Title: Vitamin D composition of Australian game products

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Abstract:

The vitamin D content of many Australian game products is unknown. These foods are potential sources of vitamin D for remote-dwelling Aboriginal and Torres Strait Islander people, of whom 39% are vitamin D deficient (serum 25-hydroxyvitamin D_3 ($25(OH)D_3$) concentrations <50 nmol/L). Vitamin D_3, $25(OH)D_3$, vitamin D_2 and $25(OH)D_2$ were measured by liquid chromatography-triple quadrupole mass spectrometry (LC-QQQ) in raw meat (camel, crocodile, emu, kangaroo), emu eggs and emu oil. Vitamin D_3 (range, 0.5-14.5 μg/100 g) was found in all products except camel and kangaroo. All samples except kangaroo contained $25(OH)D_3$; some camel samples contained relatively high concentrations (range, 0.4-5.2 μg/100 g). Vitamin D_2 was found in emu products and some kangaroo samples. We detected trace amounts of $25(OH)D_2$ in some camel and kangaroo samples. This study provides valuable insight into foods with a paucity of data on vitamin D content, showing that some are potentially useful sources of vitamin D.

Keywords: camel (*Camelus dromedaries)*; crocodile (*Crocodylus porosus)*; emu (*Dromaius novaehollandiae*); kangaroo (*Macropus giganteus* and *Macropus rufus*); vitamin D

1. Introduction

Over a quarter (27%) of the adult Australian Aboriginal and Torres Strait Islander (hereafter referred to as Aboriginal) population are vitamin D deficient (serum 25-hydroxyvitamin D (25(OH)D) concentration <50 nmol/L) (Black, Dunlop, Lucas, Pearson, & Shepherd, 2019). While this is comparable to the general Australian population (23%) (Australian Bureau of Statistics, 2014b), there is a distinct difference in the prevalence of vitamin D deficiency within the adult Aboriginal population across the geographic landscape – from 23% amongst those living in non-remote areas to 39% in remote areas (Australian Bureau of Statistics, 2014a). A number of adverse health outcomes with possible links to vitamin D status are prevalent in the Aboriginal population. Although causal associations have not been established, nationally-representative data from the 2012-2013 Aboriginal and Torres Strait Islander Health Survey have shown that diabetes and chronic kidney disease were more common in Aboriginal people with vitamin D deficiency than those who were vitamin D sufficient (Australian Bureau of Statistics, 2014a). Cross-sectional surveys have also shown greater prevalence of diabetes and other cardio-metabolic risk factors (Maple-Brown et al., 2014), gastroenteritis (Michael J. Binks, Smith-Vaughan, Bar-Zeev, Chang, & Andrews, 2014) and respiratory infection (M. J. Binks, Smith-Vaughan, Marsh, Chang, & Andrews, 2016), in Aboriginal people with low vitamin D status. Due to a lack of vitamin D food composition data for foods that are traditionally hunted and foraged by Aboriginal people, it is not possible to explore traditional and culturally beneficial food-based strategies to improve intakes and status.

Wild game products form part of the traditional and contemporary hunting activities of Aboriginal people (Bliege Bird & Bird, 2008; Kouris-Blazos & Wahlqvist, 2000) and some animals, such as kangaroo, increasingly feature in modern Australian diets (Ampt & Owen, 2008; Ratnasiri & Jayatilleke, 2017). Globally, there has been limited investigation of the vitamin D content of traditional foods (H. Kuhnlein, 2018); however, studies involving Canadian (H. V. Kuhnlein et al., 2006) and Russian (Kozlov, Khabarova, Vershubsky, Ateeva, & Ryhaenkov, 2014) Indigenous peoples and their traditional foods have indicated that traditional diets may include important sources of vitamin D.

Accurate measurement of vitamin D in food is challenging and costly. Hence, data presented in national food composition databases are frequently limited by resources and by access to the analytical methods required to measure all four of the main D vitamers found in food

(vitamin D_3, vitamin D_2, and their hydroxylated forms, 25(OH)D_3 and 25(OH)D_2). The National Measurement Institute of Australia (NMI) recently developed a liquid chromatography with triple quadrupole mass spectrometry (LC-QQQ) method (Dunlop et al., 2021; Hughes et al., 2018) with the capacity to quantitate low concentrations of vitamin D_3, vitamin D_2, 25(OH)D_3 and 25(OH)D_2 in food. We used this method to develop an analytical vitamin D food composition database for Australian retail foods (Dunlop et al., 2021); however, the vitamin D content of game animal products in Australia remains largely unexplored. There are numerous logistical barriers to direct acquisition of game products from Australia's vast remote regions. Hence, the aim was to measure vitamin D_3, 25(OH)D_3, vitamin D_2 and 25(OH)D_2 in Australian farmed and wild game products (camel, crocodile, emu and kangaroo) sourced from commercial suppliers.

