vitro *neutral detergent fibre digestibility*

For 24 and 48 h *iv*NDFd, significant effects were observed for species, season and their interaction (Table 1). *Acacia mearnsii* leaf-meal harvested in the hot-dry-season had the greatest coefficient *iv*NDFd at 24 and 48 h, with cool-wet-season leaf-meals having intermediate values regardless of species while lowest for *A. dealbata* leaf-meal harvested in the hot-dry-season ($P < 0.05$; Table 1).

Amino acid composition

A significant species × season interaction was observed for histidine, methionine, phenylalanine, arginine and proline among the essential AA (Table 2). The concentration of histidine was greater ($P < 0.05$) in *A. mearnsii* leaf-meal harvested in the hot-dry-season than *A. dealbata* × hot-dry-season leaf-meal but both were not different ($P > 0.05$) from both leaf-meal species harvested in the cool-wet-season (Table 2). Methionine concentration was lower ($P < 0.05$) in *A. dealbata* × hot-dry-season leaf-meal than in other leaf-meals ($P < 0.05$; Table 2). Phenylalanine concentration in

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Table 1. Least square means of the proximate and fibre contents, and *in vitro* digestibility of *A. mearnsii* and *A. dealbata* leaf-meals in the hot-dry and cool-wet seasons

	Hot-dry-season		Cool-wet-season			*P*-value		
Proximate components (g/kg DM)	*A. mearnsii*	*A. dealbata*	*A. mearnsii*	*A. dealbata*	S.E.M.	Species	Season	Species × season
Dry matter	955	964	893	885	1.6	0.593	0.001	0.001
Ash	40.0	50.0	38.5	29.6	0.90	0.528	0.001	0.001
Crude protein	246	209	165	199	1.9	0.502	0.001	0.001
Starch	74.7	164	129	178	1.5	0.001	0.001	0.001
Ether extract	50.2	43.7	48.6	37.0	0.40	0.001	0.001	0.001
Fibre and non-fibre components (g/kg DM)								
Neutral detergent fibre	360	367	418	381	1.6	0.001	0.001	0.001
Neutral detergent soluble fibre	640	633	582	619	1.6	0.001	0.001	0.001
Acid detergent fibre	312	356	364	379	1.7	0.001	0.001	0.001
Lignin (sa)	230	246	220	192	1.3	0.001	0.001	0.001
Non-fibrous carbohydrates	306	337	329	346	2.4	0.001	0.001	0.006
Pectin and sugars	231	173	200	168	2.6	0.001	0.001	0.001
In vitro digestibility coefficient								
24 h aNDFom	0.2	0.1	0.2	0.2	0.36	0.001	0.011	0.001
48 h aNDFom	0.2	0.2	0.2	0.2	0.19	0.001	0.001	0.001

S.E.M, standard error of means; aNDFom, neutral detergent fibre exclusive of ash.

Table 2. Least square means of the concentration of amino acids in *A. mearnsii* and *A. dealbata* leaf-meals in the hot-dry and cool-wet seasons

	Hot-dry-season		Cool-wet-season			*P*-value		
Amino acids (g/100 g DM)	*A. mearnsii*	*A. dealbata*	*A. mearnsii*	*A. dealbata*	S.E.M.	Species	Season	Species × season
Essential								
Histidine	0.36	0.26	0.28	0.34	0.026	0.409	0.991	0.005
Isoleucine	0.56	0.47	0.67	0.56	0.019	0.001	0.001	0.636
Leucine	1.08	0.85	1.33	1.11	0.034	0.001	0.001	0.799
Lysine	1.04	0.86	1.28	0.94	0.059	0.001	0.011	0.234
Methionine	0.13	0.11	0.13	0.14	0.008	0.249	0.039	0.026
Phenylalanine	1.05	0.81	1.38	0.90	0.043	0.001	0.001	0.006
Threonine	0.69	0.57	0.77	0.70	0.025	0.001	0.001	0.636
Valine	0.76	0.60	0.85	0.75	0.026	0.001	0.001	0.799
Non-essential								
Arginine	0.87	0.53	0.86	0.79	0.032	0.001	0.002	0.001
Glycine	0.65	0.53	0.78	0.69	0.023	0.001	0.001	0.453
Proline	1.48	0.78	1.18	0.90	0.044	0.001	0.047	0.001
Tyrosine	0.97	0.79	1.14	0.87	0.038	0.001	0.002	0.281
Alanine	0.80	0.65	1.04	0.93	0.041	0.003	0.001	0.566
Asparagine	1.57	1.15	1.72	1.29	0.050	0.001	0.007	0.935
Glutamine	1.37	1.24	1.70	1.43	0.058	0.001	0.001	0.245
Serine	0.77	0.68	0.83	0.70	0.027	0.003	0.068	0.583

S.E.M, standard error of means.

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Acacia leaf-meals was in the following order: *A. mearnsii* × cool-wet-season > *A. mearnsii* × hot-dry-season > *A. dealbata* × cool-wet-season > *A. dealbata* × hot-dry-season ($P < 0.05$; Table 2).

