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Nutritional composition of plants and preliminary assessment of nutrition in free-ranging bare-nosed wombats (*Vombatus ursinus*)

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ABSTRACT

Nutrition is essential for not only survival but also successful growth and reproduction. Dietary demands are increased in a diseased state due to the increased energy and nutritional requirements associated with immune response, inflammation and convalescence. The herbivorous bare-nosed wombat (*Vombatus ursinus*) is notably susceptible to sarcoptic mange, a disease caused by the mite, *Sarcoptes scabiei*, which causes debilitating pruritic skin disease and leads to secondary bacterial infections and increased wombat morbidity and mortality, as well as regionally variable population declines. It is unknown why wombats are so susceptible to sarcoptic mange and if nutrition may play a role in disease expression, particularly relating to seasonality. The objective of this study was to quantify the differences in the nutritive value, over four seasons, of plants (mostly grasses and sedges) that are available as food items for bare-nosed wombats. We collected plants over four seasons from five wombat habitats that were known to have wombats affected by sarcoptic mange. We found seasonal and site differences for macro and micronutrients in the plants analysed. Monitoring the diet quality of wombats in the wild is useful for managing their populations and understanding population dynamics in relation to food resource quality.

Keywords: Energy, faecal nitrogen, grasses, herbivore, lipids, marsupial, minerals, protein, *Sarcoptes scabiei*, sarcoptic mange, wombat health.

Introduction

Animal survival in different environments is linked to the challenges of obtaining food that provides the required macronutrients (protein, lipid and carbohydrate) (Simpson and Raubenheimer 2012). Energy intake is used for maintenance, somatic growth and reproduction (Barboza *et al.* 2009; Simpson and Raubenheimer 2012). However, when an animal is affected by illness or disease, essential physiological capabilities, such as mounting an immune response, require even more energy (Lochmiller and Deerenberg 2000; Cotter *et al.* 2011; Verma *et al.* 2016).

The bare-nosed wombat (*Vombatus ursinus*) is a herbivorous marsupial native to Australia (McIlroy 2023), currently listed as 'least concern' on the IUCN Red List (Taggart *et al.* 2016). There has been little investigation into its true population size and distribution (Buchan and Goldney 1998; Roger *et al.* 2007; Thorley and Old 2020), and one of the largest threats facing the bare-nosed wombat is sarcoptic mange (Skerratt *et al.* 1998; Old *et al.* 2018), a disease caused by the sarcoptic mite, *Sarcoptes scabiei*. Sarcoptic mange occurs throughout the geographical range of wombats (Martin *et al.* 1998; Mayadunnage *et al.* 2023) and has led to the decline of some populations (Martin *et al.* 2018) and possibly a decline in the total population.

Insight into the potential relationship between nutrition and sarcoptic mange prevalence, susceptibility, disease severity and wombat mortality could aid management of free-ranging populations affected by sarcoptic mange. Understanding dietary availability within each habitat, and the nutritional value of those plant species, could allow for the promotion of high-quality food items within the habitats of sarcoptic mange-affected populations. Improved access to high-quality food items would increase nutrient intake and may ease the impacts associated with sarcoptic mange infection, such as starvation, and support the immune system (Lochmiller and Deerenberg 2000). Also, increased nutritional availability may enhance reproductive success and survival rates of individuals (Trites and Donnelly 2003), thus supporting population maintenance.

This preliminary study aimed to determine the nutritional composition of plants (potential dietary items of the wombat) in five habitats across four seasons.

Methods

Study sites

Five study sites were chosen throughout New South Wales (NSW), with confirmed presence of wombats both with and without sarcoptic mange, including sites within or near Wolgan Valley, Badger Ground, Eagle's Drift, Robertson and Coolagolite. See Stannard *et al.* (2021) for site descriptions. Rainfall and temperature data (Supplementary Fig. S1) were obtained from the BOM (Bureau of Meteorology 2019).

