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Influence of season and phytochemicals on the elemental composition of kobs' diets in Old Oyo National Park, Nigeria

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Abstract

Phytochemicals or plant secondary metabolites (PSMs) are known to counteract mineral nutrients (MNs) utilisation in herbivorous wildlife, yet studies on the interactions between PSMs and MNs in relation to herbivory, particularly for kobs (*Kobus kob*) are scarce. Composite samples of each of five major forage plants of kobs' (*Grewia mollis, Afzelia africana, Gardenia aqualla, Maranthes polyandra* and *Andropogon gayanus*) were randomly collected in and around feeding sites of kobs in both dry and wet season. Using standard procedures, samples were analysed for PSMs (alkaloids, phenols, saponins and tannins) and MNs [copper (Cu^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), zinc (Zn^{2+}), sulphur (S^{2-}), sodium (Na^{+}) and potassium (K^{+})]. With appropriate statistical tools, data obtained were analysed, compared and correlated. Almost all plants were significantly higher in all the tested MNs and PMSs in the wet season, except for *Maranthes polyandra* which showed the inverse trend for Cu^{2+}, Mn^{2+}, S^{2-}, Na^{+} and K^{+}. Apart from tannins that were absent, the concentration of other PSMs (in *Grewia mollis*) and Ca^{2+} (in *Gardenia aqualla* and *Andropogon gayanus*) were significantly higher in the dry season. In conclusion, the concentration of Na^{+}, K^{+}, S^{2-}, Fe^{2+} and Zn^{2+} in selected forage plants of kobs were highly influenced by the presence of PSMs.

Keywords: *Kobus kob*, herbivory, mineral nutrients, plant secondary metabolites

1 Introduction

Herbivores' diet composition and their consumption under the influence of certain factors have been debated by various authors (Carmona *et al*., 2011; de Longh *et al*., 2011; Fingesi *et al*., 2017). This was made clear in the study conducted by Oates (1978), who investigated the association between mineral composition and dietary toxins in Guereza monkeys' diets. Plants, on one hand, contain plant primary metabolites (PPMs; protein, carbohydrates, lipid, minerals, and vitamins) on which herbivore animals depend for growth and development. On the other hand, plants defend themselves against pathogens and herbivores with non-nutritive substances known as plant secondary metabolites (PSMs) (Schardl, 2002; Aremu & Onadeko, 2008; Mazid *et al*., 2011; Nwauzoma & Dawari, 2013).

Many plant secondary metabolites are toxic and excessive consumption may be detrimental to the health of the consumers (Cipollini & Levey, 1997). Various studies have described PSMs as non-nutritional natural defence compounds that are capable of influencing the nutritional mechanism of herbivores, as long as the consumption rate does not exceed the physiological capability of the wildlife. However, most herbivores have developed adaptive mechanisms that allow the consumption of these poisonous PSMs without causing side effect (Dearing & Cork, 1999). In fact, it is unlikely that wild animals will over-ingest the poisonous compounds in their diets (Dearing *et al*., 2005; Schardl, 2002); rather,

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ingestion of PSMs continues until a threshold is reached and the animal stops eating instinctively (Dearing *et al*., 2005). The three major groups of PSMs include terpenoids (e.g. saponins), phenols (e.g. tannins), and N and S-containing compounds (e.g. alkaloids) (Mazid *et al*., 2011). These compounds have been explored by African ethno-medicinal practitioners for treatment of many kinds of ailments (Dearing *et al*., 2005; Carmona *et al*., 2011; Ogungbenro *et al*., 2018).

Apart from PSMs providing medication, plants also provide important minerals for various biochemical activities (Saxena *et al*., 2014). According to Robbins (1983) and Hart (2003), certain minerals like sodium (Na^+), potassium (K^+) and phosphorous (P) are important sources of electrolytes and their deficiency has been observed to cause poor appetite, urinary calculi, body stiffness, Pica and reduced growth among others. Also, calcium (Ca^{2+}), magnesium (Mg^{2+}) and manganese (Mn^{2+}) are important components of animal bones, lack of which may cause rickets and lameness. Copper (Cu^{2+}), iron (Fe^{2+}) and sulphur (S^{2-}) are not just enzyme activators but also important components for the formation of haemoglobin, inadequacy of which may cause anaemia and deformity.

