



FODDER PRODUCTION AND CHEMICAL COMPOSITION OF PIGEON PEA [*Cajanus cajan* (L.) MILLSPAUGH] VARIETIES GROWN IN THE SUBTROPICAL REGION OF SOUTH AFRICA †

[PRODUCCIÓN DE FORRAJE Y COMPOSICIÓN QUÍMICA DE VARIEDADES DE GUANDÚ [*Cajanus cajan* (L.) MILLSPAUGH] VARIEDADES CULTIVADAS EN LA REGIÓN SUBTROPICAL DE SUDÁFRICA]

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SUMMARY

Background. Pigeon peas (*Cajanus cajan*) produce large amounts of high protein leaves that make it suitable for cultivation in arid and semiarid regions. **Objective.** To evaluate the biomass production and chemical composition of three pigeon pea varieties (ICEAP 00557, ICEAP 01514 and CIMMYT 100/01). **Methodology.** A completely randomized design was used. In two growing seasons, the plots were harvested at flowering and biomass yields were measured for fresh forage and dry matter (DM). Harvested leaves were either shade or oven-dried to a constant weight before being milled for chemical analyses. The proximate composition, mineral content, phenolic, tannin and saponin contents were determined. **Results.** Varieties ICEAP 01514 and CIMMYT 100/01 yielded the highest fodder of 2620.6 and 4458.3 kg DM/ha in 2016 and 2017, respectively. Variety ICEAP 00557 produced the lowest amount of fodder in both 2016 and 2017 at 1997.6 and 2933.3 kg DM/ha, respectively. There were interactions among varieties, seasons and drying methods on proximate composition. The DM, organic matter (OM) and neutral detergent fibre (NDF) were higher for ICEAP 01514 at 96.0, 88.0 and 55.3 %, respectively and digestible dry matter (DDM) (67.8 %) and metabolizable energy (ME) (9.8 MJ/kg DM) were higher for variety ICEAP 00557 in 2017. Shade drying recorded higher crude protein (CP) values (24.2-27.0 %) across all varieties with higher values occurring in 2017 compared to 2016. The interactive effect of season, pigeon peas varieties and drying method had little effect on both macro and micro-minerals except for copper (Cu) values. The ranges in macro-minerals were: calcium (Ca) (1.21-2.35), magnesium (Mg) (0.33-0.89), sodium (Na) (0.1-0.7) and phosphorus (P) (0.13-0.31 %). The ranges in micro-minerals were: iron (Fe) (206.7-283.4) and Cu (5.94-7.95 mg/kg DM). The tannins, phenolic and saponin contents were different ($P < 0.05$) among varieties and between drying methods. Their ranges were: 2.7-8.0 mg catechin equivalent (CE)/g, 13.6-15.9 mg gallic acid equivalent (GAE)/g and 3.4-6.1 %, respectively. **Implications.** Pigeon peas are suitable for farming systems in the drylands and these varieties are suitable diets for ruminants in the subtropics, where CP in the diets are low. **Conclusions.** Air-drying under a shade was recommended to preserve the nutritional quality of pigeon peas fodder. The macro and micro-nutrients in the pigeon peas satisfied the animal requirements, except for the deficiency in Na and Cu. The concentration of the anti-nutritive compounds in the pigeon peas will not limit animal performance.

Keywords: biomass production; nutritional value; drying techniques; pigeon peas.

RESUMEN

Antecedentes. El frijol guandú (*Cajanus cajan*) produce grandes cantidades de hojas ricas en proteínas que lo hacen adecuado para el cultivo en regiones áridas y semiáridas. **Objetivo.** Evaluar la producción de biomasa y composición química de tres variedades de guandú (ICEAP 00557, ICEAP 01514 y CIMMYT 100/01). **Metodología.** Se utilizó

† Submitted May 31, 2021 – Accepted August 4, 2021. <http://doi.org/10.56369/tsaes.3816>



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ISSN: 1870-0462.

un diseño completamente al azar. En dos temporadas de crecimiento, las parcelas se cosecharon en la floración y se midieron los rendimientos de biomasa para forraje fresco y materia seca (MS). Las hojas cosechadas se secaron a la sombra o al horno hasta un peso constante antes de ser molidas para análisis químicos. Se determinó la composición próxima, contenido de minerales, contenido de fenoles, taninos y saponinas. **Resultados.** Las variedades ICEAP 01514 y CIMMYT 100/01 produjeron mayor cantidad de forraje con 2620.6 y 4458.3 kg MS/ha en 2016 y 2017, respectivamente. La variedad ICEAP 00557 produjo la menor cantidad de forraje tanto en 2016 como en 2017 con 1997.6 y 2933.3 kg MS/ha, respectivamente. Hubo interacciones entre variedades, épocas y métodos de secado sobre la composición proximal. La MS, materia orgánica (MO) y fibra detergente neutro (FND) fueron superiores para ICEAP 01514 con 96.0, 88.0 y 55.3 %, respectivamente y materia seca digestible (MSD) (67.8 %) y energía metabolizable (EM) (9.8 MJ/kg MS) fueron más altos para la variedad ICEAP 00557 en 2017. El secado a la sombra registró valores más altos de proteína cruda (PB) (24.2-27.0 %) en todas las variedades con valores más altos en 2017 en comparación con 2016. El efecto interactivo de la temporada, variedades de guandú y el método de secado tuvo poco efecto sobre los macro y microminerales excepto por los valores de cobre (Cu). Los rangos en macrominerales fueron: calcio (Ca) (1.21-2.35), magnesio (Mg) (0.33-0.89), sodio (Na) (0.1-0.7) y fósforo (P) (0.13-0.31 %). Los rangos en microminerales fueron: hierro (Fe) (206.7-283.4) y Cu (5.94-7.95 mg/kg MS). Los contenidos de taninos, fenoles y saponinas fueron diferentes ($P < 0.05$) entre variedades y entre métodos de secado. Sus rangos fueron: 2.7-8.0 mg equivalentes de catequina (CE)/g, 13.6-15.9 mg equivalentes de ácido gálico (GAE)/g y 3.4-6.1 %, respectivamente. **Implicaciones.** El frijól guandú es adecuados para los sistemas agrícolas en las tierras secas y estas variedades son adecuadas para dietas de rumiantes en los subtrópicos, donde la PC en las dietas es baja. **Conclusiones.** Se recomienda el secado al aire bajo sombra para preservar la calidad nutricional del forraje de guandú. Los macro y micronutrientes en guandú satisface los requerimientos de los animales, excepto por la deficiencia en Na y Cu. La concentración de los compuestos antinutritivos en guandú no limitará el rendimiento de los animales. **Palabras clave:** producción de biomasa; valor nutricional; técnicas de secado; guandú.

