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Research Article – Histology and Cell Biology

Climate related histological changes in the stomach papillae of *Cephalophus Niger* (Gray 1846). Implications of climate dynamics and prolonged drought

Michael O. Samuel[1*], Oliver J. Nzalak[2], Chima I. Nwaogu[3], Barnabas T. Asongo[1]

Departments of Veterinary Anatomy of [1]Federal University of Agriculture Makurdi, Benue State, [2]Ahmadu Bello University Zaria, Kaduna State, and [3]University of Nigeria Nsukka, Enugu State, Nigeria

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Abstract

This study surveyed selected tissue samples obtained from the stomach compartments of the Cephalophus niger including, rumen, reticulum, omasum and abomasum. We utilized 40 antelope's stomach compartments, comprising of 20 males and females respectively; half of the total samples were taken during the peak period of wet seasons and the other half in dry seasons. The relative dimensions of the papillae at various locations within the fore and glandular stomach, observed under a light microscope at magnifications of ×100 and ×400, revealed significant variations in heights (h), curved surface area (πrs), base area (πr^2) being a factor which determines the size of other dimensions, total surface area ($\pi r^2+\pi rs$) and cross sectional diameters between the seasons. Because prolonged drought may force the species to migrate further hinterland with risks of exposure to predators and reduced survivability of the young, the observed morphological reductive changes may be eco-adaptation for survival in the habitat in prolonged drought since they predispose the compartments to reduce fermentation capability and production and bio-utilization of volatile fatty acids. They may also contribute to the occurrence of dental abnormalities and influence disease epidemiology. The results may be used as a model for the assessment and determination of optimal season food bio-utilization index, a parameter relevant to remedial interventions for the conservation of less adaptive feeders.

Key words

Morphometry, gastric papilla, Seasonal variations, climate change.

Introduction

Climate related phenomena and their consequences on the stomach papillae of duikers have been investigated by several authors, Terje et al. (1996), studied ruminal papillae of reindeer, Hofmann (1982) the feeding strategy of ruminants and Awad and Elhadi (2010) seasonal variability in the nutritive value of ruminant diet. There is little or no information on the effects of consequences of eco-environmental changes, such as prolonged drought and shortened periods of easily available water and forage, on the histology of papillary structures within different stomach compartments of wild duikers.

Cephalophus niger (Grey, 1846), 'black duiker', belongs to the bovine family and is a member of the order Cetartiodactyla, found more commonly in tropical west

* Corresponding author. E-mail: walesamuel10@gmail.com.

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African rainforest sub regions. The species is identified by its characteristic pelage, color markings and habitat (Happolds, 1986; Stafford and Stafford, 1991; Kingdon, 1997). It is adapted to open, swampy or mountain environment. The statistical spatial ratio is 1:6, *i.e.* for every kilometer distance of home rangeland there are likely six duikers of various size and sex. The species maintains a least concern status, not endangered but reducing in population (IUCN, 2012). Presently, no evidence of conscious protection in the form of deterrent policies to conserve or preserve this species exists in Nigeria.

Seasonal climatic variations and severe changes in the tropical ecosystem, with their subduing effects on wildlife and their habitat (Terje et al., 1996), result in migrations and exposure to predators (Hernandez-Fernandez and Vrba, 2005) including man. C. niger, is an intermediate feeders gets information on canopy from primates as they follow for leftovers of fruits, in a kind of surrogate relationship to canopy itself. Alternative diet component may include flesh tissues of carcasses, duikers also nibble at the bark of some shrubs during food scarcity periods.

The socio-economic importance of C. niger derives from its genetic biodiversity and maintenance of the ecosystem; the ranching potential derives from an effective morpho-physiologic homeostasis of the gastrointestinal tract which translates into a higher meat turn over than conventional herds (Happolds, 1987). The IUCN (2012) classified the present species being resistant to extinction threats despite pressure of predation from other animals including man; it may serve as alternative protein source and bush meat delicacy.

The stomach compartments: rumen, omasum and reticulum of wild ruminants are characterized by the presence of conical projections *i.e.* 1.0-1.5 cm long, primary and secondary papillae which are part of a complex highly folded tunica mucosa (Delmann and Horst, 1981). The architecture of papillae is influenced by diet and has direct bearing on the absorption of volatile fatty acids, lactic acids, ammonia, inorganic ions and water in ruminants stomach (Hofmann and Schnorr, 1982; Awad and Elhadi, 2010).