The gap in the understanding of interactions between PSMs and MNs as components of herbivores' diets in relation to their associated benefits seems to be very wide. Little has been reported about MNs in certain wildlife diets, and much less is known about PSMs in kobs' diets in relation to their concentration of MNs. Kobs (*Kobus kob*) are one of the most common wildlife species in Old Oyo National Park (OONP) in Nigeria and are listed as least concern species (Jayeola *et al*., 2012; IUCN, 2016). Apart from kobs being threatened by habitat destruction and agricultural activities (East, 1999; Jayeola *et al*., 2012), inadequate diet composition is another challenge. Inadequate diet utilisation in kobs may be due to the presence of PSMs serving as hindrance to the utilisation of the available mineral-rich constituents. Since kobs are generalists, they will eat whatever is available for survival. This might be the reason for consuming diets rich in PSMs, even though they could be toxic (Jayeola *et al*. 2012; Parikh *et al*., 2016). However, little attention is paid to kobs' forage plants. The only work that seems to be available in Nigeria was conducted by Fingesi *et al*. (2017) in Kainji Lake National Park (KLNP); here, various forages utilised by kobs in both wet and dry season were identified. Dominant species among these forages included *Andropogon gayanus, Gardenia aqualla, Grewia mollis, Hyparrhenia rufa*, and *Afzelia africana*. Since OONP and KLNP share similar ecology (woodland savanna), the vegetation therein comprises similar species. Hence, the identified dominant forage species of kobs in KLNP can guide research in OONP to assess the mineral and phytochemical composition of forage consumed by kobs.

It is accepted knowledge that the synthesis of PSMs is costly and inversely proportional to a plant's concentration of MNs (Cipollini & Levey, 1997; Mazid *et al*., 2011; Nwauzoma & Dawari, 2013). Therefore, by correlating the mineral constituents of individual forage plants of kobs with that of their phytochemical constituents, the benefits or deficiencies of specific plants can be predicted. Also, since little is known about the mineral requirements of kobs in Nigeria, the analysis of mineral constituents of known forage plants (Fingesi, 2017) in OONP will provide baseline data for future nutritional considerations. Therefore, the aim of this study was to investigate a few selected forage plants of kobs for seasonal variation in their mineral and phytochemical constituents and the correlation between the two constituent groups.

2 Materials and methods

2.1 Study area

This study was carried out in Old Oyo National Park (OONP), one of the seventeen national parks in Nigeria. It is geographically situated between longitude 3°35' and 4°21' E and latitude 8°07' and 9°04' N in the southwestern part of Nigeria, Oyo State. With an area of 2,512 km^2, it ranks fourth among the National Parks in Nigeria. For effective coordination, the park is sectioned into 5 ranges: Yemeso, Oyo-Ile, Tede, Sepeteri, and Marguba range. The park is endowed with numerous different species of fauna, the most popular of them being the kob (Kobus kob) which is also the symbol for OONP.

2.2 Sample collection

Five top priority forage species known to be consumed by kobs as described by Fingesi (2017) and representing between 16 % to 45 % of the animals' diet composition were purposively selected and sampled. Following the method modified after Oates (1978), young and mature leaves of *Grewia mollis, Afzelia africana, Gardenia aqualla, Maranthes polyandra*, and *Andropogon gayanus* were randomly collected in and around three major feeding sites of kobs at the Marguba section of OONP. Each sample's authenticity was confirmed by a well experienced guard (botanist). For a period of about four weeks, each combination of plant samples (young/matured leaves and wet/dry season samples) were air-dried in open polyethylene bags and milled into powder with a mechanical grinder (Kenwood German Industrial 5000 W Food Crusher and Blender). Twenty grams

(20 g) of each sample were soaked in 100 ml of distilled water, shaken vigorously for at least 1 hour per day with a mechanical shaker for 3 days and thereafter filtered using Whatman filter paper. With the use of evaporating dishes and at a temperature of 35 °C, the filtrates were concentrated to aqueous extract. The latter were subjected to both qualitative and quantitative phytochemical analyses, modified from the procedures described by Harborne (1973) and Trease & Evans (1989). Following the procedure of Mirzaei (2012), the aqueous samples were analysed chemically by an atomic absorption spectrophotometer (AAS) for PSMs and MNs. The PSMs analysed included alkaloids, phenols, saponins and tannins, while MNs included Cu^{2+}, Fe^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, phosphate ($PO_4{}^{3-}$), zinc (Zn^{2+}), S^{2-}, Na^{+} and K^{+}.

2.3 Sample analysis

2.3.1 Determination of alkaloids

Presence of alkaloids was tested using Mayer's and Dragendroff's reagents. The aqueous extract (0.5 ml) of each sample was mixed with 8 ml of 1 % HCl, warmed, filtered and kept in two separate test tubes. The filtrate (2 ml) of each sample was then treated separately with 2 drops of Mayer's (potassium mercuric iodide solution) and Dragendroff's reagents. A creamy white precipitate with Mayer's reagent and a red precipitate with Dragendroff's reagents indicated the presence of alkaloids (Harborne, 1973; Trease & Evans, 1989).