INTRODUCTION

Browse species play significant roles in the mixed crop-livestock production systems, to supplement low quality feeds, fix atmospheric nitrogen and to help soil and water conservation (Murungu, 2010). Furthermore, the ability of most browse species to maintain their green fodder for a longer period, which is accredited to their deep root systems enables them to extract sufficient water and nutrients (Le Houerou, 1980). Forage from tree legumes is often used as a cushion to overcome feed gaps that arise from seasonal shifts in the productivity of native grazing in sub-Saharan Africa (Hassan *et al.*, 2014). The use of browse species is a vital component of animal production systems throughout the world (Murungu, 2010; Baloyi and Ayodele, 2013).

Tree legumes have the ability to produce large amounts of high protein leaves for animal consumption (Buthlezi *et al.*, 2019). This is very important in areas where ruminants are fed forages and crop residues of low nutritive value. Recent studies have examined the effect of supplementing these feeding resources with leaves of tree and shrub legumes, such as *Vichelleria karoo* (Mthi *et al.*, 2016; Brown *et al.*, 2018), *Sesbania grandiflora* (Makau *et al.*, 2020), *Calliandra calothyrsus*, *Gliricidia sepium* and *Leucaena leucocephala* (Maleko *et al.*, 2018). However, very little research has been conducted on planting and management systems, appropriate for

optimal leaf and wood production from these tree legumes.

Pigeon peas (*Cajanus cajan*) is relatively resistant to drought, improve soil fertility and prevent soil erosion on steep land (Shumuye *et al.*, 2016). Such properties make this crop a good candidate to bring new areas under cultivation particularly in arid and semiarid regions. Recently, studies have been focused to investigate germination, growth and yield of pigeon peas alone and with some companion intercrops at the field and under controlled conditions (Tayyab *et al.*, 2015). However, little is known about the regrowth and fodder yield of pigeon peas in arid areas. Thus, exploring the agronomic characteristics of pigeon peas is necessary to improve their fodder productivity for effective use in livestock feeding. Therefore, the objectives of this study were to determine the fodder production and chemical composition of three varieties of pigeon peas. It was hypothesized that fodder production and chemical composition do not vary among pigeon pea varieties, drying methods and between seasons.

MATERIALS AND METHODS

Description of study site

The study was conducted at the Research Farm of the University of Fort Hare, Alice. The Farm is situated in Victoria East located at a longitude of 32° 47' south, latitude of 26° 50' east and an altitude of 522 meters

above sea level. The area receives an annual average rainfall of 386 mm, which mostly occurs during summer. The mean annual maximum and minimum temperatures are 29°C and 5°C respectively. The site is classified as False Thornveld of the Eastern Cape (Acocks, 1988) with a savannah vegetation type. The predominant natural trees are *Vichellia karroo*, *Cuddia rudis*, *Grewia occidentalis* and natural grasses including *Chloris gayana* and *Panicum maximum*. The soils are Eutric Cambisol (IUSS Working Group WRB, 2006).

Field establishment and management

The land previously under dry-land *Chloris gayana* pastures was sub-divided into three large plots, ploughed using disc plough and disced using a disc harrow to achieve a fine seedbed. Each of the three large plots was subdivided into three sub-plots measuring 5 m x 5 m with a spacing of 1 m between sub-plots and the spacing between main plots was 2 m. The plants were established under dry land conditions with no supplemental irrigation. The rows were marked using a row marker and the planting spaces within sub-plots were 50 cm (inter-row spacing) and 25 cm (intra-row). All the seeds were inoculated with a rhizobium inoculant. In each sub-plot single super phosphate fertilizer was applied at a rate of 50 kg/ha. Each treatment was replicated three times. The plots were maintained free of weeds throughout the experimental period. After the first season (2016-17), the established plots were cut back to about 15 cm according to Adjei and Fianu, (1985) and allowed to grow for the second growing season (2017-18).

Measurements

Biomass yield

At flowering stage, 3 sample bushes were randomly cut at 0.3m height from the base and all leaf material (petiole and leaflets) were stripped. A 100g sample of harvested leaf material was either oven-dried at 70 °C for 48 hours or shade dried until a constant dry weight was obtained. The dry matter content (% DM) was determined by dividing the dry weight of leaf sample by the wet weight of leaf sample material multiplied by 100. The leaf dry matter yields (g/ha) were then calculated using dry weights of samples.

Chemical analyses

The harvested leaf material was either dried in an oven at 60°C for 48 hours or shade dried until a constant dry weight was obtained. The dried leaf samples were milled through a 1mm screen using a Wiley mill. The DM and ash contents were determined according to

AOAC (1990) procedures. Organic matter (OM) was calculated by difference of dry matter and ash. Crude protein (CP) content was analysed using the LECO TruSpec Nitrogen Analyser as described by (AOAC 1990, methods 7.033-7.037). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using the Van Soest fibre analysis (Van Soest *et al.*, 1991). The neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN) acid detergent lignin (ADL) hemicellulose, cellulose, total digestible nutrients (TDN), dry matter digestibility (DMD) and total non-structural carbohydrates (TNC) were determined according to Undersander (2014). For the analyses of phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn) and sodium (Na), the plant samples were individually pressed in a ring vessel to produce a pellet. The pellet was then dried to a residual moisture of less than 3% within 3-4 hours at 60 °C before X-ray fluorescence analysis (Allen *et al.*, 1974).

The phenol determination was done using the Folin-Ciocalteu's method as described by Samantha *et al.*, (2012) with some modifications. Approximately 0.5 mL of triplicate plant extracts and standard gallic acid (0.02mg/mL to 0.1 mg/mL) was pipetted in different test tubes. To this, 2.5 mL of 10% (v/v) Folin-Ciocalteu's reagent prepared in distilled water was added and the mixture was vortexed. The reaction was allowed to continue at room temperature for 5 minutes. After this 2 mL of 7.5% (w/v) anhydrous sodium carbonate was added, vortexed and incubated at 40°C for 30 minutes. A blank solution which neither had an extract nor gallic acid was used as a control. After incubation, the absorbance was measured at 765 nm using a UV- 3000 PC spectrophotometer. The phenol content was extrapolated from the gallic acid standard/calibration graph equation; $y = 8.07x + 0.1$, R^2 0.9981, and was expressed as mg gallic acid equivalent (GAE)/g of the dried pulverized sample from the equation CV/m ; where "C" is the concentration as derived from the calibration curve equation in mg/mL, "V" is the volume of the extract used in the assay in mL and "m" is the mass of the extract used in the assay in "g".