2. Methods

2.1 Sample purchase and preparation

Various cuts (Table 1), weighing between 0.3 and 3.9 kg, of wild camel (*Camelus dromedarius)*, farmed crocodile (*Crocodylus porosus)*, farmed emu (*Dromaius novaehollandiae*), wild Eastern Grey Kangaroo (*Macropus giganteus*) and wild Red Kangaroo (*Macropus rufus)* meat were purchased from Yarra Valley Game Meats (Victoria, Australia) in October 2018 and July 2021. Samples were purchased frozen and transported to NMI in chilled containers, protected from heat and light. Samples were thawed, removed from packaging and homogenised. Kangaroo tail fur was stripped prior to removing meat and fat from the bone; fur and bone were discarded. Following preparation, individual samples were stored at -20 ºC and protected from light and oxygen until analysis.

Samples of emu eggs (n=8) and emu oil (total n=6; 50 mL bottle n=4, 100 mL bottle n=2) were sourced from three emu farms located in Western Australia (Free Range Emu Farm), Victoria (Longview Emu Farm) and New South Wales (Emu Logic) in July 2021 (Table 1). The weight of each emu egg, in shell, was recorded (mean egg weight = 636 g, range 590-691 g). The entire contents of all eggs were cracked into a blending container and homogenized to create a composite sample. Following preparation, the composite sample was frozen to <-70 ºC, freeze dried to <3% moisture by weight and stored at -20 ºC, protected from light and oxygen, until analysis. The entire contents of emu oil bottles were emptied into a blending container and homogenized to create a composite sample. Following

preparation, the composite sample was stored at room temperature, protected from light and oxygen, until analysis.

2.2 Sample analysis

Details of the LC-QQQ method (ISO17025:2017) used to measure vitamin D_3, 25(OH)D_3, vitamin D_2 and 25(OH)D_2 and its validation have been published previously (Dunlop et al., 2021). In addition, we sent four samples (canned salmon, trout, feta cheese and whole chicken eggs) from our earlier study (Dunlop et al., 2021) to the Technical University of Denmark for confirmatory testing using LC-MS/MS methods detailed elsewhere (Barnkob, Petersen, Nielsen, & Jakobsen, 2018; Jakobsen, Clausen, Leth, & Ovesen, 2004; Švarc, Barnkob, & Jakobsen, 2021). Results returned were within method uncertainties. Briefly, sample aliquot weights were determined in order that the amount of saponified fat was ≤1 g, an amount that was able to be saponified within a 50 mL vessel. The sample, a known quantity of chemically labeled internal standard, 1 g sodium ascorbate, 10 mL deionised water, 30 mL ethanol, 2 g potassium hydroxide, and additional deionized water to make 50mL were placed into a capped 50 mL Falcon® tube for saponification in a shaker bath overnight. Following hydrolysis in an ethanolic potassium hydroxide solution, vitamin D analytes were extracted to diatomaceous earth solid phase extraction tubes (Chem Elut™ 10 mL unbuffered SPE cartridges, Agilent Technologies, Santa Clara, USA) and washed through with petroleum ether. After the washes were evaporated to dryness under nitrogen gas, residues were resolvated into heptane and again evaporated to dryness under nitrogen gas. The resulting residue was resolvated into 4-Phenyl-1,2,4-triazoline-3,5-dione (PTAD) in anhydrous acetonitrile for derivatisation, which was concluded after 10 minutes through addition of water.

Vitamin D analytes were isolated on a reverse phase C18 column (Supelco Ascentis® Express C18, 15 cm x 3 mm, 2.7 μm [Cat#53816-U], Sigma-Aldrich, St. Louis, USA) and were analysed, along with a range of calibration samples by LC-QQQ (1290 Infinity Series LC System and 6460 Triple Quad LC-MS, Agilent Technologies, Santa Clara, USA), which was configured in electrospray ionization mode with positive polarity. Analytes were quantitated against the calibration curve generated from calibration sample analysis.

Moisture was measured using a method developed at NMI, which was based on a previously published AOAC method (AOAC International, 2005). The Soxhlet extraction method was used to measure total fat (Food Science Australia, 1998).

2.3 Quality assurance

Analyses of proximates and all D-vitamers were duplicated, from saponification to quantitation, in all samples. The relative percent difference (RPD) between duplicate analyses was calculated as (difference between replicate values/average of replicates) x 100. Five samples were randomly allocated for recovery analyses, and spiked with a known concentration (µg/100 g of sample matrix) of each D-vitamer. In addition, three samples of an in-house control sample (infant formula and freeze-dried irradiated mushroom powder) were analysed. Measured concentrations in spiked and control samples were recorded and reported as a recovery percentage of the known concentration.