Plant and faecal sample collection

A minimum of 0.5 kg of leaves was collected from selected grasses, sedges and herbage species that were common at each study site and likely to be dietary items of the bare-nosed wombat (unpubl. data). Plant samples were collected from each site during each season from June 2018 until May 2019, except in spring from the Wolgan Valley, and identified using plant field guides (Brooker and Kleinig 1990; Auld *et al.* 1992; Jones and Clemesha 1993; Klaphake 2002; Robinson 2003; EucaLink 2004; Klaphake 2004; Fairley and Moore 2010; Klaphake 2010; Richardson *et al.* 2011; PlantNET 2020). Wombat faecal samples free of soil were collected as per Old *et al.* (2020). The site, date, time and GPS location were recorded for all faecal and plant samples.

Nutrient analysis

The nutritional composition of all plants and faecal samples collected was measured, including gross energy, proteins, lipids and minerals. Each of the plant ($n = 78$) and scat ($n = 100$) samples was analysed individually. Samples were weighed and oven dried at 60°C for 24–48 h, until reaching a constant mass. Samples were then ground into a fine powder (<1 mm) using a Foss CT 293 Cyclotec General Purpose Sample Mill for further analysis. Gross energy was measured using an Oxygen Bomb Calorimeter (Parr 6200, Parr Instrument Co, Moline, IL, USA) with a benzoic acid standard. Crude protein was determined using an ELEMENTAR vario EL cube (Elementar Australia Pty Ltd, Sydney, Australia), calculated as nitrogen × 6.25. Lipids were extracted using a BÜCHI 810 Soxhlet fat extractor (BÜCHI Labortechnik AG, Flawil, Switzerland). Mineral content was determined using a microwave digest and ICP-AES analysis at the Department of Primary Industries, Wollongbar, NSW. Samples were analysed in duplicate with 5% precision.

Wombat abundance and sarcoptic mange prevalence

A spotlighting survey was conducted once at each site during each season, winter–spring 2018 and summer–autumn 2019. Spotlighting commenced shortly after sunset, when wombats are most active (Evans 2008). Two people, each using a handheld 100 W halogen spotlight (Powa-Beam: PL – 145, 12 V), searched for wombats from opposite sides of a vehicle driven along a transect at a maximum speed of 10 km/h.

The total number of wombats observed during each survey was recorded, including whether the individual was showing visible signs of sarcoptic mange, using the sarcoptic mange scoring system described in Stannard *et al.* (2021). The time of each observation, the GPS coordinates of the vehicle on the transect, the compass bearing towards the wombat and the estimated distance from the car to the wombat were also recorded.

Statistical analysis

Two-way analysis of variance (ANOVA) and Tukey's *post hoc* tests were performed using SPSS to determine significant differences between site and season for each nutrient analysed.

Ethics

The study was approved by the Western Sydney University Animal Ethics Committee (approval no. A12033) and conducted under NSW NPWS scientific licence SL100786.

Results and discussion

In total, 78 plant samples were collected across all five study sites during each season. Common plant species collected included common couch (*Elymus repens*), kikuyu (*Cenchrus clandestinus*) and summer grass (*Digitaria ciliaris*). The list of plants included in the analysis can be found in Supplementary Table S1. Not all plant species were available at all sites. Fresh wombat faecal samples (100 in total, all pellets) were collected from the five sites across each season, 5–6 faeces per site per season.

Gross energy

There was a significant difference for site ($F = 8.78$, $P \leq 0.05$) and season ($F = 5.37$, $P \leq 0.05$) for gross energy