Arginine was lower ($P < 0.05$) in *A. dealbata* × hot-dry-season leaf-meal than in other interactions (Table 2). Concentrations of proline were greatest in the *A. mearnsii* × hot-dry-season leaf-meal followed by *A. mearnsii* × cool-wet-season leaf-meal and lowest in *A. dealbata* leaf-meals irrespective of the season ($P < 0.05$; Table 2). Species × season interaction had no effect ($P > 0.05$) on the remaining AA but were significantly influenced by both species and season except for serine which was influenced by species only (Table 2). The concentrations of isoleucine, leucine, serine, lysine, threonine, valine, glycine, tyrosine, alanine, asparagine, glutamine and serine were greater ($P < 0.05$) in *A. mearnsii* leaf-meals than in *A. dealbata* leaf-meals. The leaf-meals for the same AA except for serine had greater ($P < 0.05$) concentrations in the cool-wet-season than in the hot-dry-season (Table 2).

Mineral composition

Acacia species × season interaction influenced ($P < 0.05$) most of the analysed minerals, except for zinc ($P > 0.05$; Table 3). The content of calcium in leaf-meals was in the following order: *A. dealbata* × cool-wet-season > *A. mearnsii* × hot-dry-season > *A. dealbata* × hot-dry-season > *A. mearnsii* × cool-wet-season ($P < 0.05$; Table 3). Potassium and phosphorus leaf-meal contents were greatest in *A. mearnsii* × hot-dry-season followed by *A. mearnsii* × cool-wet-season, *A. dealbata* × cool-wet-season and *A. dealbata* × hot-dry-season in that order ($P < 0.05$; Table 3). Regardless of the season, *A. mearnsii* leaf-meals had the greatest contents of magnesium followed by *A. dealbata* leaf-meal harvested in the cool-wet and hot-dry-season in that order ($P < 0.05$; Table 3). Sodium concentration in Acacia leaf-meals was in the order of *A. mearnsii* × cool-wet-season > *A. mearnsii* × hot-dry-season > *A. dealbata* leaf-meals irrespective of the season ($P < 0.05$; Table 3).

Copper content was greatest in *A. mearnsii* leaf-meal in the cool-wet-season and lowest in *A. dealbata* leaf-meal in the hot-dry-season ($P < 0.05$; Table 3). The content of selenium was greater ($P < 0.05$) in *A. dealbata* leaf-meal in the cool-wet-season compared to the other interactions (Table 3). Iron was greatest in *A. dealbata* leaf-meal harvested in the cool-wet-season and lowest in *A. mearnsii* leaf-meal harvested in the hot-dry-season ($P < 0.05$; Table 3). The manganese content in the leaf-meals was in the order of *A. mearnsii* leaf-meal × cool-wet-season > *A. dealbata* × cool-wet-season > *A. mearnsii* × hot-dry-season > *A. dealbata* × hot-dry-season ($P < 0.05$; Table 3). The content molybdenum in leaf-meals followed the order of *A. dealbata* × cool-wet-season > *A. dealbata* × hot-dry-season > *A. mearnsii* × hot-dry-season > *A. mearnsii* × cool-wet-season ($P < 0.05$; Table 3). Aluminium content in leaf-meals was greatest in the *A. dealbata* × cool-wet-season leaf-meal, intermediate in the *A. dealbata* × hot-dry-season and lowest in *A. mearnsii* leaf-meals regardless of season ($P < 0.05$; Table 3). Regardless of the season, the zinc content of the leaf-meals was greater ($P < 0.05$) in *A. mearnsii* than *A. dealbata.* Cool-wet-season leaf-meal had greater ($P < 0.05$) zinc content than the hot-dry-season leaf-meal (Table 3).

Fatty acid composition

Acacia species × season interaction had a significant effect on all the analysed FA (Table 4). Palmitic (C16:0) and linoleic acid (C18:2*n*6) proportions in Acacia leaf-meals were greatest in *A. mearnsii* × hot-dry-season, intermediate for *A. mearnsii* × cool-wet-season and lowest in *A. dealbata* leaf-meals regardless of season ($P < 0.05$). The proportion of stearic acid (C18:0) was greatest in *A. mearnsii* leaf-meals irrespective of the season and lowest in *A. dealbata* leaf-meal in the cool-wet-season ($P < 0.05$). *Acacia dealbata* leaf-meal in the hot-dry-season had the greatest proportions of arachidic acid (C20:0) with *A. mearnsii* leaf-meal in the cool-wet-season having the lowest proportions ($P < 0.05$). The proportion of behenic acid (C22:0) was greatest in *A. mearnsii* leaf-meal harvested in the hot-dry-season and lowest in the leaf-meals harvested in the cool-wet-season regardless of species ($P < 0.05$). Total saturated fatty acids (SFA) were greatest in *A. mearnsii* leaf-meal harvested in the hot-dry-season followed by *A. mearnsii* × cool-wet-season and lowest in *A. dealbata* × cool-wet-season ($P < 0.05$). The proportions of oleic acid (C18:1*n*9) in leaf-meals followed the order of *A. mearnsii* × hot-dry-season > *A. dealbata* × hot-dry-season > *A. dealbata* × cool-wet-season > *A. mearnsii* × cool-wet-season ($P < 0.05$). Alpha-linolenic acid (C18:3*n*3) was greatest in *A. dealbata* leaf-meal in the cool-wet-season, and lowest in *A. mearnsii* leaf-meal in the hot-dry-season ($P < 0.05$). The proportion of γ-linolenic acid (C18:3*n*6) was greatest for leaf-meals harvested in the hot-dry-season irrespective of species and lowest in *A. dealbata* leaf-meal in the cool-wet-season ($P < 0.05$). Total polyunsaturated fatty acids (PUFA) were greatest in leaf-meals of *A. dealbata* × cool-wet-season and lowest in *A. mearnsii* × hot-dry-season ($P < 0.05$).