Materials and methods

Animals

Twenty adult antelopes (C. niger) were obtained from peasant farmers in the dry seasons (November-April); they were ten females and ten males. A further sample similar in number and composition was acquired from hunters in the wet season between the months of May and September over a time period of three years. These animals were taken from their ecological environment in the south-western part of Nigeria in compliance with ethics on wildlife preservation and game hunting procedures.

This investigation was carried out upon authorization, in the College of Veterinary Medicine University of Ibadan, Department of Veterinary Anatomy. Since policies regulating access to wildlife patrolling officers or veterinary personnel empowered to prevent or seize illegally captured species do not presently exist in Nigeria, verbal,permission to acquire animals through purchase or coercion from hunters on expeditions was obtained from Village chiefs (unlettered) and the chair persons of the respective local government areas (Egbeda and Akinyele LGA).

Grouping was based on sex before examination of the digestive tract from mouth to rectum for gross pathological lesions. Stomach chambers were dissected out and rinsed in normal saline. All animals used for the study had no signs of gastrointestinal disorders.

Tissue sampling and processing

Samples of 2.5c m^3 were taken from each of the following sites as soon as the compartments were removed from normal saline; they were fixed in 10% formalin for 24 hours and then into 70% alcohol for 72 h before paraffin embedding and sectioning.

From rumen: two samples rich in ruminal papilla from the dorsal sac at the ruminal atrium, and two samples from the ventral sac; from reticulum: two samples from papilla endowed regions at the base and two samples from the upper part of the reticular grooves; from omasum: two samples from the reticulo-omasal opening; from abomasum: two samples.

Sections were stained with haematoxylin and eosin (H&E) and viewed under a light microscope (Olympus, Southend-on-Sea, UK) with ×100 and ×400 objectives. The localization of papillae was assessed at ×100 while linear dimensions of structures and randomly selected various cellular components were studied at ×400.

Morphometry

The tallest papillae on each slide were photographed at 500 dpi resolution using Moticam 1000® camera with Motic Images 2.0 software (both from Motic, Hong Kong, China).

Papillary maximum height was measured from the base of the papilla, at the junction with the tunica sub mucosa, to the tip of the structure while maximum width was taken at the widest part of the neck. An average of six such structural entities was evaluated on each slide.

The data were recorded as mean ± standard deviations and subjected to statistical analysis (ANOVA) with Bartlett's test of equal variance (post test) using Graphpad prism 4.00 for Windows (Graphpad Software, San Diego, CA). $P< 0.0001$ was considered as highly significant.

Results

Representative findings are depicted in figures 1-3 and quantitative data are summarized in figures 4-8. The curved surface area (area of a cone) and total surface area (base area of a cone + curved surface area) of papillae in dorsal rumen, reticulum, and abomasums were significantly higher in females during wet seasons. A reverse situation occurred for the ventral sac of rumen and omasum, with male showing higher values. Statistical interactions of factors assessed for this group (influence of season on dimensions depending on sex) accounted for 18.06% of the total variance, an effect to be considered highly significant ($P < 0.0001$).

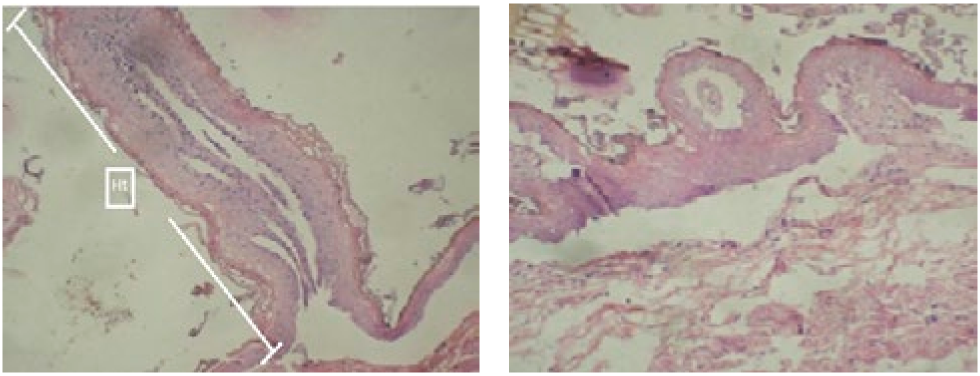

Figure1 – (A) Adult rumen papilla in the wet season; (B) rumen papilla in the extended dry season. Haematoxylin and eosin, x100.