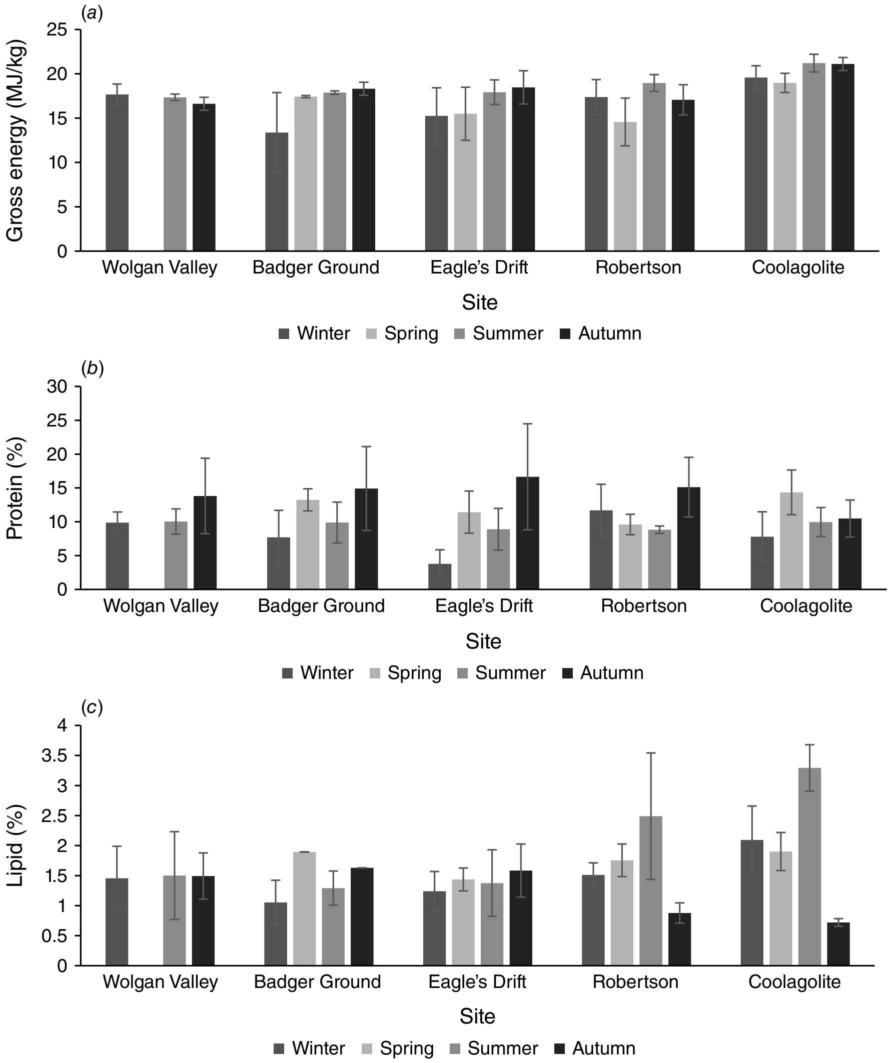

Fig. 1. Mean ± s.d. nutrient composition of plants collected in each season at the study sites. (*a*) Gross energy. (*b*) Protein. (*c*) Lipid.

content of plants. Mean gross energy content of plants was significantly higher at Coolagolite compared with the other sites (Supplementary Table S2). Robertson had the lowest energy composition of all the sites (Fig. 1*a*). There was no significant interaction between season and site for gross energy ($F = 1.73$, $P = 0.09$).

Protein

Mean protein content was significantly different across seasons ($F = 9.49$, $P \leq 0.05$) but not sites ($F = 0.39$, $P = 0.81$), and there was no significant interaction between season and site ($F = 1.60$, $P = 0.12$). Protein was significantly higher in autumn compared with winter and summer, and significantly higher in spring compared with winter (Supplementary Table S2). The mean protein content of plants was highest during autumn at four of the five sites and lowest during winter at four of the five sites (Fig. 1*b*). There was a higher-than-average rainfall during early autumn (March 2019) at most sites (Fig. 2), which likely contributed to a higher protein content during autumn. The mean protein content of plants was higher during autumn and spring than during winter and summer at all sites except Robertson, where winter had the second-highest mean protein content (Fig. 1*b*). Robertson had a higher rainfall in early winter (June 2018; Fig. 2, Supplementary Table S3), which would have contributed to plant growth and higher protein composition. It should also be noted that, at the time of sampling, there was a drought that would have affected plant growth (Supplementary Table S4).