Phytochemical composition

Acacia species × season interaction had a significant effect only on quercetin-3-galactoside, *p*-Coumaroyltrifolin A and terpene lactone. *Acacia mearnsii* leaf-meal harvested in the hot-dry-season had a greater ($P < 0.05$) concentration of quercetin-3-galactoside relative to other leaf meals (Table 5). The concentration of *p*-Coumaroyltrifolin A and terpene lactone in leaf-meals were in the order of *A. mearnsii* × hot-dry-season > *A. mearnsii* × cool-wet-season > *A. dealbata* × hot-dry-season = *A. dealbata* × cool-wet-season ($P < 0.05$; Table 5). The concentration of (−)-epigallocatechin, *p*-Coumaroyltrifolin B, kaempferol 3-sophorotrioside and quercitrin were influenced by both species and season with, *A. dealbata* leaf-meals and cool-wet-season leaf-meals having greater ($P < 0.05$) concentrations than the other treatments (Table 5). For isorhamnetin, astilbin and 3-*O*-beta-glucopyranosyl-(1 > 6)-beta-glucopyranosyl-1-octen-3-ol, their concentrations were greater ($P < 0.05$) in *A. mearnsii* leaf-meals and cool-wet-season leaf-meals than the other treatments ($P < 0.05$; Table 5). Tataroside was influenced by species only with *A. mearnsii* leaf-meals having greater ($P < 0.05$) concentration than *A. dealbata* leaf-meals (Table 5).

There was a significant interaction of Acacia species × season for total flavonoids concentrations, with *A. mearnsii* and *A. dealbata* leaf-meals harvested in the hot-dry-season having greater concentration than the other interactions (Table 5). The concentrations of total phenols and total tannins were only influenced by species with *A. dealbata* leaf-meals having greater ($P < 0.05$) concentrations than *A. mearnsii* leaf-meals (Table 5). No significant interaction of Acacia species × season was observed for proanthocyanidins.

Antioxidant activity

The effects of species, season and their interaction were not significant for DPPH (Table 6). Lipoxygenase and FRAP activity

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Table 3. Least square means of the mineral contents in *A. mearnsii* and *A. dealbata* leaf-meals in the hot-dry and cool-wet seasons

	Hot-dry-season		Cool-wet-season			*P*-value		
Minerals (g/kg DM)	*A. mearnsii*	*A. dealbata*	*A. mearnsii*	*A. dealbata*	S.E.M.	Species	Season	Species × season
Macro								
Calcium	7.17	6.52	4.57	11.83	0.035	0.001	0.001	0.001
Potassium	9.18	5.50	7.82	6.16	0.095	0.001	0.007	0.001
Magnesium	2.36	1.23	2.34	1.97	0.023	0.001	0.001	0.001
Sodium	1.08	0.33	1.17	0.34	0.084	0.001	0.001	0.001
Phosphorus	0.95	0.64	0.89	0.81	0.090	0.001	0.001	0.001
Micro								
Copper[1]	8594	4154	9636	7822	122.1	0.001	0.001	0.001
Zinc[1]	16 824	10 977	19 852	15 384	510.1	0.001	0.001	0.183
Selenium[1]	58	56	60	82	1.7	0.001	0.001	0.001
Iron	0.21	0.27	0.22	0.32	0.001	0.001	0.001	0.001
Manganese	0.11	0.09	0.14	0.13	0.001	0.001	0.001	0.027
Trace								
Molybdenum[1]	39.3	52.4	28.1	77.3	0.96	0.001	0.001	0.001
Aluminium	0.27	0.33	0.28	0.37	0.006	0.001	0.001	0.033

S.E.M, standard error of means.
[1]μg/kg.