Total proanthocyanidin (CT) was determined based on the procedure of Oyedemi *et al.*, (2010). To 0.5mL approximately 1mg/mL of the extract solution and graded concentrations (0.02 mg/mL to 1 mg/mL) of the standard catechin was added 3mL of vanillin-methanol (4% w/v) and 1.5mL of hydrochloric acid and vortexed. The mixture was allowed to stand for 15 min at room temperature and absorbance measured at 500 nm using a UV- 3000 PC spectrophotometer. The determination was done in triplicate. The

proanthocyanidin content was determined using the calibration curve equation: $y = 0.9038x + 0.0449$, $R^2 = 0.9951$ and was expressed as mg catechin equivalent (CE)/g of the dried pulverized sample using the formula, CV/m as detailed above in the determination of phenol.

The saponnin content was determined as described by Obadoni and Ochuko (2001). Briefly, 20 g of the pulverized plant sample was added to 200 mL of 20% ethanol and kept on a shaker for 30 minutes and was then heated in a water bath at 55°C for 4 h. The resulting mixture was filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol. The filtrate mixture was combined and reduced to 40 ml in a water bath at 90°C. The concentrate was transferred into a separator funnel and 20 mL of diethyl ether was added, with vigorous shaking. The ether layer was decanted and discarded while the aqueous (bottom) layer was retained in a beaker. This layer was added into the separator funnel in which 60 mL of *n*-butanol was added with continuous vigorous shaking. The butanol extract which is the upper layer was decanted and retained and washed twice with 10mL of 5% aqueous sodium chloride. The remaining solution was collected and heated in a water bath and evaporated to constant weight at 40°C in an oven. The saponnin content was calculated using the equation:

$$\% \text{ Saponnin content} = \frac{\text{weight of residue}}{\text{weight of original sample}} \times 100$$

Experimental design and treatments

A completely randomised design (RCD) with three main plots was used to evaluate biomass yield of three fodder varieties of pigeon peas. Each main plot had three sub-plots for each of the varieties giving a total of nine sub-plots. Sampling was done at 50% anthesis. The treatments were the three fodder varieties of pigeon peas (ICEAP 00557, ICEAP 01514, and CIMMYT 100/01), two seasons (2016 – 2017 and 2017 – 2018) and two drying methods (oven-dried and shade-dried).

Statistical analyses

The biomass production data of the pigeon pea varieties were subjected to analysis of variance (ANOVA) using SAS Software version 9.1 (2001). Detection of differences among treatment means for significant effects was by least significant difference (LSD) at 5 % level of significance. The following statistical model was used to analyse the data:

$$Y_{ijkl} = \mu + S_i + V_j + (SV)_{ij} + E_{ijkl}$$

Where:

Y_{ijk} = response variable e.g. fresh biomass yield, dry matter yield

μ = the common mean;

S_i = season ($i = 1, 2$)

V_j = Varieties ($j = 1, 2, 3$)

$(SV)_{ik}$ = drying method and pigeon pea variety interaction

E_{ijk} = the random error

The chemical composition data were compared using the general linear model of the SAS software version 9.1 (2001) and the means were compared by the least significant difference (LSD) method at 5 % level of significance, using the following linear model:

$$Y_{ijk} = \mu + S_i + V_j + D_k + (SV)_{ij} + (SD)_{ik} + (VD)_{jk} + (SVD)_{jkl} + E_{ijk}$$

Where:

Y_{ijk} = response variable e.g. DM, Ash, CP, NDF

μ = the common mean;

S_i = season ($i = 1, 2$)

V_j = Variety ($j = 1, 2, 3$)

D_k = drying method ($k = 1, 2$)

$(SV)_{ij}$ = season and variety interaction

$(SD)_{ik}$ = season and drying method interaction

$(VD)_{jk}$ = variety and drying method interaction

$(SVD)_{jkl}$ = three-way interaction

E_{ijk} = the random error

RESULTS

Weather

Figure 1 shows 2016/17 and 2017/18 monthly rainfall (mm) patterns at the Research Farm of the University of Fort Hare, Alice. The highest rainfall (mm) was recorded during the month of January, 2017 (89.92 mm) and the lowest was received during May, 2016 (7.62 mm). The average monthly temperatures (°C) were also recorded as shown in Figure 2.

Seasonal temperature differences ($P < 0.05$) were observed in summer (December, January, and February) and winter (June, July and August). The summer months were much hotter in 2016 compared to 2017 while the winter months were cooler in 2017 compared to 2016.

Biomass yield

The biomass production of the three varieties of pigeon pea are shown in Table 1. The fodder yield was significantly ($P < 0.05$) influenced by the season, and variety. CIMMYT 100/01 and ICEAP 01514 showed higher ($P < 0.05$) biomass yields in both seasons.