Alkaloid concentration was determined in defatted samples. To remove crude fat, 100 ml of diethyl ether were mixed with 2 g of ground sample and then heated at 65 °C for 2 hours in a Bosch Soxhlet apparatus with heating mantle and reflux condenser (model B127RE), made by Bosch Instruments (Germany), whereby the diethyl ether extracted the fat and allowed it to escape to the atmosphere through evaporation. A 0.2 molar NaOH was used to test if the fat remains, by scrubbing it on the sample in the jacket using a scrubbed patch. When the scrubbed patch was slippery, then there were still some fat in the sample; otherwise, the fat was considered to have been totally removed and then the sample was retrieved and allowed to dry. Alkaloid concentration was determined according to Harborne (1973): In a 250 ml beaker, a mixture of 5 g of defatted sample and 200 ml of acetic acid dissolved in ethanol (10 %) was left for a period of 4 hours to allow for normal complete dissolution of the sample to form a homogenous mixture. The homogenous mixture was then filtered through a dry Whatman no. 9 filter paper into a 100 ml volumetric flask. Ten millilitres (10 ml) of the filtrate were transferred into a separator funnel and the alkaloids present were extracted vigorously by shaking it with five successive portions of chloroform. The residue obtained was dissolved in 10 ml hot distilled water (at 100 °C) and transferred into a Kjeldahl tube. After addition of 0.20 g sucrose, 10 ml concentrated H_2SO_4 and 0.02 g selenium; it was wet-digested to a colourless solution in order to determine nitrogen (N) using Kjeldahl method; digestion, distillation and titration of the samples.

In the Kjeldahl digestion tubes, 0.5 g of each finely ground dried sample, 1 Kjeldahl catalyst tablet and 10 ml of concentrated H_2SO_4 were carefully poured. After a period of 4 hours, a clear colourless solution appeared in the tube. The digest was cooled and carefully transferred into 100 ml volumetric flask. The digestion tube was thoroughly rinsed with distilled water and added to the mixture in the volumetric flask until it was made up to the mark. Distillation was carried out using Markham distillation apparatus, as it allows volatile substances such as ammonia to be steam distilled with complete collection of the distillate. Into the apparatus, pipetted 5 ml portion of the digest and 5 ml of 40 % (W/V) NaOH added. The mixture was steam-distilled for 2 minutes into a 50 ml conical flask containing 10 ml of 2 % Boric acid plus mixed indicator solution placed at the receiving tip of the condenser. The Boric acid plus indicator solution changes colour from red to green showing that all the ammonia liberated have been trapped. The green colour solution obtained was then titrated against 0.01 N HCL contained in a 50 ml burette. At the end point, the green colour turns to wine colour. An indication that all the nitrogen, trapped as ammonium borate [$(NH_4)_2BO_3$], has been removed as ammonium chloride (NH_4CL). The % nitrogen in this analysis was calculated using the formula:

$$\% \text{ N} = \text{titre value} \times \text{atomic mass of nitrogen} \times \text{normality of HCL used} \times 4$$

The % nitrogen obtained was converted to % total alkaloids by multiplying it by a factor of 3.26 (i.e. total alkaloids = % N × 3.26).

2.3.2 Determination of tannins

A sub sample (0.5 ml) of the above-mentioned aqueous extract was mixed with distilled water (20 ml) and shaken vigorously with a mechanical shaker for 1 minute. The mixture was then boiled at a temperature of 80 °C for 2 to 3 minutes, filtered and separated into two test tubes. A gelatinous precipitate was observed when 3 drops of lead acetate solution were added to 1 ml of the extract in one test tube, indicating the presence of tannins. To the other test tube, 1 ml of bromine water was added and a pale brown precipitate indicated the presence of tannins (Trease & Evans, 1989). Afterwards, the tannin content of the defatted sample was determined according to the method of Van-Burden &

Robinson (1981). A sub-sample (0.5 g) of each forage material was weighed into a 50 ml plastic bottle containing 50 ml of distilled water and shaken vigorously for about 1 hour in a mechanical shaker. The mixture was filtered into a 50 ml volumetric flask and completed to 50 ml mark with distilled water. A total of 5 ml of filtrate was pipetted into a test tube and a 2 ml of 0.1 molar $FeCl_3$ in 0.1 molar NH_4Cl and 3 ml of 0.008 molar of $K_2Fe(CN)_6$ were added. The absorbance was taken at 720 nm wavelength spectrophotometrically using Labomed Spectronic 21, made by Labomed Instruments UK, within 10 min after equipment standardisation (following calibration standard).

2.3.3 Determination of saponins

Five millilitres of distilled water were added to 0.5 g of the above-described aqueous extract of each sample in a test tube and then placed in a water bath to boil for 2 to 3 minutes. The mixture was vigorously shaken with a mechanical shaker for 1 minute and observed for development of a stable persistent froth. Presence of emulsion was also observed when 2 drops of olive oil were added to 1 ml of the filtrates extract in another test tube. These tests indicated presence of saponins.