Table 4. Least square means of fatty acid contents in *A. mearnsii* and *A. dealbata* leaf-meals in the hot-dry and cool-wet seasons

	Hot-dry-season		Cool-wet-season			*P*-value		
Fatty acids (g/100 g of total fatty acids)	*A. mearnsii*	*A. dealbata*	*A. mearnsii*	*A. dealbata*	S.E.M.	Species	Season	Species × season
C16:0	27.4	20.8	24.6	21.6	0.61	0.001	0.130	0.005
C18:0	3.7	3.0	3.8	2.2	0.14	0.001	0.022	0.004
C20:0	1.46	2.19	0.44	1.38	0.046	0.001	0.001	0.032
C22:0	3.10	2.69	1.52	1.74	0.070	0.001	0.001	0.001
Total SFAs	35.7	28.6	30.4	26.9	0.62	0.001	0.001	0.001
C18:1*n*9	7.5	6.6	5.0	5.8	0.16	0.869	0.001	0.001
C18:3*n*3	30.2	43.8	42.8	48.9	0.47	0.001	0.001	0.001
C18:2*n*6	21.2	14.4	16.4	14.3	0.39	0.001	0.001	0.001
C18:3*n*6	3.01	2.95	2.59	1.90	0.063	0.001	0.001	0.001
Total PUFAs	54.4	61.2	61.8	65.1	0.66	0.001	0.001	0.013

S.E.M., standard error of means; SFAs, Saturated fatty acids (sum of C16:0, C18:0, C20:0 and C22:0); PUFAs, Polyunsaturated fatty acids (sum of C18:3*n*3, C18:2*n*6 and C18:3*n*6).

were not affected by Acacia species × season interaction ($P > 0.05$; Table 6), but individual effects of species and season were significant. Lipoxygenase activity was greater ($P < 0.05$) for *A. mearnsii* leaf-meals and hot-dry-season leaf-meals than in their counterparts (Table 6). Relative to their counterparts, *A. dealbata* leaf-meals and hot-dry-season leaf-meals had greater ($P < 0.05$) FRAP activity (Table 6). There was a significant interaction of species × season for APC index with the following order: *A. dealbata* × hot-dry-season > *A. mearnsii* × hot-dry-season > *A. mearnsii* × cool-wet-season > *A. dealbata* × cool-wet-season.

Discussion

The greater content of CP observed for *A. mearnsii* leaf-meal harvested in the hot-dry-season compared to other leaf-meals could be related to the interconnected effects of genetics and primary environmental stressors (i.e. high temperature, solar radiation and water deficit). Heat, solar radiation and water stress in the hot-dry-season might have induced specific genes in *A. mearnsii* to synthesise and elevate the accumulation of stress-specific proteins such as proline (Ul Haq *et al.*, 2019; Karmous and Verma,

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Table 5. Least square means of the concentrations of phenols in *A. mearnsii* and *A. dealbata* leaf-meals in the hot-dry and cool-wet seasons

Phenolic compound (mg/kg catechin)	Sub-class	Formula	Average retention time	Hot-dry-season		Cool-wet-season		S.E.M	*P*-value		
				A. mearnsii	*A. dealbata*	*A. mearnsii*	*A. dealbata*		Species	Season	Species × season
(−)-Epigallocatechin	Flavonols	$C_{15}H_{14}O_7$	8.17	13.9	17.1	17.2	21.7	0.72	0.013	0.001	0.546
Isorhamnetin	Flavonols	$C_{16}H_{12}O_7$	7.69	36	15	55	27	2.9	0.001	0.001	0.402
Kaempferol 3-sophorotrioside	Flavonols	$C_{33}H_{40}O_{22}$	14.61	35	56	78	48	3.8	0.001	0.005	0.417
Quercetin 3-galactoside	Flavonols	$C_{21}H_{20}O_{12}$	17.58	11.6	1.9	3.1	2.1	0.34	0.001	0.001	0.001
p-Coumaroyltrifolin A	Hydroxycinnamic acid	$C_{27}H_{30}O_{15}$	10.95	4.2	0.7	1.6	0.5	0.17	0.001	0.001	0.001
p-Coumaroyltrifolin B	Hydroxycinnamic acid	$C_{30}H_{26}O_{13}$	11.39	39	91	52	122	5.8	0.001	0.011	0.130
Quercitrin	Glycoside	$C_{15}H_{10}O_7$	19.77	36	57	48	79	2.7	0.001	0.001	0.189
3-O-beta-glucopyranosyl-(1 > 6)-beta-glucopyranosyl-1-octen-3-ol	Glycoside	$C_{26}H_{32}N_2O_5$	11.61	196	131	222	157	11.7	0.001	0.035	0.989
Terpene lactone	Terpenoid	$C_{23}H_{28}O_8$	13.47	10.9	4.7	7.7	5.2	0.49	0.001	0.013	0.001
Tataroside	Sesquiterpenoids	$C_{21}H_{32}O_{10}$	11.22	35	0	36	0	1.9	0.001	0.664	0.713
Astilbin	Dihydroflavonols	$C_{21}H_{22}O_{11}$	19.10	41	19	69	26	6.7	0.001	0.015	0.127
Polyphenols (g/kg DM)											
Total flavonoids[1]				0.35	0.35	0.29	0.26	0.016	0.388	0.454	0.001
Total phenols[2]				307	323	295	338	9.5	0.037	0.919	0.311
Total tannins[2]				285	307	269	313	9.4	0.016	0.719	0.409
Proanthocyanidins[3]				58.0	58.0	57.0	54.0	0.25	0.581	0.454	0.502

S.E.M., standard error of means.
[1]Expressed as catechin equivalent.
[2]Expressed as gallic acid equivalent.
[3]Expressed as leucocynadin equivalent.