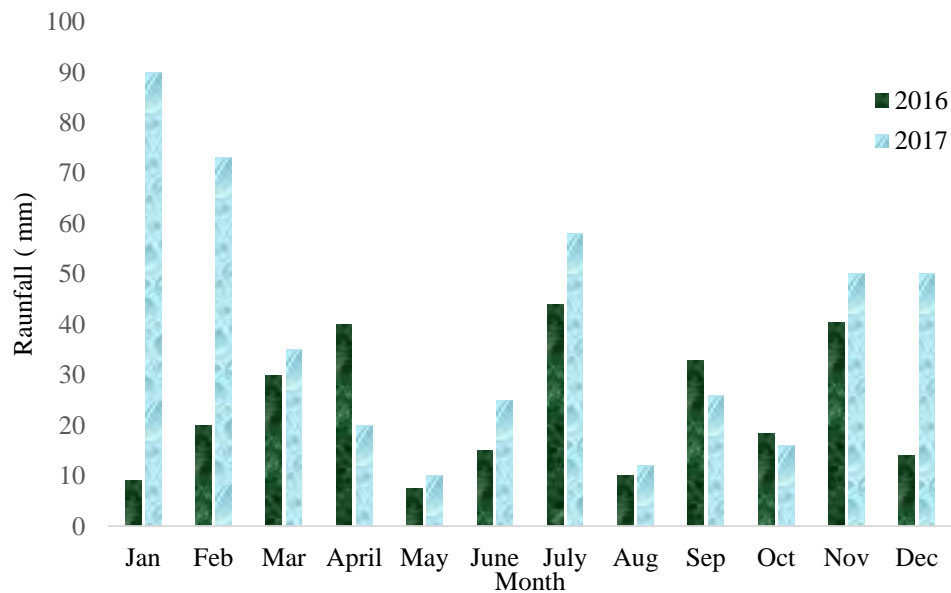


Figure 1. The monthly rainfall (mm) of the Research Farm of the University of Fort Hare situated in Alice, Eastern Cape Province of South Africa from April 2016 to April 2017.

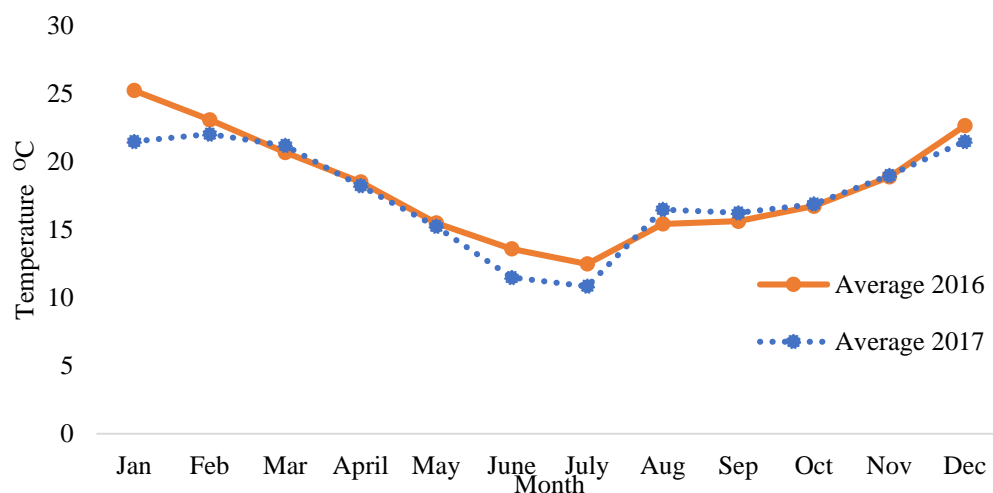


Figure 2. The monthly average temperature (°C) of the Research Farm at the University of Fort Hare, Alice from 2016/17 to April 2017/18 season.

Table 1. Biomass yield (kg/ha) of three pigeon peas varieties grown in the subtropics, harvested at anthesis stage.

Year (S)	Parameters	Pigeon pea varieties (V)			SEM	P values		
		CIMMYT 100/01	ICEAP 00557	ICEAP 01514		S	V	SV
2016	Fresh Yield	4908.1 ^{ab}	4836.9 ^b	5953.0 ^a	118.35	0.001	0.001	0.543
	DM Yield	2100.39 ^b	1997.62 ^c	2620.58 ^a	48.10	0.054	0.047	0.678
2017	Fresh Yield	12230.00 ^a	8060.00 ^c	9500.0 ^b	30.75	0.001	0.001	0.876
	DM Yield	4458.33 ^a	2933.33 ^c	3542.67 ^b	56.03	0.001	0.001	0.987

^{abc} Means within the same row having different superscripts were significantly different ($P < 0.05$) SEM= Standard error of mean.

Chemical composition

The proximate analyses results are shown in Table 2. There were interactions among seasons, varieties and drying methods on DM, OM, CP, Fat, hemicellulose, cellulose and ADIN. There was a season and variety interaction ($P < 0.05$) on ADF, ADL, DDM, ME and TDN. The DM was higher for ICEAP 01514 while the OM, DDM, ME and TDN were higher for ICEAP 00557 variety ($P < 0.05$) in 2017. Higher ADF, ADL NDIN values were obtained in 2016 for all varieties ($P < 0.05$) and these were associated with lower TDN and ME values. Shade drying showed higher CP values across all varieties with higher values occurring in 2017 compared to 2016. The ash content differed ($P < 0.05$) irrespective of the drying method between seasons with higher values occurring in 2016. The NDF content differed ($P < 0.05$) among varieties with ICEAP 01514 exhibiting the highest values for oven and shade drying, respectively. The variety ICEAP 01514 exhibited higher average NDF and hemicellulose, while CIMMYT 100/01 showed higher DM, OM, ash, ADL and ADF. ICEAP 00557 exhibited higher CP, fat, DDM, TDN and ME values compared to ICEAP 01514 and CIMMYT 100/01. The DM, CP, ADF, NDF, ADL values were higher in 2016 compared to 2017. The 2017 values for hemicellulose, cellulose OM, DDM, TDN and were higher ($P < 0.05$) compared to those recorded for 2016, respectively.

Mineral composition

Concentrations of both macro- and micro-minerals are presented in Table 3. The interactive effect of season, browse varieties and drying method had no significant ($P > 0.05$) effect on both macro and micro-minerals except the Cu values ($P < 0.05$). The highest Cu values were exhibited by ICEAP 00557 and CIMMYT 100/01 in 2016 while the highest values were recorded for oven drying in 2017. The Na and Mn content were significantly influenced ($P < 0.05$) by season with higher values being recorded in 2017. Varietal differences were observed for Ca, Mn, Cu and Fe. CIMMYT 100/01 showed significantly higher Ca, Mn and lowest Fe values compared to ICEAP 00557 and ICEAP 01514, respectively. The drying method significantly affected ($P < 0.05$) the Zn, Mg and Mn content.

Anti-nutritional composition

The concentration of anti-nutritional factors in the three varieties of pigeon peas are presented in Table 4. The CT, phenolic and saponnin content were different among varieties and between drying methods (P

< 0.05). There was an interaction between variety and drying method for all anti-nutrients ($P < 0.05$).