Protein composition of the individual plants was similar to those previously determined for native and introduced grass species (Fulkerson *et al.* 2007; Foster *et al.* 2010). However, one site and season contained lower values than previously recorded: Eagle's Drift. During winter at Eagle's Drift, mean protein was 3.8% due to low protein content in all grasses analysed in this season. Protein available for wombats would therefore be reduced at this time. This site had very low rainfall during winter, likely contributing to minimal plant growth and reduced protein composition.

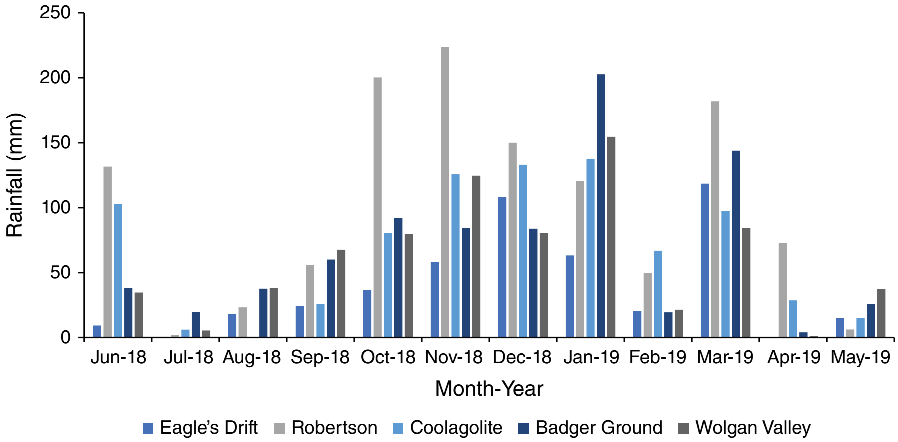

Fig. 2. Monthly total rainfall (mm) at each study site from June 2018 to May 2019.

Lipid

Lipid concentration ranged from 0.7 to 3.6%, with the higher values recorded in Robertson and Coolagolite in summer, 2.5 and 3.3%, respectively (Fig. 1c). Mean lipid content was significantly different across seasons ($F = 5.97$, $P < 0.05$) and sites ($F = 3.19$, $P < 0.05$), and there was a significant interaction between season and site ($F = 4.14$, $P < 0.05$). Mean lipid concentration was significantly higher at Coolagolite compared with Wolgan Valley, Eagle's Drift and Badger Ground. Seasonally, mean lipid concentration was higher for summer compared with winter and autumn (Supplementary Table S2). Lipid concentrations were within the expected range for grasses, based on published data (Fulkerson *et al.* 2007).

Minerals

Potassium content in the plants was significantly different across seasons ($F = 3.26$, $P < 0.05$) but not sites ($F = 0.81$, $P = 0.53$). Phosphorus content in the plants was also significantly different across season ($F = 5.71$, $P < 0.05$) but not site ($F = 2.53$, $P = 0.054$). There were no significant differences between site and season for the other minerals analysed – calcium, iron, magnesium and sodium (Supplementary Table S5).

Wombat abundance and sarcoptic mange prevalence

Lower numbers of wombats were observed at Badger Ground and Eagle's Drift per spotlighting survey ($\bar{x} = 9.0$ and $\bar{x} = 6.0$, respectively) compared with the Wolgan Valley site ($\bar{x} = 36.5$). No wombats were observed during spotlighting at Robertson in any season. One wombat was observed in spring and two in summer at Coolagolite with no signs of sarcoptic mange, and no wombats were observed during spotlighting surveys in autumn or winter at this site.