The limit of quantitation (LOQ) represents the lowest concentration that can be quantitated by the specified method. It remains constant and accounts for variations in bias and precision that may occur between analytical runs. The LOQ for all vitamers was estimated as 0.10 µg/100 g for all samples except camel hump and emu oil. A LOQ of 0.25 µg/100 g was estimated for camel hump and emu oil, due to their high fat content and consequential requirement of a reduced analytical sample. The limit of detection (LOD) represents the lowest concentration that can be detected on a specific day during a specific analytical run and may vary due to a number of factors, including food matrix. LODs were calculated as: (standard deviation (SD) of seven replicate analyses carried out on low-level spiked sample matrix) x (t-test value at 99% confidence interval (CI)), where the spike concentration was related to the least amount of analyte discernible in comparison to multiple readings of a blank reagent. Detected values >LOD and <LOQ, that were subject to greater uncertainty, were reported as ‘trace’.

2.4 Data handling

Analytical values for duplicate analyses were averaged to give mean values for each sample. For samples that were analysed individually, mean concentrations are reported: camel meat, crocodile (lower-fat cuts and body trim meat were separated due to fat content differences), emu meat, kangaroo meat, emu heart and camel hump. For samples that were composited for

analysis (emu oil and eggs), the mean of duplicate analyses is reported. Concentrations of D vitamers per 100 g fat were calculated as follows: (D vitamer per 100 g sample/fat per 100 g sample) x 100. All values, other than those per 100 g fat, are reported per wet weight.

Total vitamin D was calculated as the sum of vitamers for samples containing vitamin D_3 and/or vitamin D_2 only. As 25(OH)D may be between one and five times more bioactive than vitamin D (Cashman et al., 2012; Jakobsen, Melse-Boonstra, & Rychlik, 2019; Ovesen, Brot, & Jakobsen, 2003), we have presented a range for samples containing quantifiable concentrations of 25(OH)D. The lower bound was calculated based on equal bioactivity of all four vitamers; the upper bound was calculated using a bioactivity factor of five for $25(OH)D_3$ and $25(OH)D_2$.

3.0 Results

In all samples except camel hump and emu oil, for which the LOD was 0.1 μg/100 g, LODs ranged between 0.01-0.06 μg/100 g for all vitamers. The mean RPD was 11, 9, 17 and 7% for vitamin D_3, $25(OH)D_3$, vitamin D_2 and $25(OH)D_2$, respectively. Recovery from spiked samples ranged from 74 to 126%. Recovery of known amounts from three control samples was 94, 101 and 102%.

All camel cuts contained $25(OH)D_3$; however, hump was the only camel cut with detectable vitamin D_3 (Table 2, Supplementary Table 1). The mean concentration of $25(OH)D_3$ was greater for camel hump compared to other camel meat cuts; however, when calculated per 100 g fat, the concentration of this vitamer was more than 25 times greater in meat cuts compared to hump (Table 3). Vitamin D_2 was not detected in any camel samples, but some did contain trace amounts of $25(OH)D_2$.

The mean concentration of vitamin D_3 was three times greater in crocodile body trim than in lower-fat crocodile cuts, but was not greatly different when calculated by 100 g fat. $25(OH)D_3$ was detected in trace amounts in lower-fat crocodile cuts and found in concentrations close to the LOQ in body trim samples. Neither vitamin D_2 nor $25(OH)D_2$ was detected in crocodile samples.

All emu samples contained vitamin D_3, 25(OH)D_3 and vitamin D_2. For all three vitamers, concentrations were greatest in emu oil and lowest in emu meat cuts; for vitamin D_3 and vitamin D_2, this was reversed when calculated by 100 g fat.

Neither vitamin D_3 nor 25(OH)D_3 was detected in kangaroo samples. Vitamin D_2 and 25(OH)D_2 were detected in trace amounts in some kangaroo samples.

4.0 Discussion

At least one of the four main D vitamers found in food was detected in each game animal product. Our results provide valuable insight into foods with little to no previously published data on vitamin D content. Some of these foods are potentially useful dietary sources of vitamin D, particularly for Aboriginal people living in remote areas.

The vitamin D profile of camel products was unique among products included in this study and our previous studies of Australian foods. Meat and hump samples contained the greatest concentrations of 25(OH)D_3 of all foods included in our vitamin D studies to date (Dunlop et al., 2021; Hughes et al., 2018); however, vitamin D_3 was not detected in camel meat and was found in only one camel hump sample. Where 25(OH)D_3 and 25(OH)D_2 are detected in meat, they are often accompanied by their respective non-hydroxylated forms, vitamin D_3 and vitamin D_2 (Barnkob et al., 2018; Dunlop et al., 2021; Liu, Greenfield, Strobel, & Fraser, 2013; Purchas, Zou, Pearce, & Jackson, 2007). The absence of vitamin D_3 in camel meat samples may reflect the adaptation of this animal for survival under harsh and arid conditions (Faye & Bengoumi, 2000), whereby storing 25(OH)D_3 rather than vitamin D_3 provides a quicker or localised metabolic path to the active form, 1,25-dihydroxyvitamin D_3 (Chun et al., 2019). We found the greatest concentration of 25(OH)D_3 in hump flesh; however, when expressed per 100 g fat, the concentration of 25(OH)D_3 was much lower in hump flesh compared to camel meat, which has a low mean fat content compared to other commonly-consumed meats. Jakobsen and Saxholt (2009) have proposed the greater polarity of 25(OH)D_3 as an explanation for its presence in fat-free food matrices, while muscle has been demonstrated to act as a storage site for 25(OH)D (Abboud et al., 2013), and may have greater uptake potential of 25(OH)D than fat cells (Abboud et al., 2014). The 25(OH)D_3 content of camel meat has previously been measured by radioimmunoassay in Moroccan dromedary camel (*Camelus dromedarius*), with 0.42 µg/100 g found in muscle meat (El Khasmi et al., 2013). The vitamin D_3 content was not reported in that study and, to our