Subsequently, the method of Obdoni & Ochuko (2001) was adopted to determine the content of saponins. Of each defatted sample, 20 g were added to 100 cm^3 of 20 % aqueous ethanol (prepared by dilution in water) in a conical flask. The mixture was heated to 55 °C in a water bath for 4 hours with constant stirring. After cooling, the mixture was filtered to obtain the residue which was re-extracted by mixing it with 200 ml of 20 % ethanol. The obtained extract was then concentrated to 40 ml in a water bath at about 90 °C. The concentrate was purified by adding it to 20 ml of diethyl ether in a 250 ml separating funnel and vigorously shaken to recover the aqueous layer (while the ether layer was discarded). This was repeated, but this time 60 ml of n-butanol was added. The extract containing n-butanol was washed two times with 10 ml of 5 % NaCl. The resulting solution was evaporated in a water bath and later dried in the oven to constant weight for calculation of the percentage saponin content: W1-W2 / W1; where W1 is the initial weight of sample; W2 is the oven dried weight of the residue.

2.3.4 Determination of total phenols

To 10 mg of each of the above described sample extracts, 2 drops of ferric chloride solution were added. A blue-black colour formation indicated the presence of phenols. Afterwards, the spectrophotometric method was used for the determination of total phenols in the defatted sample. Of the defatted sample, 5 g were dissolved in 50 ml of ether and the solution was boiled for 15 minutes to extract the phenolic constituent. Of the extract containing the phenolic constituents, 5 ml were pipetted into a 50 ml flask, together with 10 ml of distilled water, 5 ml concentrated amyl-alcohol and 2 ml of ammonium hydroxide. The mixture was allowed to settle for 30 minutes for thorough reaction and colour development, after which the total phenol concentration was measured at 505 nm on the spectrophotometer.

2.4 Statistical analysis

All data were analysed statistically using SPSS (2017) software package. Seasonal variation in PSM and MN constituents of each forage sample were determined using Analysis of Variance (ANOVA). Means were compared by Tukey's test at $p < 0.05$ level of significance. Pearson correlation (r) analysis was used to determine the relationship between the phytochemical and mineral constituents of kobs' forage plants.

3 Results

3.1 Seasonal variation in the quality of forage plants

Table 1 shows the concentration of MNs in five major forage plants of kobs in the wet and dry season. Except for *Maranthes polyandra* that showed no significant seasonal difference for Cu^{2+}, Mn^{2+}, S^{2-}, Na^+ and K^+, the other plants showed significant differences in all tested minerals between dry and wet season. Also, apart from the concentration of Cu^{2+} that was significantly lower in *Gardenia aqualla* and *Andropogon gayanus*, Fe^{2+}, Mn^{2+}, Ca^{2+} and Mg^{2+} concentrations were significantly higher in *Grewia mollis, Afzelia africana, Gardenia aqualla* and *Andropogon gayanus* in the wet season than in the dry season. Similarly, with the exception of Na^+ in *Afzelia africana* and Zn^{2+} in *Andropogon gayanus*, dry season concentrations of Zn^{2+}, S^{2-}, Na^+ and K^+ in the tested plants were significantly higher than wet season concentrations. Table 2 shows the concentration of PSMs in the five forage plants. Of the four PMS groups tested, only tannins were not detected in any plant in the dry season. With the exception of *Maranthes polyandra*, the concentration of all groups of PMSs were significantly higher in the wet season than in the dry season (except alkaloids in *Grewia mollis*).

3.2 Correlation between phytochemical and mineral constituents

Table 3 and Table 4 show the correlation matrix for phytochemicals and mineral concentrations in five forage plants of kobs in the dry and wet season. In the dry season, a significant negative relationship existed between Cu and Fe

Table 1: *Concentration of selected minerals in five forage plants of kobs in the dry and wet season.*