Table 6. Least square means of antioxidant activity of *A. mearnsii* and *A. dealbata* leaf-meals in the hot-dry and cool-wet seasons

	Hot-dry-season		Cool-wet-season			*P*-value		
Compounds	*A. mearnsii*	*A. dealbata*	*A. mearnsii*	*A. dealbata*	S.E.M.	Species	Season	Species × season
DPPH	22.7	25.5	25.5	23.8	0.75	0.514	0.485	0.064
Lipoxygenase[1]	44	41	40	35	1.7	0.012	0.003	0.426
FRAP	52	56	48	50	1.2	0.002	0.001	0.301
APC index	93.5	97.7	92.0	86.8	0.00	0.001	0.001	0.001
Rank[2]	2	1	3	4				

S.E.M, standard error of means; DPPH, 2-diphenyl-1-picrylhydrazyl expressed as % radical scavenging activity; FRAP, ferric reducing antioxidant power expressed as micromolar Trolox equivalent per gram DM; APC, antioxidant potency composite index.
[1]Expressed as % inhibition of lipoxygenase activity.
[2]Ranked according to the APC index, with 1 being higher antioxidant activity.

2020), which was high in the same leaf-meal in the current study. The elevated CP content observed for *A. mearnsii* leaf-meal in the hot-dry-season could also be partly related to the low fibre and tannin contents reported for the same leaf-meal, which may have led to low fibre-protein and tannin-protein complexes, hence increased free unbound protein molecules (Grabber, 2005; Naumann *et al.*, 2017). If fed as a sole diet, the range of CP content (165–249 g/kg DM) in the current study is sufficient to meet the CP (110–175 g/kg DM) requirements for productive ruminants (Freer *et al.*, 2007).

The observation that *A. mearnsii* leaf-meal in the hot-dry-season had the greatest EE contents could be possibly related to genetics and seasonality in plant energy and fatty acid metabolism. The greater content of EE in *A. mearnsii* could be explained by a high content of chloroplasts due to its dark-olive green leaves compared to *A. dealbata* silvery-grey green leaves (Falcone *et al.*, 2004; Aid, 2019). Biosynthesis of FA occurs mostly in chloroplasts and in the plastids of non-photosynthetic tissues (Falcone *et al.*, 2004; Aid, 2019). The seasonal differences in EE can be explained by the fact that plants in the hot-dry-seasons optimise their energy metabolism by sequestering toxic FA degraded by membrane lipids that supply energy, while excess lipids are transformed into cutin and wax that prevent water loss and subsequently increases the lipid content of leaves (Falcone *et al.*, 2004; Kim, 2020). This is evident in the high unsaturated FA contents observed for *A. mearnsii* leaf-meal in hot-dry-season. The reported EE values (3.7–5.0 g/kg DM) in Acacia leaves are in the same range with those required for maintenance (i.e. 4.0–4.5 g/kg DM) in ruminants (Freer *et al.*, 2007).

The observation that starch was greatest in *A. dealbata* × cool-wet-season leaf-meal compared to other interactions, could be possibly related to their differences in leaf morphology and seasonal response to light intensity and water availability which influence photosynthesis. *Acacia dealbata* has larger leaves (Weeds of Australia, 2016), hence large photon-harvesting surface and more stomata than *A. mearnsii* (Yan *et al.*, 2017). Under low-light conditions prevalent in the cool-wet-season, plants develop a higher specific leaf area, which consequently increases photon-harvesting surface and stomata to optimise light capture and carbon gain for photosynthesis (Steinger *et al.*, 2003; Dong *et al.*, 2014; Liu *et al.*, 2016). Also, sufficient water in the cool-wet-season may increase stomata openings and subsequently increase carbon dioxide concentration in the mesophilic tissue that accelerates photosynthesis (Yan *et al.*, 2017). The process of photosynthesis has been reported to increase the content of non-structural carbohydrates in leaves that lead to greater starch reserves in plants (Thompson *et al.*, 2017). The present values for starch (75–178 g/kg DM) are lower than the readily fermentable carbohydrates values (230–280 g/kg) required for ruminant production (Freer *et al.*, 2007).

The elevated plant fibre contents noted for *A. mearnsii* leaf-meal harvested in the cool-wet-season could be related to this species' superior ability to increase formation and deposition of phenolic polymers (i.e. lignin and suberin) in the cell wall to prevent itself from chilling injury and cell collapse under cold stress (Sharma *et al.*, 2019). The greater content of aNDFom in *A. mearnsii* may also be ascribed to its faster growth rate compared to *A. dealbata,* which could have increased the accumulation of cell wall (Van Soest, 1994). The aNDFom values for the Acacia leaf meals reported in the current study are above the acceptable range of 100–210 g/kg DM aNDFom required for optimal DMI (319 g/kg DM) and digestibility (520 g/kg DM) of browse legume forages in small ruminant diets (Hassan *et al.*, 2015). Elevated lignin (sa) content observed for *A. dealbata* leaf-meal in the hot-dry-season may be attributed to high temperatures and light intensity in the hot-dry-season that stimulates lignin synthesis in plants along with an increased concentration of phenylalanine ammonia lyase (PAL), a key enzyme in the biosynthesis of structural and defence compounds in plants (Nelson and Moser, 1994; Bhardwaj *et al.*, 2014).