knowledge, there are no other data on the vitamin D profile of camel meat with which to compare our findings. Further research into meat from *Camelus dromedaries* and other camel species from other geographical locations is needed to confirm whether this 25(OH)D_3-dominated vitamin D profile is usual in these species.

Crocodile meat contained vitamin D_3 and 25(OH)D_3, similarly to Australian chicken and pork (Dunlop et al., 2021). We found both vitamers in greater concentrations in the higher-fat body trim than in other lower-fat cuts. The vitamin D_3 (0.6-10.9 μg/100 g) and 25(OH)D_3 (0.1-0.3 μg/100 g) content of two samples of crocodile egg yolk has been measured previously; however, we found no other studies on the vitamin D content of crocodile meat products. Crocodiles are not expected to purposefully ingest fungi or other matter that might provide vitamin D_2; chicken heads and kangaroo meat are commonly used as feed for farmed crocodiles in Australia (Peucker & Jack, 2006). Therefore, the absence of vitamin D_2 and 25(OH)D_2 in the meat of crocodiles may be due to their diet.

We found vitamin D_3, 25(OH)D_3 and vitamin D_2 in all emu product samples included in this study. Animals do not synthesise vitamin D_2; rather it is produced in fungi. It is thought that UVB-exposure of fungal-contaminated plant-based animal feed may explain the presence of vitamin D_2 and 25(OH)D_2 in animal products (Barnkob et al., 2018; Jäpelt, Didion, Smedsgaard, & Jakobsen, 2011). Emus are farmed outdoors and are opportunistic omnivores. In addition to feed provided, they may consume a wide range of plant matter, insects and small animals. Vitamin D_2 and 25(OH)D_2 have been found in ruminant animal meats (beef and lamb) commonly consumed in Australia (Dunlop et al., 2021). It is unknown whether these vitamers are obtained from fungal-contaminated feed provided to farmed animals or from other food sources accessible in the local environment.

Our findings for emu meat contradict those of a previous Canadian study that found no detectable vitamin D_3 in emu thigh and leg meat (Pegg, Amarowicz, & Code, 2006). However, that study used an older high performance liquid chromatography method that lacked the sensitivity (LOD 2.1 μg/100 g) to detect the concentrations of vitamin D_3 found in our emu meat samples (up to 1.1 μg/100 g) and did not examine other D vitamers. To our knowledge, no other studies have investigated the vitamin D content of emu products.

We found that emu eggs contained more than twice the mean concentration of vitamin D_3 found in chicken eggs (Dunlop et al., 2017; Dunlop et al., 2021), and the vitamer profiles differ due to the presence of vitamin D_2 in emu eggs. Emu oil had the greatest concentrations of vitamin D_3 and vitamin D_2 of all products included in this study. Emu oil is applied topically for cosmetic purposes due to its moisturising and purported anti-inflammatory properties and to promote hair and skin growth (Jeengar et al., 2015). It may also be ingested, and is thought to lower cholesterol and assist in treatment of internal inflammatory conditions such as inflammatory bowel syndrome and mucositis (Jeengar et al., 2015). Despite being a richer source of vitamin D by concentration than other emu products, it is likely to be consumed in much lower amounts (commonly available capsules contain 750-1000 mg emu oil), and may, therefore provide a similar amount of vitamin D per usual dose/serving.

In contrast to other animal products, kangaroo meat is interesting due to its relative lack of vitamin D. Amongst a variety of meat from mammalian, avian, aquatic vertebrate and reptilian species previously sourced in Australia and analysed for vitamin D content (Dunlop et al., 2017; Dunlop et al., 2021), this marsupial animal's meat is unusual for the absence or scarcity of all four D vitamers measured. Neither vitamin D_3 nor 25(OH)D_3 were detected in any kangaroo meat samples despite low LODs ranging from 0.01-0.05 μg/100 g. These results are consistent with data from our previous study (Dunlop et al., 2021) that included a composite of six kangaroo steak samples purchased from supermarkets in Melbourne. Kangaroos are herbivores and are not farmed for meat in Australia; meat available for purchase is from wild-caught animals. Serum 25(OH)D concentrations in healthy, free-ranging koalas with no evidence of metabolic bone disease have previously been reported as being much lower than non-marsupials, prompting speculation that the koala's vitamin D requirements may be relatively low (Pye, Ellis, FitzGibbon, Opitz, Keener, & Hollis, 2013). Similarly, low serum 25(OH)D concentrations have been recorded in other marsupials, specifically wombats and brushtail possums (Fowler & Fraser, 1993). It does not yet appear to be known why the D vitamer profile of kangaroo meat differs to other animal types, particularly to those that are also herbivores, or whether the D vitamer profile observed in kangaroo meat is specific only to macropods or to marsupials in general.