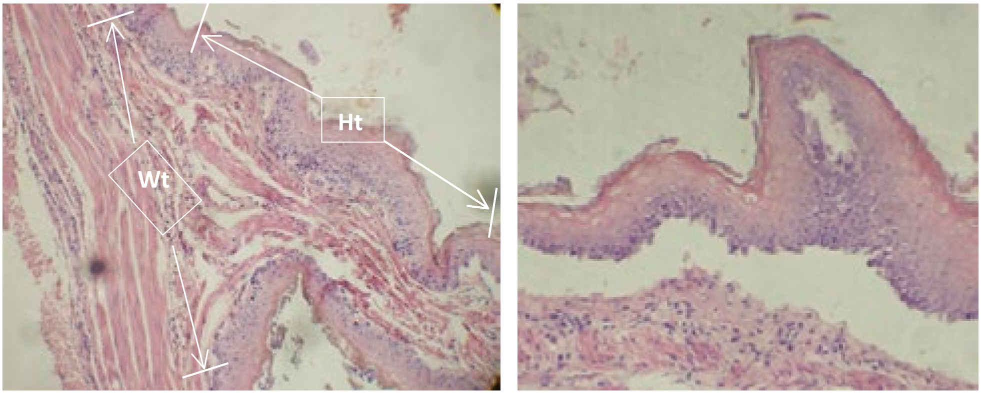

Figure 2 – (A) Adult C. niger omasum papilla in the wet season; (B) omasum papilla in the extended dry season. Haematoxylin and eosin stain, x100.

Papillary structural parameters (height, base diameter, base radius, curved and total surface areas) at various stomach locations during extended dry season periods was higher in females, except for papillary height and base diameter in omasum and all papillary parameters in abomasum, which were higher in males.

Analysis of interaction among measured parameters, season and sex revealed a highly significant effect ($P< 0.0001$). Analysis restricted to males showed curved and total surface area higher but with higher variance and hence standard error of the mean (SEM) in wet periods than in extended dry season ($P < 0.0001$) except for reticulum and abomasum which did not show significant differences.

Analysis of interaction among between season and papilla height, base radius, diameter curved and total surface area gave highly significant results ($P < 0.0001$).

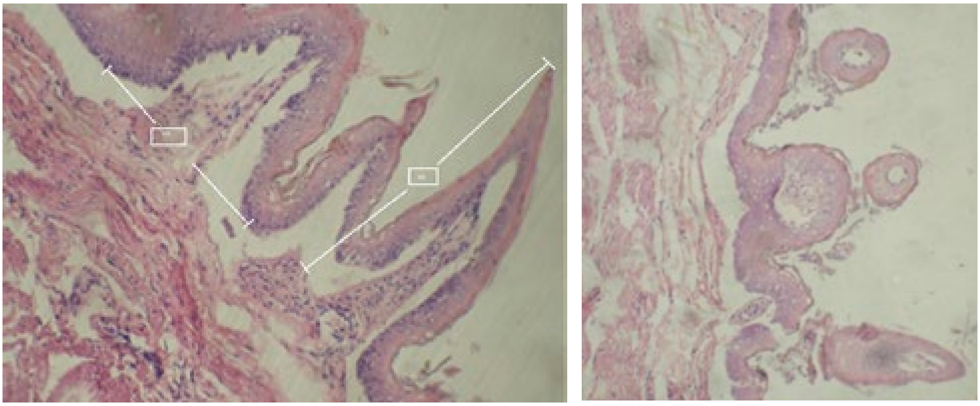

Figure 3 – (A) Adult C. niger reticular papilla in the wet season; (B) reticular papilla in the extended dry season. Haematoxylin and eosin stain, x100.