The CT values were higher for all varieties under shade drying except for ICEAP 00557 in 2017 which was higher by 1mg CE/g. The phenolic content was higher ($P < 0.05$) for ICEAP 00514 and ICEAP 00557 under oven drying method while CIMMYT 100/01 showed higher ($P < 0.05$) values under shade drying in both seasons. Amongst all varieties the saponnin content was higher for oven drying compared to shade drying ($P < 0.05$).

The CT concentration ranged from 2.76 mg CE/g in variety CIMMYT 100/01 to 8.0 mg CE/g in variety ICEAP 00514. The saponnin concentration ranged from 3.77 for ICEAP 00557 to 6.1 % in ICEAP 01514. Variety ICEAP 00557 showed the highest ($P < 0.05$) phenol content of 15.9 mg GAE/g compared to CIMMYT (14.96 mg GAE/g) and ICEAP 01514 (14.73 mg GAE/g).

DISCUSSION

Biomass yield

The differences in fresh and DM yield of pigeon peas observed between the two seasons was expected. The low yields recorded in 2016 season was because it was a drought year. Drought has been reported to influence biomass yield for legumes (Nadeem *et al.*, 2019). However, pigeon pea was found to experience lower drought-induced yield reduction compared to cowpea (*Vigna unguiculata*) and green gram (*Vigna radiata*). The difference in yield recorded in the current study was high because the drought may have occurred at the anthesis stage of the pigeon peas. Greater yield reductions have been reported to occur when drought coincided with the reproductive stage compared to the vegetative stage in forage legumes (Daryanto *et al.*, 2015). Despite the effects of drought, the DM yield recorded was within the acceptable range for pigeon peas (Netsanet and Yonatan, 2015; Feedipedia, 2016). Achieving such DM yields even in a drought year indicates that the pigeon peas are suitable for small scale farming systems which rely on dry land agriculture.

The suitability of pigeon peas under drought conditions is because they have stomata regulation and osmotic adjustment, which is less energy demanding, allowing root growth to proceed under drought condition (Nunes *et al.*, 2008). Pigeon peas maintain their leaf water content, thereby avoiding tissue dehydration, which is generally achieved by controlling stomatal conductance and closure (Pinheiro

Table 2. Proximate composition (DM basis) of either oven- or shade-dried pigeon peas varieties grown in the subtropics.

Season (S)	2016						2017						SE	P values			
Variety (V)	ICEAP 00557		ICEAP 01514		CIMMYT 100/01		ICEAP 00557		ICEAP 01514		CIMMYT 100/01			S	V	D	SVD
Drying method (D)	Oven	Shade	Oven	Shade	Oven	Shade	Oven	Shade	Oven	Shade	Oven	Shade					
DM (%)	93.3 ^{ab}	90.4 ^c	93.7 ^{ab}	89.9 ^c	94.0 ^{ab}	90.9 ^c	92.9 ^b	89.3 ^c	76.0 ^d	96.0 ^a	94.8 ^{ab}	91.1 ^c	0.230	0.001	0.001	0.267	0.001
CP (%)	24.0 ^{bc}	24.4 ^{ab}	24.5 ^{ab}	25.7 ^a	23.5 ^c	24.6 ^{ab}	25.3 ^a	26.9 ^a	19.2 ^d	27.0 ^a	22.9 ^c	24.2 ^{bc}	0.371	0.947	0.132	0.001	0.028
Fat (%)	6.9 ^{ab}	5.7 ^b	6.4 ^{ab}	6.0 ^b	7.3 ^a	5.9 ^b	6.2 ^b	6.4 ^{ab}	4.8 ^c	6.5 ^{ab}	7.5 ^a	5.5 ^c	0.270	0.012	0.534	0.019	0.050
Ash (%)	9.1 ^a	8.4 ^a	8.9 ^a	9.0 ^a	9.48 ^a	9.1 ^a	5.9 ^c	7.1 ^b	7.9 ^{ab}	8.0 ^a	8.9 ^a	8.8 ^a	0.450	0.004	0.074	0.095	0.286
NDF (%)	51.3 ^b	49.3 ^{ab}	50.1 ^b	49.4 ^{ab}	52.5 ^b	51.2 ^b	39.5 ^d	42.4 ^c	55.3 ^a	54.4 ^a	51.7 ^b	50.8 ^b	1.170	0.190	0.008	0.101	0.209
ADF (%)	46.4 ^a	46.1 ^a	45.4 ^a	44.4 ^b	46.2 ^a	46.5 ^a	27.0 ^f	32.2 ^e	40.3 ^c	37.3 ^d	41.9 ^c	46.2 ^a	1.181	0.001	0.001	0.887	0.131
ADL (%)	23.3 ^a	22.7 ^a	18.0 ^{bc}	21.4 ^a	19.5 ^{ab}	19.1 ^{ab}	15.2 ^c	8.8 ^d	13.8 ^{cd}	13.9 ^{cd}	16.4 ^c	18.8 ^{ab}	1.831	0.002	0.006	0.496	0.206
NDIN (%)	2.4 ^b	2.5 ^b	2.2 ^{bc}	2.4 ^b	2.4 ^b	2.3 ^b	1.9 ^c	2.3 ^{bc}	3.1 ^a	2.6 ^{ab}	2.7 ^{ab}	1.9 ^c	0.080	0.491	0.016	0.580	0.478
ADIN (%)	2.0 ^d	2.3 ^c	1.9 ^e	2.1 ^d	2.0 ^a	2.0 ^d	2.8 ^b	2.8 ^b	3.3 ^{ab}	1.6 ^f	2.4 ^c	3.5 ^a	0.080	0.013	0.521	0.171	0.027
TNC (%)	1.0	1.1	1.3	1.3	1.1	1.3	1.2	2.0	1.9	1.5	1.4	1.9	0.110	0.110	0.211	0.085	0.277
Hemicellulose (%)	4.9 ^d	3.2 ^f	4.6 ^d	4.9 ^d	6.3 ^c	4.6 ^d	5.5 ^{cd}	10.7 ^b	15.7 ^a	5.4 ^d	6.5 ^c	4.3 ^e	0.721	0.001	0.001	0.001	0.001
Cellulose (%)	23.1 ^b	23.4 ^b	27.4 ^a	23.0 ^b	26.7 ^a	27.4 ^a	22.1 ^b	22.4 ^b	20.4 ^{bc}	20.3 ^{bc}	27.2 ^a	27.0 ^a	1.760	0.001	0.001	0.413	0.003
OM (%)	83.2 ^b	81.8 ^b	84.1 ^b	83.0 ^b	84.8 ^{ab}	82.4 ^b	86.9 ^a	82.3 ^b	68.2 ^c	88.0 ^a	85.9 ^a	82.4 ^b	0.738	0.033	0.001	0.015	0.001
DDM (%)	51.9 ^c	55.6 ^b	52.7 ^{bc}	54.5 ^b	52.9 ^{bc}	53.0 ^b	67.8 ^a	63.8 ^a	57.6 ^{ab}	59.8 ^a	56.2 ^b	52.9 ^{bc}	1.395	0.001	0.001	0.887	0.131
TDN (%)	41.7 ^e	47.2 ^{cd}	42.9 ^e	45.5 ^{de}	43.2 ^e	43.4 ^e	65.3 ^a	59.3 ^b	50.1 ^c	53.4 ^c	48.1 ^{cd}	43.3 ^e	2.059	0.001	0.001	0.887	0.131
ME (MJ/kgDM)	6.5 ^c	6.5 ^c	6.3 ^c	7.1 ^{bc}	6.5 ^c	6.9 ^c	9.8 ^a	8.9 ^a	7.5 ^{bc}	8.1 ^b	7.2 ^{bc}	6.5 ^c	0.310	0.001	0.001	0.887	0.131