The findings that *A. mearnsii* leaf-meal in the hot-dry-season had greatest NDSF could be attributed to its low total tannin and fibre contents which could lessen the fibre-to-protein and tannin-to-protein ratios, resulting in less interlinking of soluble fibre with resistant protein and unbound tannins, thus increasing NDSF (Llobera and Cañellas, 2007). Furthermore, increased NDSF in hot-dry-season could be possibly due to plants ability to synthesise more soluble sugar-alcohols that substitute for water molecules in the hydration layer of proteins and membranes, thus allowing the functionality of enzymes at extremely low water concentrations (Figueroa *et al.*, 2020). The higher coefficient *iv*NDFd observed at 24 and 48 h for *A. mearnsii* leaf-meal in the hot-dry-season compared to other leaf-meals could be related to its higher CP and lower aNDFom contents. A higher CP content supplies nitrogenous compounds that stimulate microbial growth on the fibrous carbohydrates, which leads to increased aNDFom digestibility (Souza *et al.*, 2010).

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The finding that *A. mearnsii* leaf-meal harvested in the hot-dry-season contained high proline content was expected since plants in the hot-season releases proline because of its osmoprotective properties and ability to detoxify reactive oxygen species (ROS), enabling plants to cope with environmental stresses and sustain plant growth (Kavi Kishor and Sreenivasulu, 2014; Khan *et al.*, 2020). Generally, the cool-wet-season contained higher concentrations of most AA that may be attributed to the plants' ability to maintain basal levels of cellular metabolism at low temperatures by increasing dehydrogenase activity that leads to higher enzymatic activities, which accelerates protein synthesis (Guy, 1990). The observation that *A. mearnsii* contained greater contents of AA may be an indication it is more thermally sensitive and code for more stress-tolerant genes as demonstrated by its capacity to produce more heat-shock proteins and protective proteins compared to *A. dealbata* (Ul Haq *et al.*, 2019). Greater lysine content observed in *A. mearnsii* leaf-meals may be attributed to its higher proline content that also protects cellular structures, stability and integrity of proteins and increase enzyme activity (Karmous and Verma, 2020). Furthermore, the observation that lysine was lowest in the hot-dry-season may be related to its response to osmotic stress, which triggers its degradation to generate glutamic acid that act as a precursor of proline (Nakayama *et al.*, 1961; Arruda *et al.*, 2000). The lysine and methionine are usually limiting in ruminant production and contents reported in the current study do not meet the required levels for rumen microbes in growing ruminants for meat production at 0.32–0.9 and 0.26–2.9 g/day, respectively (Han *et al.*, 1996; Klemesrud *et al.*, 2000; Lin *et al.*, 2016) and milk production at 18.7–22 and 59.3–68 g/kg DM, respectively (Rapetti and Bava, 2005; Wang *et al.*, 2010).

The elevated contents of calcium and micro-minerals (i.e. selenium, iron, molybdenum and aluminium) observed for *A. dealbata* in the cool-wet-season leaf meal could be attributed to its observed higher contents of total phenols and phenolic compounds, particularly flavonols. In plants, phenolic compounds and their metabolites improve mineral absorption via chelation of metallic ions, enhancing active absorption sites and soil porosity, that accelerates the mobilisation of minerals (Halvorson *et al.*, 2009; Sharma *et al.*, 2019). Furthermore, it is reported that at low temperatures and high contents of soil moisture plants increase the production of minerals to suppress oxidative stress (Sharma *et al.*, 2019). Also, *A. dealbata* silvery-grey green leaves compared to *A. mearnsii* dark-olive green leaves, may explain the higher content of immobile minerals such as iron that are observed to be high in leaves with less chlorophyll (Marschner, 1997).

The increased contents of potassium, magnesium and phosphorus reported for *A. mearnsii* leaf-meal in the hot-dry-season may be associated with high temperatures and evapotranspiration rates, which may have enhanced the uptake and translocation of mineral elements from the roots to the leaves (Marschner, 1997). Moreover, increased accumulation of the aforementioned minerals in plants during the hot-dry-season could be attributed to stomatal regulation by potassium ions that maintain water retention in plant tissues and corresponding higher rates of photosynthesis and mineral uptakes (Wang *et al.*, 2013; Dias *et al.*, 2017; Malhotra *et al.*, 2018). It may also be ascribed to faster growth rates of *A. mearnsii* compared to *A. dealbata* as mentioned earlier (Fangji *et al.*, 1994; Stelling, 1998). As the age and size of the plants increase, the relative importance of the evapotranspiration rate, particularly for the translocation of mineral elements increases (Marschner, 1997). Regardless of the season, *A. mearnsii* leaf-meals contained greater magnesium concentrations compared to *A. dealbata,* which could be related to the plant's magnesium remobilisation capacity by a breakdown of cell structures (e.g. chloroplasts) and enzyme proteins thereby transforming structurally bound mineral nutrients (e.g. magnesium in chlorophyll) into a soluble form in leaves (Marschner, 1997). It is noteworthy, that the calcium, phosphorus, magnesium and potassium contents of *A. mearnsii* and *A. dealbata* leaf-meals are within the recommended maintenance requirements for sheep and cattle (1.15–11.0, 0.9–3.8, 0.56–2.2 and 0.93–5.0 g/kg DM), respectively; (Freer *et al.*, 2007; Pulina *et al.*, 2008; Gomes *et al.*, 2011).