This study has shown that some Australian game products, that may be hunted and foraged for in remote areas, offer nutritionally important amounts of vitamin D. Availability of foods in remote communities has considerable influence on nutritional intake (Scelza, 2012).

Nutritious foods are often in limited and unreliable supply, relatively expensive (Harrison et al., 2007; Northern Territory Government: Department of Health and Community Services, 2007; Pollard, Landrigan, Ellies, Kerr, Lester, & Goodchild, 2014) and are frequently passed over in favour of more energy-dense foods with a lower cost per unit of energy (Brimblecombe & O'Dea, 2009). Prior to European settlement, traditional diets based on bush tucker would likely have provided a reasonable supply of vitamin D as they included offal and fish (Kouris-Blazos et al., 2000), both of which are good sources of vitamin D. There is generally less reliance on bush tucker now than in the past, with colonisation marking the most pronounced decline. Despite this, bush tucker contributes up to 50% of food intake for some remote-dwelling people (Bliege Bird et al., 2008). Our findings have identified some Australian game products as useful sources of vitamin D. One serving (120 g) of emu egg would provide approximately half of the Estimated Average Requirement (EAR) of 10 µg/day (Institute of Medicine, 2011). Assuming a bioactivity factor of five for 25(OH)D_3, one serving of camel meat (100 g raw weight) would provide approximately 5 µg total vitamin D. Kangaroo, while being nutritious in other ways and commonly hunted (Bliege Bird et al., 2008), does not contain nutritionally useful amounts of vitamin D. As well as contributing to vitamin D intake, game products are a good source of protein and are often lower in total fat compared to cheaper commercial meats (Food Standards Australia New Zealand, 2019). Encouraging the consumption of bush tucker may be nutritionally and culturally beneficial (Brimblecombe et al., 2014).

A major strength of our study was the use of a highly sensitive and specific LC-QQQ method to measure the four main dietary D vitamers in camel, crocodile, emu and kangaroo products. Given the complexity of measuring D vitamers in food, few international food composition databases include all four vitamers. Due to logistical barriers to collection of samples from remote areas, samples were sourced from commercial suppliers, and included products from both farmed and wild animals. It was not possible to investigate the effect of the animals' diets on the vitamin D content and profile of their products. Future studies could include wider sampling of a greater range of products, and could examine factors that may affect vitamin D content (e.g. seasonal and geographical variation (Liu et al., 2013), animals' diets and the effect of cooking).

4.0 Conclusions

We investigated the four major D vitamers in Australian camel, crocodile, kangaroo and emu products, revealing interesting variations in profiles and concentrations of D vitamers between species. Identifying good sources of vitamin D provides important evidence for developing dietary strategies to promote vitamin D sufficiency in Aboriginal and Torres Strait Islander people living in remote areas.

Abbreviations

25(OH)D	25-hydroxyvitamin D
LC-QQQ	Liquid chromatography with triple quadrupole mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantitation
NMI	National Measurement Institute of Australia
PTAD	4-Phenyl-1,2,4-triazoline-3,5-dione
RPD	Relative percent difference
SRM	Standard Reference Material
UVB	Ultraviolet-B

Chemical compounds studied in this article:

Vitamin D_3/cholecalciferol (PubChem CID: 5280795); Vitamin D_2/ergocalciferol (PubChem CID: 5280793); 25-hydroxyvitamin D_3/25-hydroxycholecalciferol (PubChem CID: 5283731); 25-hydroxyvitamin D_2/25-hydroxyergocalciferol (PubChem CID: 5710148).

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366 **Authors' contributions*:*** Conceptualization: LJB, JC; Funding acquisition: LJB; Formal
367 analysis: NS; Data curation: ED; Methodology: LJB, JC, NS: Project administration: ED;
368 Supervision: LJB, JC; Writing – original draft: ED; Writing – review & editing: CCJS, JC,
369 NS, RML, LJB. LJB had primary responsibility for the final content. All authors read and
370 approved the final version of the manuscript.

371 **References**

372 Abboud, M., Gordon-Thomson, C., Hoy, A. J., Balaban, S., Rybchyn, M. S., Cole, L., . . .
373 Mason, R. S. (2014). Uptake of 25-hydroxyvitamin D by muscle and fat cells.
374 *Journal of Steroid Biochemistry and Molecular Biology, 144*, 232-236.
375 https://doi.org/10.1016/j.jsbmb.2013.10.020.