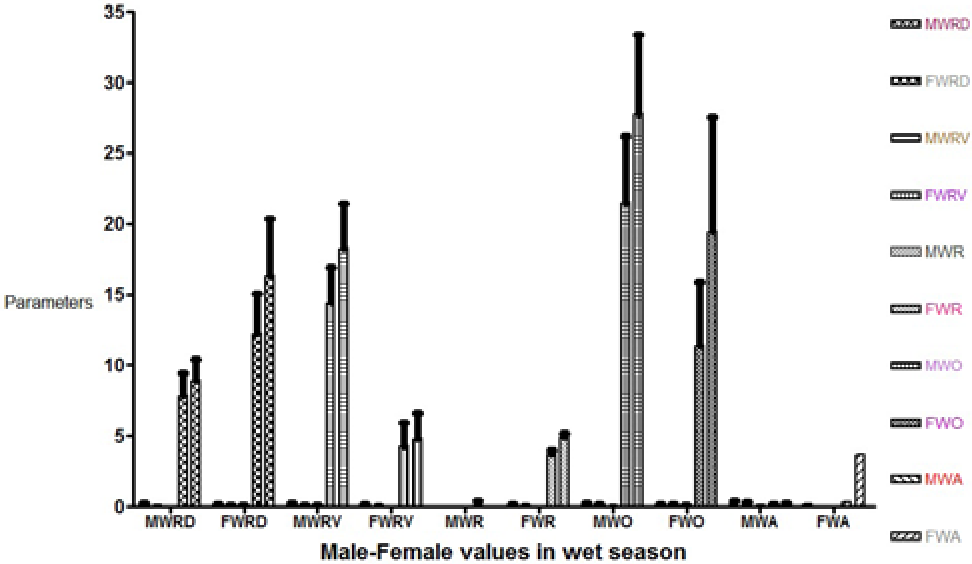

Figure 4 – Comparisons between sexes for the parameters estimated in the wet season. MWRD/FWRD: dorsal sac of rumen for males and females respectively. MWRV/FWRV: male and respectively female ventral sac of rumen in the wet season. MWR/FWR: male and respectively female wet season reticulum. MWO/FWO: male and respectively female wet season omasum. MWA/FWA: male and respectively female wet season abomasum. MDRD/FDRD: male and respectively female dry season rumen dorsal sac. MDRV/FDRV: male and respectively female dry season rumen ventral sac. MDR/FDR: male and respectively female dry season reticulum. MDO/FDO: male and respectively female dry season omasum. MDA/FDA: male and respectively female dry season abomasum.

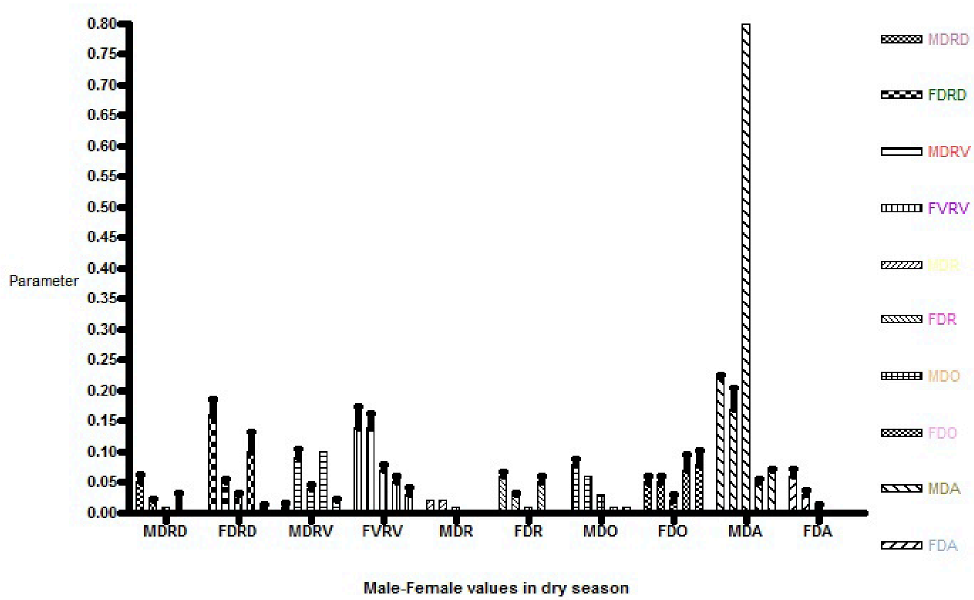

Figure 5 – Comparison between sexes of the estimated parameters for the extended dry season. Abbreviations as in figure 4.