Mineral nutrients	*Season*	Grewia mollis	Afzelia africana	Gardenia aqualla	Maranthes polyandra	Andropogon gayanus
Cu^{2+} (mg kg^{-1})	Dry	4 ± 0.1^{a}	7 ± 0.1^{a}	5 ± 0.1^{a}	8 ± 0.2^{a}	7 ± 0.1^{a}
	Wet	12 ± 0.1^{b}	13 ± 0.2^{b}	9 ± 0.1^{b}	8 ± 1.0^{a}	10 ± 0.2^{b}
Fe^{2+} (mg kg^{-1})	Dry	1400 ± 7.8^{a}	1001 ± 3.1^{a}	1270 ± 4.9^{a}	762 ± 6.3^{a}	1331 ± 1.1^{a}
	Wet	15663 ± 67.1^{b}	12018 ± 87.6^{b}	17779 ± 21.8^{b}	8371 ± 1.0^{b}	16865 ± 200.3^{b}
Mn^{2+} (mg kg^{-1})	Dry	35 ± 0.2^{a}	35 ± 0.2^{a}	27 ± 0.3^{a}	27 ± 0.2^{a}	236 ± 3.7^{a}
	Wet	64 ± 0.8^{b}	60 ± 1.0^{b}	30 ± 1.4^{b}	26 ± 1.0^{a}	272 ± 3.9^{b}
Ca^{2+} (mg kg^{-1})	Dry	3312 ± 2.7^{a}	60 ± 1.0^{a}	15023 ± 126.7^{a}	2607 ± 18.5^{a}	13099 ± 23.5^{a}
	Wet	15000 ± 149.8^{b}	13013 ± 62.3^{b}	9538 ± 37.5^{b}	26 ± 1.0^{b}	2600 ± 24.9^{b}
Mg^{2+} (mg kg^{-1})	Dry	1164 ± 45.4^{a}	918 ± 3.3^{a}	1029 ± 20.9^{a}	916 ± 0.8^{a}	683 ± 2.9^{a}
	Wet	7243 ± 445.8^{b}	4631 ± 3.7^{b}	4889 ± 1.9^{b}	3226 ± 1.0^{b}	3993 ± 7.3^{b}
Zn^{2+} (mg kg^{-1})	Dry	190 ± 3.3^{a}	203 ± 3.0^{a}	173 ± 3.7^{a}	124 ± 1.8^{a}	125 ± 1.9^{a}
	Wet	176 ± 1.9^{b}	123 ± 1.0^{b}	131 ± 1.5^{b}	62 ± 1.0^{b}	152.3 ± 3.5^{b}
S^{2-} (%)	Dry	9.1 ± 0.18^{a}	5.4 ± 0.02^{a}	3.0 ± 0.09^{a}	2.7 ± 0.04^{a}	5.6 ± 0.10^{a}
	Wet	2.7 ± 0.03^{b}	2.2 ± 0.02^{b}	2.7 ± 0.03^{b}	2.5 ± 1.00^{a}	3.1 ± 0.02^{b}
Na^{+} (%)	Dry	0.5 ± 0.10^{a}	0.1 ± 0.01^{a}	0.6 ± 0.01^{a}	0.3 ± 0.02^{a}	0.3 ± 0.01^{a}
	Wet	0.2 ± 0.02^{b}	0.1 ± 0.01^{b}	0.2 ± 0.01^{b}	1.1 ± 1.00^{a}	0.1 ± 0.01^{b}
K^{+} (%)	Dry	2.3 ± 0.03^{a}	2.7 ± 0.08^{a}	4.0 ± 0.09^{a}	1.6 ± 0.04^{a}	1.8 ± 0.06^{a}
	Wet	1.7 ± 0.03^{b}	1.4 ± 0.02^{b}	2.3 ± 0.03^{b}	2.1 ± 1.00^{a}	1.5 ± 0.26^{b}

Values are means ± S.D. of triplicate determinations; values within the same row with different superscript letters differ at $p \leq 0.05$ (across the plant species).

Table 2: *Concentration of selected groups of secondary metabolites in five forage plants of kobs in the dry and wet season.*

Phytochemical group	*Season*	Grewia mollis	Afzelia africana	Gardenia aqualla	Maranthes polyandra	Andropogon gayanus
Alkaloids	Dry	0.22 ± 0.00^{a}	0.22 ± 0.01^{a}	0.52 ± 0.01^{a}	0.51 ± 0.02^{a}	0.22 ± 0.01^{a}
	Wet	0.26 ± 0.26^{a}	0.33 ± 0.02^{b}	0.25 ± 0.00^{b}	1.26 ± 1.00^{a}	0.31 ± 0.02^{b}
Phenols	Dry	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.40 ± 0.02^{a}	0.35 ± 0.02^{a}	0.14 ± 0.12^{a}
	Wet	0.16 ± 0.01^{b}	0.17 ± 0.01^{b}	0.15 ± 0.01^{b}	1.16 ± 1.00^{a}	0.20 ± 0.01^{b}
Tannins	Dry	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Wet	0.01 ± 0.00^{a}	0.08 ± 0.00^{a}	0.01 ± 0.00^{a}	1.01 ± 1.00^{a}	0.13 ± 0.06^{b}
Saponins	Dry	0.25 ± 0.02^{a}	0.23 ± 0.02^{a}	0.60 ± 0.02^{a}	0.63 ± 0.01^{a}	0.23 ± 0.01^{a}
	Wet	0.34 ± 0.01^{b}	0.45 ± 0.01^{b}	0.38 ± 0.00^{b}	1.37 ± 1.00^{a}	0.46 ± 0.02^{b}

Values are means ± S.D. of triplicate determinations; values within the same row with different superscript letters differ at $p \leq 0.05$ (across the plant species).