376 Abboud, M., Puglisi, D. A., Davies, B. N., Rybchyn, M., Whitehead, N. P., Brock, K. E., . . .
377 Mason, R. S. (2013). Evidence for a specific uptake and retention mechanism for
378 25-hydroxyvitamin D (25OHD) in skeletal muscle cells. *Endocrinology, 154*,
379 3022-3030. https://doi.org/10.1210/en.2012-2245.

380 Ampt, P., & Owen, K. (2008). Consumer attitudes to kangaroo meat. Retrieved from:
381 https://www.agrifutures.com.au/wp-content/uploads/publications/08-026.pdf.
382 Accessed July 10, 2021.

383 AOAC International. (2005). Dairy samples-determination of total solids and moisture.
384 In W. Horwitz (Ed.), *Official methods of analysis of AOAC International*. Atlanta,
385 GA, USA: AOAC International.

386 Australian Bureau of Statistics. (2014a). 4727.0.55.003 - Australian Aboriginal and
387 Torres Strait Islander Health Survey: biomedical results, 2012-13: vitamin D.
388 Retrieved from:
389 http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/by%20Subject/4727.0.55.0
390 03~2012-13~Main%20Features~Vitamin%20D~133. Accessed July 17, 2021.

391 Australian Bureau of Statistics. (2014b). Australian Health Survey: biomedical results
392 for nutrients, 2011-2012. Retrieved from:
393 https://www.abs.gov.au/statistics/health/health-conditions-and-
394 risks/australian-health-survey-biomedical-results-nutrients/latest-release.
395 Accessed July 17, 2021.

396 Barnkob, L., Petersen, P., Nielsen, J., & Jakobsen, J. (2018). Vitamin D enhanced pork
397 from pigs exposed to artificial UVB light in indoor facilities. *European Food*

Research and Technology, 245, 411-418. https://doi.org/10.1007/s00217-018-3173-6.

Binks, M. J., Smith-Vaughan, H. C., Bar-Zeev, N., Chang, A. B., & Andrews, R. M. (2014). Vitamin D insufficiency among hospitalised children in the Northern Territory. *Journal of Paediatrics and Child Health, 50*, 512-518. https://doi.org/10.1111/jpc.12623.

Binks, M. J., Smith-Vaughan, H. C., Marsh, R., Chang, A. B., & Andrews, R. M. (2016). Cord blood vitamin D and the risk of acute lower respiratory infection in Indigenous infants in the Northern Territory. *Medical Journal of Australia, 204*, 238.e231-238.e237. https://doi.org/10.5694/mja15.00798.

Black, L. J., Dunlop, E., Lucas, R. M., Pearson, G., & Shepherd, C. (2019). Prevalence and predictors of vitamin D deficiency in a nationally-representative sample of Australian Aboriginal and Torres Strait Islander adults. *Annals of Nutrition & Metabolism, 75*, 207. https://doi.org/10.1017/S0007114520003931.

Bliege Bird, R., & Bird, Douglas W. (2008). Why women hunt. Risk and contemporary foraging in a western desert Aboriginal community. *Current Anthropology, 49*, 655-693. https://doi.org/10.1086/587700.

Brimblecombe, J., Maypilama, E., Colles, S., Scarlett, M., Dhurrkay, J. G., Ritchie, J., & O'dea, K. (2014). Factors influencing food choice in an Australian Aboriginal community. *Qualitative Health Research, 24*, 387-400. https://doi.org/10.1177/1049732314521901.

Cashman, K. D., Seamans, K. M., Lucey, A. J., Stöcklin, E., Weber, P., Kiely, M., & Hill, T. R. (2012). Relative effectiveness of oral 25-hydroxyvitamin D3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults. *American Journal of Clinical Nutrition, 95*, 1350-1356. https://doi.org/10.3945/ajcn.111.031427.

Chun, R. F., Shieh, A., Gottlieb, C., Yacoubian, V., Wang, J., Hewison, M., & Adams, J. S. (2019). Vitamin D binding protein and the biological activity of vitamin D. *Frontiers in Endocrinology, 10*, 718. https://doi.org/10.3389/fendo.2019.00718.

Dunlop, E., Cunningham, J., Sherriff, J. L., Lucas, R. M., Greenfield, H., Arcot, J., . . . Black, L. J. (2017). Vitamin D3 and 25-hydroxyvitamin D3 content of retail white fish and eggs in Australia. *Nutrients, 9*, 1-15. https://doi.org/10.3390/nu9070647.

Dunlop, E., James, A. P., Cunningham, J., Strobel, N., Lucas, R. M., Kiely, M., . . . Black, L. J. (2021). Vitamin D composition of Australian foods. *Food Chemistry, 358*, e129836. https://doi.org/10.1016/j.foodchem.2021.129836.

El Khasmi, M., Bargaâ, R., Riad, F., Safwate, A., El Hassane, T., Farh, M., . . . Faye, B. (2013). Meat levels of 25-hydroxyvitamin D3 in Moroccan one-humped dromedary camels (Camelus dromedarius). *Emirates Journal of Food and Agriculture, 25*, 267-273. https://doi.org/10.9755/ejfa.v25i4.15495.