Sarcoptic mange prevalence ranged from 0 to 50% per survey in our study, and relatively low numbers of wombats were observed at two of the sites. Eagle's Drift had the highest prevalence of sarcoptic mange in winter, summer and autumn compared with the other sites (Table 1). At Wolgan Valley and Badger Ground, the highest prevalence of sarcoptic mange was in spring and the lowest in winter, whereas Eagle's Drift had the highest prevalence of sarcoptic mange in summer and the lowest in spring (Table 1). Compared with previous data collected by Stannard *et al.* (2021), similarly lower total numbers of wombats were observed at Badger Ground and Eagle's Drift compared with Wolgan Valley. Wombats in Wolgan Valley are likely habituated to humans doing spotlighting throughout the site regularly because it is an ecoresort. Sarcoptic mange prevalence was lower at Wolgan Valley (0–15%), higher at Eagle's Drift (20–50%) and varied greatly at Badger Ground (0–29%), compared with previous studies at these sites (16.1–40.7%; 7.0–20.8%; 12.0–22.5% respectively) (Stannard *et al.* 2021). However, the present study only observed sarcoptic mange prevalence on one night per season, compared with numerous survey nights in Stannard *et al.* (2021).

Relationship between plant nutrient composition and wombat nutrition

At four of the five study sites, faecal protein increased in concentration from winter to spring. A prominent peak in faecal protein concentration was particularly evident at Coolagolite (Fig. 3). Although winter faeces had the lowest protein content, at Badger Ground (6.6%) and Eagle's Drift (6.2%), faecal protein concentration was relatively consistent across seasons at these sites. At Wolgan Valley, faecal protein was highest in winter followed by autumn, spring and summer (Supplementary Table S6). Wombats have a low requirement for protein and apparent digestibility for protein of approximately 80% (Barboza and Hume 1992; Barboza *et al.* 1993); therefore, the level of protein available to the wombats in the habitats we studied would be approximately 6.8–11.4%, depending on season. Because grasses senesce nitrogen content (and protein) reduces while fibre increases (Barboza 1989; Hume 1999). These nutrient changes of plants in differing stages of growth would affect

Table 1. Number and prevalence (%) of wombats showing signs of sarcoptic mange at the Wolgan Valley, Badger Ground and Eagle's Drift sites during all seasons.

Sarcoptic mange	Wolgan Valley		Badger Ground		Eagle's Drift	
	N	%	*N*	%	*N*	%
Winter						
No	30	100.0	6	85.7	3	75.0
Yes	0	0.0	0	0.0	1	25.0
Unknown	0	0.0	1	14.3	0	0.0
Spring						
No	32	78.0	5	71.4	4	80.0
Yes	6	14.6	2	28.6	1	20.0
Unknown	3	7.3	0	0.0	0	0.0
Summer						
No	37	72.5	9	75.0	3	50.0
Yes	5	9.8	2	16.7	3	50.0
Unknown	9	17.6	1	8.3	0	0.0
Autumn						
No	18	75.0	8	80.0	5	55.6
Yes	3	12.5	1	10.0	3	33.3
Unknown	3	12.5	1	10.0	1	11.1

No wombats were observed at Robertson, and no wombats with visible signs of sarcoptic mange were observed at Coolagolite.

wombat nutrient intake. More targeted plant sampling and including quantities of plant consumed would provide further insights into the availability and digestibility of nutrients to wombats in these habitats. Further studies should be undertaken to assess grazing preferences, which are likely influenced by floristic availability and abundance in different seasons, as occurs in other wombat species (Casey *et al.* 2023). A vegetation survey conducted at the same time as collection of material for nutritional analysis would also likely be beneficial to understanding more about dietary preferences throughout different seasons.