The high proportion of C18:3*n*3 acid and low proportions of C18:2*n*6, C18:1*n*9 and SFAs reported for *A. dealbata* leaf-meal in the cool-wet-season could be ascribed to low temperatures and light intensity that increases the synthesis of trienoic FA from dienoic, monoenoic and saturated FA by the respective FA desaturases (FADs) to attenuate oxidative stress by scavenging ROS (Cao *et al.*, 2011; Das *et al.*, 2014; Dar *et al.*, 2017). At low temperature, the cell membrane changes from the solid-gel to liquid-crystalline phase, which is correlated to a higher ratio of unsaturated to SFAs, providing higher tolerance under cold climate (Routaboul *et al.*, 2000; Cao *et al.*, 2011). Contrary, proportions of C18:3*n*3 acid were low, while C18:2*n*6, C18:1*n*9 and SFAs were high for the *A. mearnsii* leaf-meal in the hot-dry-season, which might be associated with increased temperatures above 15°C that causes mutations or transformations in the FADs pathways (Falcone *et al.*, 2004; Wiley *et al.*, 2008; Dar *et al.*, 2017). These changes modulate the level of desaturase activity at several steps within the lipid biosynthetic pathway as well as controlling inter-compartmental flux between the chloroplastic and cytosolic pathways, increasing FA production that is transformed into cutin and wax, enabling plants to tolerate and protect themselves against increased temperatures and light intensity (Falcone *et al.*, 2004; Aid, 2019; Kim, 2020). Furthermore, water deficit and heat stress accelerate the kinetic energy and movement of molecules across membranes thereby loosening chemical bonds within molecules of biological membranes (Wahid *et al.*, 2007; Salehi-Lisar and Bakhshayeshan-Agdam, 2016). This makes the lipid bilayer of biological membranes more fluid by either denaturation of proteins or an increase in unsaturated FA (Wahid *et al.*, 2007; Salehi-Lisar and Bakhshayeshan-Agdam, 2016) as observed for *A. mearnsii* in the hot-dry-season leaf-meal. The increased SFAs in *A. mearnsii* leaf-meal in the hot-dry season may also be due to *A. mearnsii*'s faster growth rate (Fangji *et al.*, 1994; Stelling, 1998) as discussed earlier. Fully developed leaves exposed to high temperatures produce more SFAs that increase the melting temperature of plasma membranes and thus reduces heat tolerance of the plants (Xalxo *et al.*, 2020). Such changes in FA composition, which are mediated mainly by the activity of FADs, allow the maintenance of the membrane fluidity at low and high environmental temperatures (Upchurch, 2008; Jerónimo *et al.*, 2020). The greater proportions of C18:3*n*3 acid in *A. dealbata* leaf-meal in the cool-wet-season and C18:2*n*6 in *A. mearnsii* leaf-meal in the hot-dry-season provides an opportunity for its inclusion in ruminant diets. That may increase duodenal flow and tissue deposition of PUFA [i.e. C18:3*n*3, C18:2*n*6 and their biohydrogenation intermediates (i.e. rumenelic, rumenic and vaccenic acids)], which seem to have positive human health effects (Frutos *et al.*, 2020; Vahmani *et al.*, 2020).

The high concentration of phenolic compounds for *A. mearnsii* leaf-meal in the hot-dry-season may be due to its chlorophyll

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content, since phenolics are biosynthesised in the chloroplast and mesophyll cells (Agati *et al*., 2013). The phenolics concentration are further exacerbated by high temperature, water and/or nutrient deficiency increasing stomatal closure that depresses the photosynthesis rates and activate PAL activity (Agati *et al*., 2013; Sharma *et al*., 2019; Šamec *et al*., 2021). The increased amounts of flavonoids, glucosides, terpenoid and hydroxycinnamic acid observed for *A. mearnsii* leaf-meal in the hot-dry-season is essential in the photoprotection of plants to prevent cell membrane damage, oxidative stress and cell death during hot-dry-seasons (Di Ferdinando *et al*., 2014). They act as antioxidants by scavenging superoxide, hydrogen peroxide, hydroxide, singlet oxygen, or peroxy radicals (Agati *et al*., 2013; Di Ferdinando *et al*., 2014). To exemplify, *p*-Coumaroyltrifolin A, a hydroxycinnamate that was high in *A. mearnsii* leaf-meal harvested in the hot-dry-season is effective in absorbing ultraviolet-B radiation than flavonoids that have a greater ability to absorb visible light and UV-A wavelengths (Agati *et al*., 2013; Sharma *et al*., 2019).