(r = −0.77***), Mg (r = −0.75***), Zn (r = −0.53**), S (r = − 0.67***) and Na (r = −0.74***), and between Mn and Mg (r = − 0.803***) as well as Mn and Zn (r = − 0.54**). Iron was significantly and positively correlated with S (r = 0.63**) and Na (r = 0.55**), so was Ca with K (r = 0.55**) and K with Zn (r = 0.52**) as well as K with Na (r = 0.63**). Mg had a significant positive relationship with Zn (r = 0.64**) and Na (r = 0.59**) but was negatively correlated with PO_4^{3-}. Meanwhile, of all the elements, only S has a significant negative relationship with alkaloids (r = − 0.819**), phenols (r = −0.805**) and saponins (r = − 0.794**), and K only had a significant positive relationship with phenols (r = 0.515**).

In the wet season, a significantly positive correlation existed between Cu and Ca (r = 0.805**), Mg and Zn (r = 0.777**) and Ca and Mg (r = 0.825**). Iron was significantly and negatively correlated with Na (r = –0.589**), alkaloids (r = –0.624**), saponins (r = –0.620**), phenols (r = –0.609**) and tannins (r = –0.606**), but positively correlated with Zn (r = 0.812**). Likewise, Zn was significant and negatively correlated with Na (r = –0.623**), alkaloids (r = –0.641**), saponins (r = –0.650**), phenols

Table 3: *Correlation coefficients (r) between mineral concentrations and plant secondary metabolite groups in five forage plants of kobs in the dry season.*

	Cu^{2+}	Fe^{2+}	Mn^{2+}	Ca^{2+}	Mg^{2+}	PO_4^{3-}	Zn^{2+}	S^{2-}	Na^{+}	K^{+}	ALK	PHE	SAP	TAN
Cu^{2+}	1													
Fe^{2+}	-0.767***	1												
Mn^{2+}	0.236^{ns}	0.392^{ns}	1											
Ca^{2+}	0.070^{ns}	0.410^{ns}	0.429^{ns}	1										
Mg^{2+}	-0.751***	0.170^{ns}	-0.803***	-0.411^{ns}	1									
PO43-	0.725***	-0.350^{ns}	0.358^{ns}	0.584**	-0.703***	1								
Zn^{2+}	-0.531**	0.270^{ns}	-0.541**	0.031^{ns}	0.642**	-0.599**	1							
S^{2-}	-0.676***	0.634**	0.130^{ns}	-0.303^{ns}	0.349^{ns}	-0.851***	0.467^{ns}	1						
Na^{+}	-0.739***	0.552**	-0.259^{ns}	0.207^{ns}	0.591**	-0.156^{ns}	0.133^{ns}	0.035^{ns}	1					
K^{+}	-0.350^{ns}	0.212^{ns}	-0.488^{ns}	0.548**	0.453^{ns}	0.121^{ns}	0.521**	-0.294^{ns}	0.628**	1				
ALK	0.206^{ns}	-0.453^{ns}	-0.443^{ns}	0.045^{ns}	0.160^{ns}	0.500^{ns}	-0.348^{ns}	-0.819***	0.437^{ns}	0.448^{ns}	1			
PHE	0.156^{ns}	-0.377^{ns}	-0.424^{ns}	0.123^{ns}	0.168^{ns}	0.503^{ns}	-0.314^{ns}	-0.805***	0.497^{ns}	0.515**	0.992***	1		
SAP	0.210^{ns}	-0.490^{ns}	-0.082^{ns}	-0.044^{ns}	0.183^{ns}	0.468^{ns}	-0.371^{ns}	-0.794***	0.414^{ns}	0.380^{ns}	0.993***	0.976***	1	
TAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-

***, **, * significant at $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$ respectively; ns: not significant; ALK: alkaloids; PHE: phenols; SAP: saponins; TAN: tannins

Table 4: *Correlation coefficients (r) between mineral concentrations and plant secondary metabolite groups in five forage plants of kobs in the wet season.*