^{abcdef} row means having different superscripts were significantly different (P< 0.05).

Table 3. Macro and micro mineral content (DM basis) of either oven- or shade-dried pigeon peas varieties grown in the subtropics.

Season (S)	2016						2017						SE	P values			
Variety (V)	ICEAP 00557		ICEAP 01514		CIMMYT 100/01		ICEAP 00557		ICEAP 01514		CIMMYT 100/01			S	V	D	SVD
Drying method (D)	Oven	Shade	Oven	Shade	Oven	Shade	Oven	Shade	Oven	Shade	Oven	Shade					
Ca (%)	1.27 ^d	1.21 ^d	1.23 ^d	1.29 ^c	1.40 ^b	1.35 ^b	1.31 ^c	1.22 ^d	1.30 ^c	1.30 ^c	2.35 ^a	1.35 ^b	0.184	0.076	0.011	0.065	0.057
Mg(%)	0.37 ^b	0.35 ^b	0.33 ^b	0.35 ^b	0.37 ^b	0.36 ^b	0.54 ^{ab}	0.35 ^b	0.40 ^b	0.41 ^b	0.89 ^a	0.41 ^b	0.063	0.097	0.100	0.033	0.098
K(%)	1.42	1.40	1.51	1.46	1.50	1.48	1.64	1.35	1.58	1.53	1.30	1.45	0.630	0.078	0.531	0.085	0.974
Na(%)	0.01 ^c	0.02 ^c	0.02 ^c	0.01 ^c	0.02 ^c	0.01 ^c	0.35 ^b	0.35 ^b	0.70 ^a	0.65 ^a	0.03 ^c	0.08 ^c	0.150	0.003	0.507	0.776	0.186
K/(Ca+Mg)	0.39 ^c	0.42 ^b	0.44 ^b	0.41 ^b	0.39 ^c	0.41 ^b	0.45 ^b	0.45 ^b	0.50 ^a	0.43 ^b	0.38 ^c	0.34 ^c	0.026	0.957	0.001	0.872	0.836
P(%)	0.26	0.25	0.27	0.30	0.27	0.28	0.31	0.15	0.25	0.28	0.30	0.22	0.034	0.068	0.192	0.476	0.308
Ca:P	0.21 ^{bc}	0.21 ^{bc}	0.22 ^b	0.23 ^a	0.19 ^b	0.21 ^{bc}	0.24 ^b	0.20 ^c	0.34 ^a	0.30 ^a	0.17 ^d	0.14 ^d	0.200	0.828	0.001	0.429	0.963
Zn (mg/kg DM)	32.25 ^a	29.33 ^b	30.11 ^a	30.64 ^a	30.87 ^a	28.6 ^b	32.7 ^a	28.8 ^b	31.01 ^a	30.1 ^a	30.94 ^a	29.2 ^b	3.187	0.065	0.093	0.010	0.110
Cu (mg/kg DM)	7.64 ^a	7.62 ^a	5.94 ^e	6.21 ^d	7.87 ^a	6.72 ^d	7.95 ^a	6.60 ^d	6.35 ^d	7.20 ^{ab}	7.05 ^c	7.82 ^a	0.190	0.143	0.001	0.079	0.001
Mn (mg/kg DM)	113.8 ^a	103.4 ^b	102.1 ^{bc}	99.54 ^c	111.2 ^a	106.6 ^b	114.2 ^a	101.9 ^c	102.7 ^{bc}	105.6 ^b	114.1 ^a	111.2 ^a	1.292	0.002	0.001	0.001	0.059
Fe (mg/kg DM)	245.7 ^b	218.8 ^b	259.7 ^b	283.4 ^a	226.7 ^b	215.5 ^c	248.9 ^b	215.3 ^c	276.6 ^a	206.7 ^d	223.7 ^c	220.6 ^c	17.39	0.406	0.001	0.983	0.112

^{abcd} means within the same row having different superscripts were significantly different (P< 0.05).

Table 4. Phytochemical composition of either oven- or shade-dried pigeon peas varieties grown in the subtropics.

Season (S)	2016								2017				SE	P values			
Variety (V)	ICEAP 00557		ICEAP 01514		CIMMYT 100/01		ICEAP 00557		ICEAP 01514		CIMMYT 100/01			S	V	D	SVD
Drying method (D)	Oven	Shade	Oven	Shade	Oven	Shade	Oven	Shade	Oven	Shade	Oven	Shade					
Tannins (mg CE/g)	6.9 ^{ab}	5.8 ^{bc}	5.2 ^a	7.5 ^a	2.7 ^b	4.8 ^c	7.4 ^a	6.4 ^{ab}	5.7 ^{bc}	8.0 ^a	3.3 ^{cd}	5.3 ^{bc}	1.07	0.309	0.001	0.001	0.998
Phenols (mg GAE/g)	14.9 ^{ab}	13.7 ^{bc}	15.4 ^a	13.6 ^{bc}	14.8 ^{ab}	14.9 ^{ab}	15.5 ^a	14.3 ^{ab}	15.9 ^a	14.2 ^{ab}	15.3 ^a	15.5 ^a	1.12	0.317	0.032	0.001	0.978
Saponins (%)	5.2 ^a	3.4 ^{bc}	5.9 ^a	5.6 ^a	5.1 ^a	4.3 ^b	5.5 ^a	3.7 ^{bc}	6.1 ^a	5.8 ^a	5.3 ^a	4.5 ^b	0.47	0.438	0.001	0.001	0.897