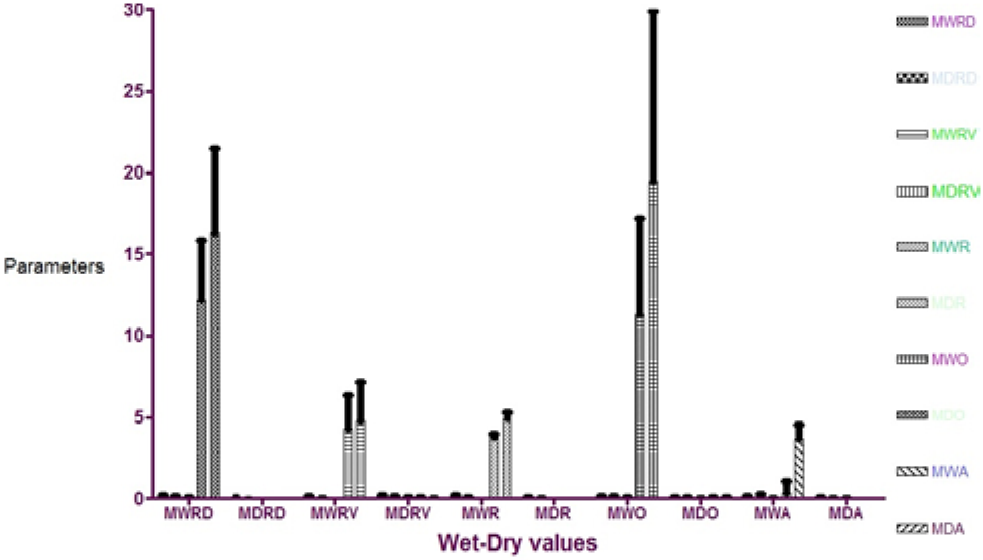

Figure 6 – Behaviour of the estimated parameters for the stomach of males in the extended dry season. Abbreviations as in figure 4.

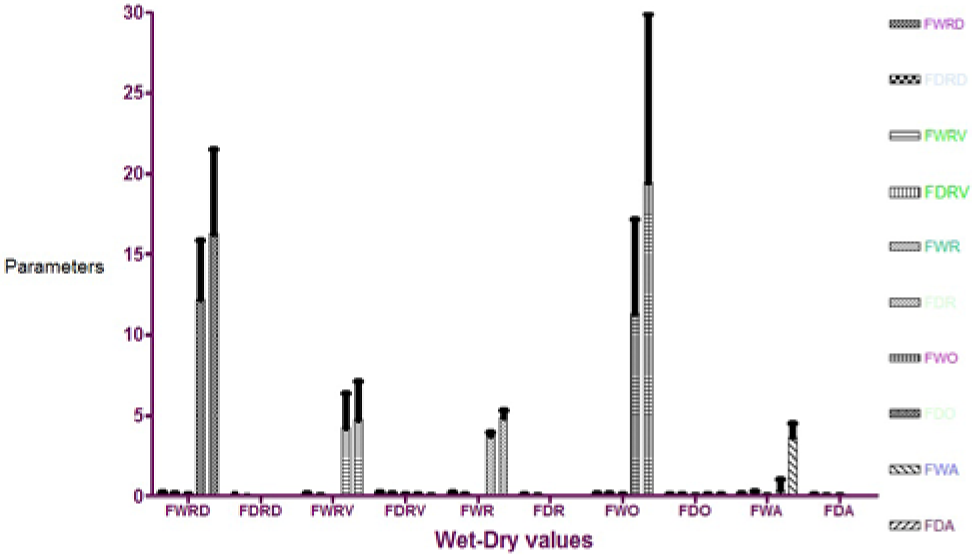

Figure 7 – Behaviour of the estimated parameters for the stomach of females in extended dry season. Abbreviations as in figure 4.