Relationship between nutrition and health of wombats

The relationship between seasonal and site variance in plant nutritional status and wombat health was not examined in this study, but this would aid future studies. Previous studies on wombats and other mammals have only anecdotally suggested a winter increase in the prevalence of sarcoptic mange possibly due to lower nutritional availability (Martin *et al.* 1998; Hartley and English 2005; Alasaad *et al.* 2012). Respondents to a survey by Martin *et al.* (1998) from across Australia anecdotally reported, based on observations and opinions of respondents surveyed, an increased prevalence of sarcoptic mange in wombat populations during times of low food availability, including drought, as well as in areas impacted by habitat degradation, and with high numbers of wombats. Hartley and English (2005) assessed the health of 23 (plus four recaptured) wombats in the Southern Highlands of NSW (from August 2001 to March 2002) and suggested that sarcoptic mange may be more prevalent in July to August (50% – 12) than December to January (22% – 9), but the numbers were small and not significant. In addition, the first recorded outbreak of sarcoptic mange in wild giraffes (*Giraffa camelopardalis reticulata*) occurred during a time of severe drought and was potentially influenced by immune suppression resulting from malnutrition(Alasaad *et al.* 2012). Stannard *et al.* (2021) found no significant correlation between season and sarcoptic mange prevalence at Wolgan Valley, Eagle's Drift or Badger Ground from 2011 to 2017, but they did find a correlation between higher rainfall years and higher sarcoptic mange occurrence.

There is evidence that sarcoptic mange has a deleterious effect on the mineral status of the host. Compared with healthy individuals, sarcoptic mange-affected animals have reduced levels of Zn, Fe, Cu, Ca, Cl and Se in their blood (Skerratt *et al.* 1999; Hartley and English 2005; Nwufoh *et al.* 2019; Abdel-Saeed 2020). Although it may not be a direct reflection of a deficiency in those specific minerals, ensuring maintenance nutrient requirements are met is essential for supporting immune function and recovery from disease. Significant seasonal variation in mineral content of plants was determined at our study sites for potassium and phosphorus and may influence intake of these minerals by wombats seasonally. These minerals are

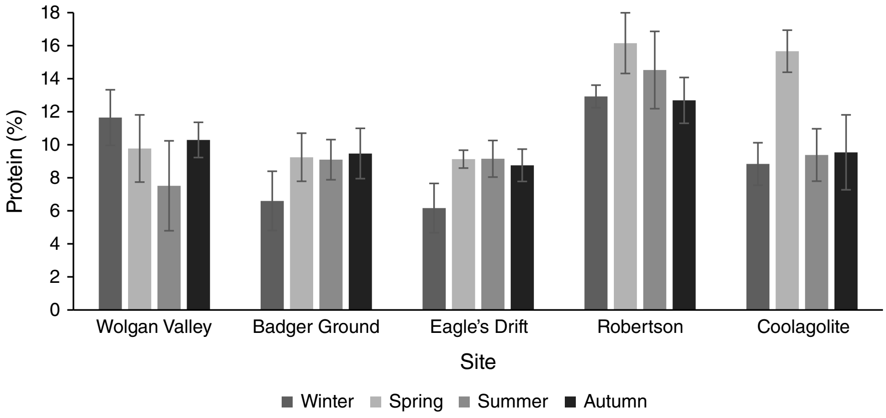

Fig. 3. Protein (%) content of faeces collected from each study site across four seasons.

important for nerve and immune function, water balance, bone formation and energy metabolism (Spears 1994).

Conclusion

Energy, protein, lipid and some mineral compositions of plants varied significantly across sites and/or seasons. Not all plant species known to be consumed by bare-nosed wombats were assessed in this study; however, we chose plant species that were common at each study site and known to be eaten by wombats. Seasonal changes in nutrient levels suggest wombats may need to alter their dietary intake to match their nutrient needs as seasonal changes occur. Wombats are likely meeting their nutrient needs by consuming a wide variety of plants with varying nutritive values. Further studies are required to understand the impacts, if any, of seasonal and site variation in nutrition and its impact on sarcoptic mange susceptibility and outcome in affected wombats, in combination with a floristic survey to compare dietary preferences and dietary item availability. Further understanding of the specific plants consumed by (e.g. through molecular barcoding), and nutrient requirements of, healthy wombats versus mange-affected wombats may aid management plans to better support wombats and potentially increase the opportunity for recovery after treatment.

Supplementary material

Supplementary material is available online.

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Data availability. The data that support this study are available in the article and accompanying online supplementary material.

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