^{abcd} Means within the same column having different superscripts were significantly different (P<0.05).

et al., 2001). Stomatal closure has been reported to lead to a decrease in internal carbon dioxide (CO₂) concentrations, consequently limiting photosynthesis and shoot growth. Chickpea, cowpea, common bean and pigeon pea have been reported to allocate solutes to their roots to lower their osmotic potentials enabling the roots to continue extracting water at low soil water potentials (a trait known as low epidermal conductance) (Chaves *et al.*, 2003). Drought resistance by leguminous plants has been reported to involve the interaction between nitrogen fixing bacteria (rhizobia) and arbuscular mycorrhiza (Daryanto *et al.*, 2015). Such an interaction may have reduced the effects of drought on DM yield in the current study since the pigeon pea seeds were inoculated with rhizobia at planting.

Proximate composition

The range of CP content for all varieties (23-25 %) obtained in this study are consistent with reports in the literature (Gbenga, (2014); Haftay and Kebede, (2014); Liu *et al.*, (2014); Abebe and Tamir, (2016); Bode *et al.*, (2018). These levels were higher than those reported by Netsanet and Yonatan, (2015) and Abebe and Tamir, (2016). The higher CP suggests that the varieties studied have the potential to improve the diets of ruminants in the subtropics, where CP concentrations of grasses are generally low during the dry season. Thus, pigeon peas are ideal supplements to traditional veld pasture based diets which are protein-deficient for a greater part of the season. The effects of drying method on CP levels obtained in the current study were not expected. However, Buthelezi *et al.*, (2019) reported similar observations when oven dried pigeon pea leaves had higher CP levels compared to shade dried leaves. In agreement Da Silva *et al.*, (2016) reported that shade drying altered the essential oil in *Psidium guajava* leaves. These result are in contrast to those obtained by Sengul *et al.*, (2019). A possible reason could be that the proximate components are generally affected by heat as suggested by Oni *et al.*, (2018).

The range of ash content was higher than the 5.8% reported by Dzowela *et al.*, (1995a). This could be as a result of varietal effects, compounded by climatic conditions, soil and management conditions and stage at harvest as all these are known to affect the plant nutrient accumulation (Adjolohoun *et al.*, 2013). The NDF, ADF, NDIN and ADIN values in the present study are considered normal (NRC 2001). According to Washaya *et al.*, (2016); Abebe and Tamir, (2016) and Diribsa, (2017) as NDF values increase, total feed intake will decrease. Rivera and Parish, (2010), showed that NDF values between 47 to 53% are

suitable and would promote digestibility in ruminants. The threshold NDF content, for ruminant animals, beyond which feed intake and digestibility is adversely affected is 600g NDF/kg DM (Brown *et al.*, 2018). Nonetheless all the three varieties of pigeon peas provided enough NDF to meet a minimum acceptable range of between 25-33% for dairy cows (NRC, 2001). Oni *et al.*, (2018) reported that browse species that have an NDF content above 50%, have high proportions of soluble carbohydrates which are beneficial for proper rumen function. In the current study varieties ICEAP 00557 and CIMMYT 100/01 would be less preferred relative to their NDF and ADF values while varieties ICEAP 00557 and ICEAP 00514 would be preferred because of their high TDN values.

The higher fibre and lignin components produced by oven-drying method in the current study is consistent with the results obtained by other researchers (Dzowela *et al.*, 1995b). The higher fibre and lignin may be due to the formation of artefact lignin (Buthelezi *et al.*, 2019). The differences in chemical constituents between oven-dried and shade-dried samples are primarily attributed to non-enzymatic browning effect, Maillard reactions and the formation of insoluble polymers (Ramsumair *et al.*, 2014). These differences may, also, simply be due to loss of organic matter (Andueza *et al.*, 2019). In such circumstances, freeze-drying would be advisable as it gives results that are ideal, because enzymatic activity is low and ice crystals may not disrupt membranes and cell walls. However, the practicality of this method in small scale farming systems is probably non-existent. On the other hand, sun-air drying exposes the material to ultra-violet radiation, which reacts with forage constituents to again increase ADF and NDF. Furthermore, ultra-violet radiation can cause the browning or Maillard effects (Dzowela *et al.*, 1995b) limiting its selection as drying and preservation method for forages. To counter this, the effect of ultra-violet radiation could be avoided by air-drying under a shade, and this influenced our decision to use shade drying in the current study. The higher ADIN values obtained in the current study for oven drying testified to the fact that Maillard products increased the amount of N bound to fibre, measured as ADIN. Evidently the variety, climatic conditions, and drying method affected the plant nutrient composition. Additionally, sampling site, soil and management conditions and stage of harvest affect plant nutrient composition (Adjolohoun *et al.*, 2013).

The effect of drying methods on forages has been reported by Alomar *et al.*, (2003). The choice of any drying method is premised on the fact that it rapidly

reduces plant metabolic activity and preserves macromolecular structures after harvesting. Such a method would be considered the most efficient to reflect the chemical composition of fresh samples (Ramsumair *et al.*, 2014). The interaction between season, variety and drying method in the current study warrants further studies on the three varieties of pigeon peas and is a basis for nutritional evaluation *in vivo*.

Mineral composition

The mineral composition results indicate that the Ca content of the three varieties of pigeon peas satisfied the animal requirements as specified by the Agriculture Research Council (ARC) (1980), National Research Council (NRC) (2001; 2007). The range of mineral concentration was within the range of (1.2-1.6 %) reported for legumes (Feedipedia, 2016). The range of Mg content in the three varieties of pigeon peas were higher than the dietary requirements (0.1-0.2 %) of sheep and cattle, as recommended by the ARC (1980). This indicates that the forage plants grown in the subtropics are not deficient to the point of hypomagnesemic tetany confirming reports by NRC, (2001). The Na content of all the three varieties of pigeon peas was less than 0.22% recommended by NRC (2001; 2007). Inadequacy of Na is implicated in suboptimal voluntary feed intake, hence dietary inclusions are necessary in the subtropics (Abrahams and Steismajer, 2003).