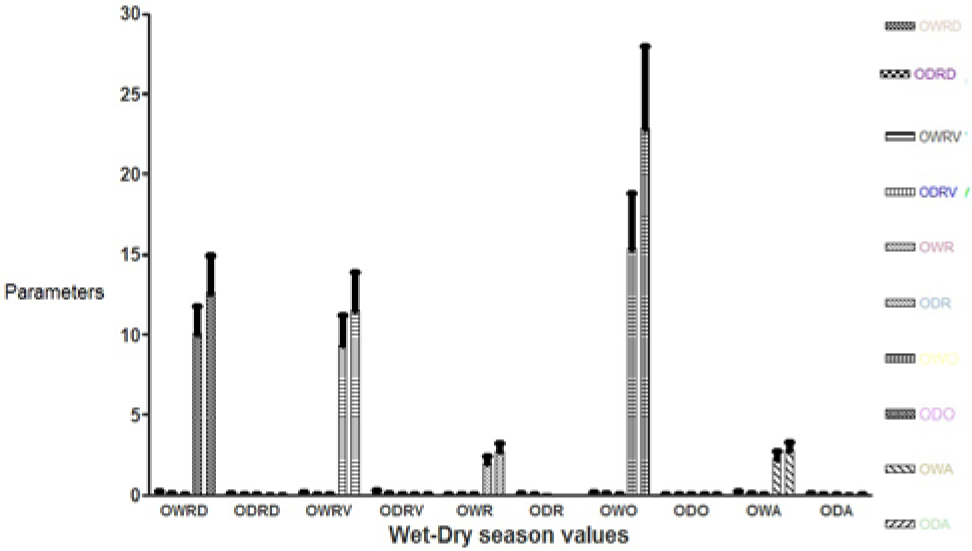

Figure 8 – Comparison between sexes of the parameters estimated for the wet and the extended dry seasons. Abbreviations as in figure 4.

In females, curved and total surface area were higher and and with higher SEM in wet seasons ($P<0.0001$) contemporary with reduction in cross sectional diameter of the fore stomach, while papillary curved surface area in abomasum was similar between seasons.

Papillary height, base radius, base diameters, curved and total surface areas were highly significantly higher in the wet seasons ($P<0.0001$).

Discussion

Wildlife species respond differently to extreme and unusual weather phenomenon such as sustained increase in environmental temperatures (National Research Council, 2013), largely determined by variations in rainfall and oscillatory movements of inter-tropical discontinuities (Nigerian Meteorological Agency, 2010). The objective of this study were to: (1) examine the difference in stomach compartmental papilla morphology between the wet and prolonged dry season (Stafford and Stafford, 1991); (2) compare between sexes the variations assessed between seasons: and (3) determine the effects of prolonged drought and climate dynamics on gastric homeostasis and survivability of adults and young of the ruminant species *C. niger* (Ita, 1994).

The tropical hinterland climate zone where *C. niger* lives (Iliasu and Alsop, 1987) has recently experienced increasing drought conditions considered as a consequence of shifts in intercontinental discontinuities (Nigerian Meteorological Agency, 2010); these conditions are characterized by earlier onset of rainfall anomaly (February/March) with usually high year cumulative output (above 4500 mm) and mean temperature departure from normal of 1.9-2.9ºC/month. A longer stretch of drought concomitantly results.

Females can utilize the wet season better than males as demonstrated by the surface area of papillae in the rumen. Adult females, often pregnant and lactating, utilize more feed in the lush vegetation (Beier and McCollough, 1990; Artle et al., 2001) during breeding periods thereby altering the dimensions of the digestive structural tools used for the generation of volatile fatty acids for metabolic activities (Terje et al., 1996).

Observations in extreme and unusual dry weather conditions showed that most parameters measured were significantly lower compared to samples obtained in the wet season, indicating severe reduction in papillary size and density. However, papillary density in abomasum remained approximately constant even during extreme season, perhaps expressing an adaptation to preserve nutrition. A coefficient expressing the extent of papillary surface per unit surface area of the mucosa, *i.e.* (100+papillary length x perimeter x number)/(cm^2 x 100) (Terje et al., 1996; Awad and Elhadi, 2010), was quite constant for the abomasum, indicating resilience of the papillae for that particular location. Our observation may also be as a result of an increase in the home range area (perhaps thereby gaining access to more food and water) when Western highlands watersheds and other water bodies dry up (Ayeni, 2000; Bashares and Arcese, 2000). This adaptation would be important in supporting pregnancy, lactation and the consequently increased metabolic demand, at variance with larger ruminants which range less in extreme dry weather conditions as a result of slower fat depletion rate (Artle, 2001).