	Cu^{2+}	Fe^{2+}	Mn^{2+}	Ca^{2+}	Mg^{2+}	PO_4^{3-}	Zn^{2+}	S^{2-}	Na^{+}	K^{+}	ALK	PHE	SAP	TAN
Cu^{2+}	1													
Fe^{2+}	0.105^{ns}	1												
Mn^{2+}	-0.036^{ns}	0.419^{ns}	1											
Ca^{2+}	0.805***	0.205^{ns}	-0.480^{ns}	1										
Mg^{2+}	0.648***	0.474^{ns}	-0.176^{ns}	0.825***	1									
PO_4^{3-}	-0.899***	0.245^{ns}	0.014^{ns}	-0.613**	-0.404^{ns}	1								
Zn^{2+}	0.574**	0.812***	0.421^{ns}	0.513^{ns}	0.777***	-0.300^{ns}	1							
S^{2-}	-0.096^{ns}	0.356^{ns}	0.479^{ns}	-0.335^{ns}	-0.012^{ns}	0.315^{ns}	0.256^{ns}	1						
Na^{+}	-0.290^{ns}	-0.589**	-0.274^{ns}	-0.375^{ns}	-0.400^{ns}	0.232^{ns}	-0.623**	0.462^{ns}	1					
K^{+}	-0.354^{ns}	0.030^{ns}	-0.360^{ns}	-0.150^{ns}	-0.115^{ns}	0.588**	-0.278^{ns}	0.585**	0.711***	1				
ALK	-0.273^{ns}	-0.624**	-0.229^{ns}	-0.404^{ns}	-0.450^{ns}	0.189^{ns}	-0.641**	0.450^{ns}	0.995***	0.660***	1			
PHE	-0.295^{ns}	-0.609**	-0.227^{ns}	-0.414^{ns}	-0.438^{ns}	0.216^{ns}	-0.634**	0.463^{ns}	0.998***	0.673**	0.999***	1		
SAP	-0.283^{ns}	-0.620**	-0.208^{ns}	-0.426^{ns}	-0.478^{ns}	0.198^{ns}	-0.650***	0.457^{ns}	0.993***	0.659***	0.999***	0.997***	1	
TAN	-0.286^{ns}	-0.606**	-0.183^{ns}	-0.436^{ns}	-0.466^{ns}	0.201^{ns}	-0.631**	0.479^{ns}	0.992***	0.655***	0.998***	0.997***	0.999***	1

***, **, * significant at $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$ respectively; ns: not significant; ALK: alkaloids; PHE: phenols; SAP: saponins; TAN: tannins

($r = -0.634^{**}$) and tannins ($r = -0.631^{**}$). Sodium was significant and positively correlated with K ($r = 0.711^{***}$), alkaloids ($r = 0.995^{***}$), saponins ($r = 0.993^{**}$), phenols ($r = 0.998^{***}$) and tannins ($r = 0.992^{**}$). Similarly, K was significantly and positively correlated with alkaloids ($r = 0.660^{**}$), saponins ($r = 0.659^{**}$), phenols ($r = 0.673^{**}$) and tannins ($r = 0.655^{**}$).

4 Discussion

The seasonal variation in both mineral nutrients and phytochemical constituents of kobs' selected forage plants as recorded in this study is not an exceptional occurrence. Seasonal variations in the quality of wildlife diets has been well documented in several studies, although they were not specifically related to the relationship between PSMs and MNs (Chaves *et al.* 2013; Irwin *et al.*, 2014; Capoani, 2019). The work of Fingesi *et al.* (2017), which seems to be the only study on kobs' diets in Nigeria, concentrated on plant species utilised by kobs in Kainji Lake National Park, for which there was no significant difference between dry and wet season. Rduch (2013) described how kobs' diets overlapped with other bovids' diets in Kasanka National Park and Kafue National Park, Zambia. Teng *et al.* (2022) recently described the occurrence of seasonal changes in diet composition of chipmunk (Tamias sibiricus) in Liangshui National Nature Reserve, China. Kos *et al.* (2012) gave account of seasonal variation in diets of elephant (Loxodonta africana) and impala (Aepiceros melampus) in Kruger National Park, South Africa.

In this study, the studied plants' concentration of the elements Zn, S, Na and K was significantly higher in the dry