The reduced concentration of quercetin-3-galactoside in *A. mearnsii* leaf-meal harvested in the cool-wet-season probably illustrates restoration of *A. mearnsii* plant growth after light, heat and water stress events inflicted in the hot-dry-season. Increased quercetin production in plants is associated with water inadequacy and UV light stress (Olsen *et al*., 2009; Sharma *et al*., 2019). Generally, decreased temperature and increased UV-B radiation may cause plant cells to produce ROS that may damage lipids, deoxyribonucleic acid, structural proteins and other cellular structures (Cirak and Radusiene, 2019). As a result, higher UV-B radiation and lower temperature can stimulate the production of secondary metabolites with UV-B absorbing and ROS-scavenging qualities, such as phenolic acids, proanthocyanidins and flavonoids (Cirak and Radusiene, 2019). The greater concentrations of flavanols observed in the cool-wet-season than in hot-dry-season could be ascribed to differences in the metabolic response of the shikimate pathway and biosynthesis of phenolic compounds (Ancillotti *et al*., 2015). In the cool-wet-season, lower temperatures induce PAL activity that increases the biogenesis of phenolic compounds (Rezende *et al*., 2015). This also corresponds with the high copper content reported for the cool-wet-season leaf-meals, which also activates the PAL pathway (Rezende *et al*., 2015).

The higher phenolic compounds reported for *A. mearnsii* might be attributed to its defence mechanism that probably code for more phenolic biosynthesis stress resistance genes (Ul Haq *et al*., 2019). The finding that *A. dealbata* leaf-meals respectively contained 8.5 and 10% more total phenol and tannins further confirms its increased phenotypic plasticity to multiple abiotic stresses compared to *A. mearnsii* (Sharma *et al*., 2019). The proanthocyanidin values observed in the current study are within the recommended threshold of 20–50 g/kg DM that positively affect DMI and nutrient digestibility (Patra and Saxena, 2011; Naumann *et al*., 2017), suppress helminths (⩾20 g/kg DM; Huang *et al*.,. 2018) and alleviate bloat (5–30 g/kgDM; Li *et al*., 1996; Sottie *et al*., 2014) in ruminants.

The result that *A. dealbata* leaf-meal harvested in the hot-dry-season was more efficient in chelating iron and scavenging ROS according to APC index using lipoxygenase, and FRAP is mostly attributed to its polyphenolic contents, particularly flavonoids (Farag *et al*., 2020). At high temperatures, the plant's antioxidant system is activated via the stimulation of the phenylpropanoid biosynthetic pathway that induces the synthesis of phenolic acids (Šamec *et al*., 2021). Increased flavonoids and phenolic acids in plants are associated with the deactivation of endogenous oxidative enzymes that prevent enzymatic oxidation (Jannat *et al*., 2010), reduce ROS by quenching oxygen ions (Maffei *et al*., 2007) and/or act as reducing agents, hydrogen donors, pro-oxidants and metal ion chelators (Pfukwa *et al*., 2019). The increased antioxidant activity reported for *A. dealbata* leaf-meal could be ascribed to its increased phenotypic plasticity (Sharma *et al*., 2019), as emphasised previously. Overall, the observed antioxidant activity in the current study is comparable to Xiong *et al*. (2016) who reported antioxidant activity of *A. mearnsii* leaves. The polyphenols (i.e. proanthocyanidin) values reported in the current study are within the range of 20–50 g/kg DM reported to extend oxidative shelf-life of meat (Luciano *et al*., 2011; Gesteira *et al*., 2018) and increase the antioxidant activity of milk (Di Trana *et al*., 2015; Cabiddu *et al*., 2019; Delgadillo-Puga *et al*., 2019). Research to evaluate worm burdens, fatty acid profile and oxidative shelf-life of meat and milk from ruminants fed diets containing these Acacia leaf-meals is indispensable.

Conclusions

Relative to other leaf-meals, *A. mearnsii* leaf-meal harvested in the hot-dry-season had the lowest aNDFom and ADFom content and the greatest contents of CP, EE, NDSF, 24 and 48 h *iv*NDFd, macro-minerals, individual polyphenols, C18:1*n*9 and C18:2*n*6. *Acacia dealbata* leaf-meal harvested in the cool-wet-season had the highest starch, NFC, microminerals and C18:3*n*3 whereas *A. dealbata* leaf-meal harvested in the hot-dry-season had the highest APC index. The greatest AA concentrations were contained in *A. mearnsii* leaf-meals irrespective of the season. Regardless of species, hot-dry-season leaf-meals had the highest total flavonoids concentration. Overall, *A. mearnsii* leaf-meal harvested in the hot-dry-season had the most desirable nutritional, polyphenolic and antioxidant profiles, making it a potentially suitable nutraceutical and preservative in ruminant production and product preservation, respectively. Further studies are warranted to determine the optimum inclusion levels of these Acacia leaf-meals in ruminant diets and their effects on nutrient digestibility and utilisation, animal production and product quality, which is necessary for the adoption and utilisation of these alternative ruminant feed resources.

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Conflict of interest. None.

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