Table 1 – Measures obtained for the wet season (mean± S.D.).

	No.	Males					Females				
		MWRD	MWRV	MWR	MWO	MWA	FWRD	FWRV	FWR	FWO	FWA
Height (mm)	6	0.22±0.13	0.20±0.06	0.02±0.00	0.22±0.11	0.38±0.02	0.17±0.04	0.13±0.11	0.12±0.05	0.11±0.06	0.14±0.03
Base diameter	6	0.06±0.00	0.10±0.04	0.03±0.00	0.14±0.03	0.25±0.10	0.10±0.05	0.03±0.02	0.06±0.02	0.11±0.12	0.04±0.02
Base radius (r)	6	0.03±0.00	0.05±0.02	0.01±0.00	0.07±0.01	0.12±0.00	0.05±0.03	0.00±0.00	0.03±0.00	0.05±0.06	0.01±0.01
Curved surface area (πrs)	6	7.98±4.84	14.45±7.82	0.34±0.03	21.44±14.95	0.15±0.02	12.22±8.96	4.29±5.11	3.76±0.57	11.35±14.24	0.31±1.72
Total surface area (πr2+πrs)	6	8.89±4.83	18.27±9.93	0.01±0.00	27.79±17.53	0.20±0.20	16.32±12.75	4.81±5.67	4.93±0.83	19.49±25.43	3.65±2.10

Table 2 – Measures obtained for the wet season for both sexes together (mean± S.D.).

	No	OWRD	OWRV	OWR	OWO	OWA
Height(s) (mm)	12	0.20±0.10	0.16±0.09	0.07±0.06	0.15±0.11	0.20±0.11
Base diameter	12	0.08±0.04	0.07±0.05	0.04±0.02	0.12±0.09	0.09±0.09
Base radius (r)	12	0.04±0.02	0.03±0.02	0.02±0.01	0.06±0.05	0.04±0.05
Curved surface area (πrs)	12	10.10±7.50	9.37±8.33	2.05±1.76	15.38±15..34	2.30±1.94
Total surface area (πr2+πrs)	12	12.61±10..34	11.54±10.52	2.74±2.27	22.81±22..96	2.79±2.35

Table 3 – Measures obtained for the prolonged/extended dry season (mean± S.D.).

	No	Males					Females				
		MDRD	**MDRV**	**MDR**	**MDO**	**MDA**	**FDRD**	**FDRV**	**FDR**	**FDO**	**FDA**
Height (mm)	6	0.05±0.04	0.01±0.02	0.02±0.00	0.08±0.03	0.22±0.02	0.16±0.08	0.14±0.11	0.06±0.02	0.05±0.03	0.06±0.04
Base diameter	6	0.02±0.01	0.09±0.05	0.02±0.00	0.06±0.00	0.17±0.11	0.05±0.02	0.14±0.07	0.03±0.01	0.05±0.03	0.03±0.02
Base radius (r)	6	0.01±0.00	0.04±0.02	0.01±0.00	0.03±0.00	0.08±0.00	0.03±0.01	0.07±0.03	0.01±0.00	0.02±0.03	0.01±0.01
Curved surface area (πrs)	6	0.00±0.00	0.01±0.00	0.00±0.00	0.01±0.00	0.05±0.20	0.01±0.01	0.05±0.03	0.00±0.00	0.07±0.08	0.00±0.00
Total surface area (πr2+πrs)	6	0.00±0.00	0.02±0.01	0.00±0.00	0.01±0.00	0.07±0.10	0.01±0.01	0.03±0.04	0.00±0.00	0.08±0.07	0.00±0.00

Table 4 – Measures obtained for the prolonged/extended dry season for both sexes together (mean± S.D.).