Faye, B., & Bengoumi, M. (2000). Le dromadaire face à la sous- nutrition minérale: un aspect méconnu de son adaptabilité aux conditions désertiques. *Sécheresse, 11*, 155-161. https://www.jle.com/en/revues/sec/e-docs/le_dromadaire_face_a_la_sous_nutrition_minerale_un_aspect_meconnu_de_son_adaptabilite_aux_conditions_desertiques_230070/article.phtml.

Food Science Australia. (1998). Crude fat determination - Soxhlet method. Retrieved from: https://meatupdate.csiro.au/infosheets/Crude%20Fat%20Determination%20-%20Soxhlet%20Method%20-%201998.pdf. Accessed July 20, 2021.

Food Standards Australia New Zealand. (2019). Australian Food Composition Database - Release 2. Retrieved from: https://www.foodstandards.gov.au/science/monitoringnutrients/afcd/Pages/default.aspx. Accessed 15 February, 2022.

449 Fowler, A. M., & Fraser, D. R. (1993). Vitamin D metabolism in nocturnal marsupials.
450 *Proceedings of the Wildlife Disease Association (Australasian Section) Annual*
451 *Conference*, 21-22.

452 Harrison, M. S., Coyne, T., Lee, A. J., Leonard, D., Lowson, S., Groos, A., & Ashton, B. A.
453 (2007). The increasing cost of the foods required to promote health in
454 Queensland. *Medical Journal of Australia, 186*, 9-14.
455 https://doi.org/10.5694/j.1326-5377.2007.tb00778.x.

456 Hughes, L. J., Black, L. J., Sherriff, J. L., Dunlop, E., Strobel, N., Lucas, R. M., & Bornman, J. F.
457 (2018). Vitamin D content of Australian native food plants and Australian-grown
458 edible seaweed. *Nutrients, 10*, E876. https://doi.org/10.3390/nu10070876.

459 Institute of Medicine. (2011). Dietary reference intakes for calcium and vitamin D. (Vol.
460 2020). Washington, DC, USA: National Academies Press.

461 J. K. Brimblecombe, & O'Dea, K. (2009). Role of energy cost in food choices for an
462 Aboriginal population in northern Australia. *Medical Journal of Australia, 190*,
463 549-551. https://doi.org/10.5694/j.1326-5377.2009.tb02560.x.

464 Jakobsen, J., Clausen, I., Leth, T., & Ovesen, L. (2004). A new method for the
465 determination of vitamin D3 and 25-hydroxyvitamin D3 in meat. *Journal of Food*
466 *Composition and Analysis, 17*, 777-787.
467 https://doi.org/10.1016/j.jfca.2003.10.012.

468 Jakobsen, J., Melse-Boonstra, A., & Rychlik, M. (2019). Challenges to quantify total
469 vitamin activity: How to combine the contribution of diverse vitamers? *Current*
470 *Developments in Nutrition, 3*, nzz086. https://doi.org/10.1093/cdn/nzz086.

471 Jakobsen, J., & Saxholt, E. (2009). Vitamin D metabolites in bovine milk and butter.
472 *Journal of Food Composition and Analysis, 22*, 472-478.
473 https://doi.org/10.1016/j.jfca.2009.01.010.

Jäpelt, R. B., Didion, T., Smedsgaard, J., & Jakobsen, J. (2011). Seasonal variation of provitamin D2 and vitamin D2 in perennial ryegrass (Lolium perenne L.). *Journal of Agricultural and Food Chemistry, 59*, 10907-10912. https://doi.org/10.1021/jf202503c.

Jeengar, M. K., Kumar, P. S., Thummuri, D., Shrivastava, S., Guntuku, L., Sistla, R., & Naidu, V. G. M. (2015). Review on emu products for use as complementary and alternative medicine. *Nutrition, 31*, 21-27. https://doi.org/10.1016/j.nut.2014.04.004.

Kouris-Blazos, A., & Wahlqvist, M. (2000). Indigenous Australian food culture on cattle stations prior to the 1960s and food intake of older Aborigines in a community studied in 1988. *Asia Pacific Journal of Clinical Nutrition, 9*, 224-231. https://doi.org/10.1046/j.1440-6047.2000.00189.x.

Kozlov, A., Khabarova, Y., Vershubsky, G., Ateeva, Y., & Ryhaenkov, V. (2014). Vitamin D status of northern indigenous people of Russia leading traditional and "modernized" way of life. *International Journal of Circumpolar Health, 73*, 26038. https://doi.org/10.3402/ijch.v73.26038.

Kuhnlein, H. (2018). Vitamin D intake by Indigenous Peoples in the Canadian Arctic. *Public Health Nutrition, 21*, 1986-1987. https://doi.org/10.1017/S1368980018000411.

Kuhnlein, H. V., Barthet, V., Farren, A., Falahi, E., Leggee, D., Receveur, O., & Berti, P. (2006). Vitamins A, D, and E in Canadian Arctic traditional food and adult diets. *Journal of Food Composition and Analysis, 19*, 495-506. https://doi.org/10.1016/j.jfca.2005.02.007.