The P contents were slightly lower than those reported by Feedipedia (2016) but they were similar to those reported by Høgh-Jensen *et al.*, (2007). However, the P values recorded in the current study were higher than those reported by Adjolohoun *et al.*, (2013). Tropical soils are known to be deficient in P (Khouri *et al.*, 2015). Furthermore, the NRC (2001) reported that the mineral contents of forages were lower during the rainy season, and this was related to leaching out of the minerals from the soil. The ratio of Ca: P in the three varieties of pigeon peas was lower than the recommended range of 1 to 2 (NRC, 1981). However, the recorded ratios in the current study were considered adequate for growth and bone development for livestock (NRC 2007).

The concentrations of micro-minerals varied among varieties and drying methods and the differences were most pronounced for Cu and Fe. The range of Cu concentration obtained in this study was lower than the requirements for cattle (10 g/kg DM). However, the range of Fe concentration was higher than the recommended amount of 50 g/kg DM (NRC, 2001). The Fe content was above the minimum requirement for dairy (NRC 2001), beef cattle (NRC, 1978) and

sheep (NRC, 1984), for both the seasons studied. The NRC (1981) recommended that dietary iron should not exceed 1000 mg/kg DM. The high content and the variation among the three pigeon peas varieties could be as a result of genetic difference, variation in uptake of minerals among pigeon peas, soil fertility and the soil mineral status, stage of maturity and the proportion of leaf samples taken for analysis. As much as 250 to 500 mg of iron/kg dietary DM is known to deplete copper in cattle (NRC, 2001). Only variety ICEAP 01514 had Fe values within this range after oven drying. Iron has been reported to interfere with the absorption of other minerals, primarily copper and zinc (NRC 2001; 2007; Mc Donald *et al.*, 2002). The absorption of dietary iron depends on the binding capacity of transferrin and lactoferrin in the blood and tissues and an excess of Fe leads to surplus unbound free iron. This free iron is very reactive and causes generation of reactive oxygen, lipid peroxidation and free radical production. All these reactions result in oxidative stress, thereby increasing anti-oxidant requirements of the animal (NRC, 2001). The effects of the drying method on mineral content in feed has been documented and freeze drying recommended to minimize these effects. However, the practicality of freeze drying of feeds in the developing nations and for small scale farmers is questionable.

Anti-nutritional composition

The presence of condensed tannins (CT) and phenols is beneficial to livestock production. Tannins tend to lower forage degradability (Washaya *et al.*, 2018) and rumen enteric methane production (Hammond *et al.*, 2016). However, if tannins exceed 5% of the total diet they lower the dry matter intake (Washaya *et al.*, 2016). Lascano *et al.*, (2001) showed that CT concentration in foliar tissue of legume species increased with environmental temperature. This explains the higher CT values for 2016 which was a drought year compared to 2017. In principle CTs precipitate protein to form stable complexes at the rumen pH, which adversely affects protein and fibre degradation in the rumen (Lascano *et al.*, 2001). Higher condensed tannin levels of leaves have been reported in woody plants, which usually have high ADF and NDF, resulting in lower dry matter digestibility (Dzowela *et al.*, 1995b). In certain woody plants, however, leaves are generally higher in tannins than stems or woody parts, thereby potentially depressing leaf digestibility.

Elevated levels of polyphenolics and condensed tannins have been reported in other forage legume (Baloyi and Ayodele, 2013 and Washaya *et al.*, 2018). The higher tannin values for oven-drying have been

reported elsewhere and it is caused by the formation of tannin-cell wall complexes during drying. However, the current study indicated that the oven-drying temperature did not cause higher tannin concentration in all the varieties except ICEAP 00557.

The high saponins values in the current study indicates that pigeon peas will enhance the flow of microbial protein from the rumen, improve feed utilization and decrease protozoal populations that have a tendency of sequestration within the rumen (Jayanegara *et al.*, 2012). The effects of variety and drying method on total phenols and saponins in the current study affirmed reports by Oni *et al.*, (2018). These reports showed that sun drying method reduced the concentration of anti-nutritional factors. In agreement with these reports, the current study showed that the levels of anti-nutrients were lower under shade drying compared to oven drying. The effects of drying methods on anti-nutrients have also been reported by Hove *et al.* (2003) who concluded that shade-dried leaves had higher extractable proanthocyanidins compared to sun or oven dried in *Acacia angustissima*, *Calliandra calothyrsus* and *Leucaena leucocephala* leaves.

CONCLUSIONS

The fodder yield was influenced by the pigeon peas variety and the season. Varieties ICEAP 01514 and CIMMYT 100/01 yielded the highest fodder in 2016 and 2017, respectively. Variety ICEAP 00557 produced the lowest amount of fodder in both 2016 and 2017. There were interactions among varieties, seasons and drying methods on proximate composition. Shade drying showed higher CP values across all varieties with higher values occurring in 2017 compared to 2016. The interactive effect of season, pigeon peas varieties and drying method had little effect on both macro and micro- minerals except for the Cu values. The tannins, phenolic and saponin contents were different among varieties and between drying methods.

The DM yields achieved indicated that the pigeon peas are suitable for small scale farming systems which rely on dry land agriculture. The high CP content suggested that the varieties studied have the potential to improve the diets of ruminants in the subtropics, where CP concentrations of grasses are generally low during the dry season. Air-drying under a shade was recommended to preserve the nutritional quality of pigeon peas fodder. The macro and micronutrients contents of the three varieties of pigeon peas satisfied the animal requirements, except for the deficiency in Na and Cu. The concentration rate of the anti-nutritive

compounds in the three varieties of pigeon peas will not limit animal performance.

Acknowledgements

Funding. Authors would like to express their gratitude to the National Research Foundation of South Africa - Research and Technology Fund Grant 98715 for financial support.

Conflict of interest statement. Authors declare no conflicts.

Compliance with ethical standards. The nature of the work does not require approval by a (bio) ethical committee

Data availability. Data are available with Washaya S (email: washayas@gzu.ac.zw) upon reasonable request.

Author contribution statement (CRediT). L.S Buthelezi – Conceptualization, J. Mupangwa – Funding acquisition, Supervision, S.Washaya– Methodology Data curation, Supervision.

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