than in the wet season. In the wet season, only Na exhibited an inverse relationship with Zn, whereas in the dry season, Fe, Mg, Zn, S and Na showed an inverse relationship with Cu, while Mg and Zn had an inverse relationship with Mn. This suggests a proportional decrease in the concentration of Cu and Mn due to an increase in the concentration of Fe, Mg, Zn, S and Na. Copper and Mn are components of haemoglobin, bones and enzymes. A decrease in Cu and Mn in the diet may therefore lead to deficiency and cause kobs to suffer from diarrhoea, weight loss, anaemia, deformation of bones and reproduction problems during the dry season (Robbin, 1983; Hart, 2003). The findings differ from those of Estevez *et al*. (2010) who did not observe significant temporal changes in the elemental compositions of wild red deer diets in a subtropical region. According to Oates (1978), mineral deficiency in herbivores' diets is not uncommon and mostly due to seasonal changes in diet composition. For example, except for dry season diets, which are concentrated in minerals due to moisture loss, fresh lush forage in the early wet season is usually higher in certain minerals than dry season diets (Mirzaei, 2012). This is often accounted for by seasonal migration or geophagic behavior of wild herbivores, either in search of greener pastures or deficient minerals (Kreulen, 1975; Oates, 1978). According to Robbin (1983), surplus accumulation of certain minerals or surplus production of secondary metabolites in a forage plant may produce an antagonistic effect on other minerals by forming insoluble complexes. Although this study determined elevated concentrations of Fe^{2+}, Mn^{2+}, Ca^{2+} and Mg^{2+} and Cu^{2+} in wet season diets of kobs, only Fe^{2+} showed a significant negative relationship with all the tested groups of PSMs. This negative relationship implies that the wildlife consuming such forage plant may be deprived of the benefit of Fe^{2+} due to the presence of the tested groups of PSMs. As Fe^{2+} is a very important component of enzymes and haemoglobin, deficiency may result in anaemia. On the other hand, the increase in alkaloids, phenols and saponins in the kobs' forage plants during the dry season was accompanied by a decrease in the concentration of S^{2-}, which may have an adverse effect on wildlife consuming such forage plants. As S^{2-} is an important macro mineral for the synthesis of protein and production of enzymes, hair, hormones, hemoglobin, vitamins and milk, its deficiency may lead to weakness and poor general performance (Robbin, 1983; Hart, 2003).

Optimum availability of Na and K are very crucial to the survival of wildlife herbivores. The concentration of Na required by wildlife is dictated by the amount of K available in the forage plants and the kind of stress the wildlife is subjected to in a particular moment (Robbin, 1983). In this study, the increased concentration of both K and Na in the studied plants was paralleled by an increased concentration of all tested groups of PSMs in the wet season. This was however not the case in the dry season, when only K concentration showed a parallel relationship with the concentration of phenols. This suggests that the environmental stress (high temperature and low forage moisture content) in the dry season may excessively enhance the content of phenols, which could lead to an increased K concentration in the forage plants at the expense of Na. This may consequently lead to an increase in the demand of Na by the wildlife to counteract the excess intake of K. According to Robbin (1983), excess availability of K in forage plants usually resulted to a corresponding higher demand for Na when consumed by wildlife in an unbalanced ratio. For this reason, diseases like Pica (geophagy), urinary calculi and stiffness of muscles may ensue in kobs during the dry season (Robbin, 1983; Hart, 2003), due to lack of adequate Na supply, as Na is critical to the physiological needs of wildlife. The balance of K and Na in the diet of wildlife has been stressed by Robbin (1983), stating that an increased K ingestion, especially during the wet season when plant water content is high, may exacerbate the corresponding Na demand. In contrast, this study indicates that there is a positive relationship between Na and K in kobs' diets, which can be influenced by the diets' concentration of certain phytochemicals, such as alkaloids, phenols, saponins and tannins.

Although PSM consumption is thought to be inversely proportional to available nutrients, it is still possible that kobs may have an opportunity to benefit more from the mineral content of wet season forage than from dry season forage. Villalba & Provenza (2009) argued that PSMs may have less antagonistic effects on the bioavailability of nutrients if animals consume a variety of forages. Studies on the dietary diversity of kobs in the face of competing PSMs may be needed to provide a conclusive picture of interactions. The work of Scogings *et al*. (2015) in Kruger National Park, South Africa, on the effect of seasonal influences of carbon-based secondary metabolites on nutrient availability was characterised by inconsistencies. Nevertheless, ungulates such as kobs may still derive various benefits during this competitive season and from PSMs due to their adaptive mechanisms. This assertion is undoubtedly the subject of ongoing debate with respect to kobs and other ungulates.

5 Conclusion

This study showed that almost all of the analysed PSMs and MNs in kobs' forage plants were significantly higher in the wet than in the dry season. Since the concentration of PSMs correlates with that of certain MNs in selected forage

plants, the utilisation of minerals like S, K, Fe, Zn and Na by kobs may be negatively affected by the presence of certain PSMs, depending on season, even though some PMSs may have medicinal value.

Conflict of interest

The authors hereby declare no conflicts of interest.

Declaration of author contribution

Adewale, R. O. conceptualised the idea, designed the study and wrote the first manuscript. Alarape, A. A. supervised all the work. Womiloju, M. A. carried out the literature review and collected the kobs' dungs. Akinsorotan, O. A. and Oguntade, O. A. helped in the designing and coordination of the study. Olagunju S. O., Olayiwola, R. O. and Kolapo, M. A. helped finance, statistically analysed and interpreted the results. All authors read, reviewed and approved the final manuscript.

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