	No	ODRD	ODRV	ODR	ODO	ODA
Height (mm)	12	0.1±0.09	0.15±0.8	0.08±0.09	0.07±0.04	0.12±0.06
Base diameter	12	0.04±0.02	0.12±0.06	0.03±0.10	0.06±0.05	0.06±0.06
Base radius (r)	12	0.02±0.01	0.05±0.03	0.10±0.00	0.03±0.03	0.03±0.03
Curved surface area (πrs)	12	0.01±0.01	0.03±0.03	0.00±0.00	0.05±0.07	0.01±0.02
Total surface area (πr2+πrs)	12	0.01±0.01	0.04±0.04	0.00±0.00	0.05±0.07	0.02±0.03

Attenuated histology of male's papillae in harsh dry weather periods serves as diet index for defining climate influenced diet-responses triggered by scarcity of preferred food and water (Mitchell and Jones, 2005; Omogbai, 2010). Severe reduction in the papillary dimensions may result in profound metabolic or homeostatic imbalance and predispose to systemic pathology.

The results in both wet and extended dry periods were similar for females and males except for reticulum and abomasum, where measures were smaller for males in both seasons, probably due to a combination of factors; females may deplete reserves faster (despite heavy ruminal load) as a result of reasons given above, and duikers - being intermediate feeders - switch to alternative diet in scarcity of food of choice, which leads to adapt papillary size and density to allow for enhanced functionality at such times (Beier and McCollough, 1990). Furthermore, the different seasonal modifications between sexes may be an adaptation for survival in an altered eco environment. Males utilize more energy in fights and territorial protection but otherwise have less metabolic needs than females who have to undergo pregnancy and lactation (Brogan et al., 1997; Brashares and Arcese, 2000; Heilbronn and Ravussin, 2003; Olopade, 2006; Fernandes et al., 2008). The constancy in reticular and abomasal papillary architecture between seasons might help females to take the best advantage of eaten food. Furthermore, females will tend to venture farther in search of alternative food and water sources to cope with metabolic demands and with competition with younglings for food. This behaviour exposes females to higher risk from predators including man (IUCN, 2012).

The results which were not influenced by sex and sample location might be related to availability of fresh vegetation between the months of May and July, just before the little dry spell (LDS) (Ita, 1994; Nigerian Meteorological Agency, 2010). Papillary dimensions were markedly reduced in the periods which would reduce the food available for absorption. Such diet restriction may lead to deplete body reserves and if long lasting may impact negatively on homeostatic balance and contribute to the development of dental abnormalities, as reported by Agbo and Kene (1998), when calcium to phosphate ratio becomes distorted (Kene and Uwagie-Ero, 2001; Rochger et al., 2004; Mulder et al., 2006; O'Leary et al., 2012).

The presence of high levels of volatile fatty acids in ruminants' rumen follows to the availability of fermentable feed and is correlated with high papillary density and increased absorptive surface area in this location (Terje et al., 1996). High volatile fatty acid levels are an index of diet bio-availability in captive and free ranging ruminants. Diet of free ranging wild ruminants in dry seasons consists mainly of crude fibre with low nutritive value (Awad and Elhadi, 2010); as a result of reduced availability of fruits, the ingesta contains more dry seeds and bark of trees (Happolds, 1987).

Less gastric secretions may be present during the dry season, due to reduced water and food intake as most small rivers are seasonal, dwindling in capacity at the onset of dry season (Beier and McCollough, 1990; Ita, 1994) then drying up by late January. This in turn might contribute to a more acidic stomach.

The Southern rain forest vegetation zones have peculiar hydrological patterns in which rivers, lakes and other water bodies quickly overflow to connect with each other, peaks occurring twice in the rainy/wet season (Iliasu and Alsop, 1987; Mitchell and Jones, 2005). Notwithstanding, water discharges quickly from the hinterland at the onset of dry season and the soil water retention capacity determines extent and availability of pools and surface water in this part of the country in the latter season (Ayeni, 2000). Rainfall anomaly and extreme weather events, made worse by unavailability of underground water (Omogbai, 2010), have profound effects on wildlife preservation and regeneration. In regions where the annual precipitation exceeds 4322 mm between the rainy months of May to October, because of shifts in inter-tropical discontinuities (Mitchell and Jones, 2005; Nigerian Meteorological Agency, 2010), flooding of habitable places occurs as torrential rain continues nonstop for several days and sometimes up to two weeks, which restricts feeding and further allows predation and poaching activities.

In conclusion, the authors believe that the results of this study can be useful for diet formulation for captive animals and for feeding assistance to wild ruminants in critical dry periods.

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