Liu, J., Greenfield, H., Strobel, N., & Fraser, D. R. (2013). The influence of latitude on the concentration of vitamin D3 and 25-hydroxy-vitamin D3 in Australian red meat. *Food Chemistry, 140*, 432-435. https://doi.org/10.1016/j.foodchem.2012.10.032.

Maple-Brown, L. J., Hughes, J. T., Lu, Z. X., Jeyaraman, K., Lawton, P., Jones, G. R. D., . . . O'Dea, K. (2014). Serum vitamin D levels, diabetes and cardio-metabolic risk factors in Aboriginal and Torres Strait Islander Australians. *Diabetology & Metabolic Syndrome, 6*, 78. https://doi.org/10.1186/1758-5996-6-78.

Northern Territory Government: Department of Health and Community Services. (2007). NT Market Basket Survey 2006*.* Retrieved from: https://digitallibrary.health.nt.gov.au/prodjspui/bitstream/10137/338/1/Market basket 2006.pdf. Accessed July 10, 2021.

Ovesen, L., Brot, C., & Jakobsen, J. (2003). Food contents and biological activity of 25-hydroxyvitamin D: A vitamin D metabolite to be reckoned with? *Annals of Nutrition & Metabolism, 47*, 107-113. https://doi.org/10.1159/000070031.

Pegg, R. B., Amarowicz, R., & Code, W. E. (2006). Nutritional characteristics of emu (Dromaius novaehollandiae) meat and its value-added products. *Food Chemistry, 97*, 193-202. https://doi.org/10.1016/j.foodchem.2005.04.002.

Peucker, S. K. J., & Jack, R. H. (2006). Crocodile farming research: On-farm research of pelleted feed for crocodiles*.* Retrieved from: https://www.agrifutures.com.au/product/crocodile-farming-research-on-farm-research-of-pelleted-feed-for-crocodiles/. Accessed July 29, 2021.

Pollard, C., Landrigan, T., Ellies, P., Kerr, D., Lester, M., & Goodchild, S. (2014). Geographic factors as determinants of food security: A Western Australian food pricing and quality study. *Asia Pacific Journal of Clinical Nutrition, 23*, 703-713. https://doi.org/10.6133/apjcn.2014.23.4.12.

Purchas, R., Zou, M., Pearce, P., & Jackson, F. (2007). Concentrations of vitamin D3 and 25-hydroxyvitamin D3 in raw and cooked New Zealand beef and lamb. *Journal of Food Composition and Analysis, 20*, 90-98. https://doi.org/10.1016/j.jfca.2006.07.001.

526 Pye, G. W., Ellis, W., FitzGibbon, S., Opitz, B., Keener, L., & Hollis, B. W. (2013). Serum
527 vitamin D levels in free-ranging koalas (Phascolarctos cinereus). *Journal of Zoo*
528 *and Wildlife Medicine, 44*, 480-483. https://doi.org/10.1638/2012-0032R.1.

529 Ratnasiri, S., & Jayatilleke, B. (2017). Changing patterns of meat consumption and
530 greenhouse gas emissions in Australia: Will kangaroo meat make a difference?
531 *PLoS ONE, 12*, e0170130. https://doi.org/10.1371/journal.pone.0170130.

532 Scelza, B. A. (2012). Food scarcity, not economic constraint limits consumption in a
533 rural Aboriginal community. *Australian Journal of Rural Health, 20*, 108-112.
534 https://doi.org/10.1111/j.1440-1584.2012.01270.x.

535 Švarc, P. L., Barnkob, L. L., & Jakobsen, J. (2021). Quantification of vitamin D3 and 25-
536 hydroxyvitamin D3 in food – The impact of eluent additives and labelled internal
537 standards on matrix effects in LC-MS/MS analysis. *Food Chemistry, 357*, 129588.
538 https://doi.org/10.1016/j.foodchem.2021.129588.
539

Table 1: Characteristics of game products analysed for vitamin D_3, 25-hydroxyvitamin D_3, vitamin D_2 and 25-hydroxyvitamin D_2

Product type	*n*	Meat cuts included	Production method	Origin
Camel (*Camelus dromedarius)*			Wild	Central Australia
Meat	6	Fillet, mince[a], diced[a], eye fillet, rump, knuckle		
Hump fat/meat	4	Hump		
Crocodile meat (*Crocodylus porosus)*	8	Tail fillet, mince[a], striploin, sweet cut (cheek), body trim[b]	Farmed	Queensland
Emu (*Dromaius novaehollandiae)*			Farmed	
Meat	6	Fan fillet, flat fillet, mince[a]		Victoria
Heart	4	Heart		Victoria
Eggs	8	-		New South Wales, Victoria, Western Australia
Oil	6	-		New South Wales, Victoria, Western Australia
Kangaroo meat (*Macropus giganteus* and *Macropus rufus*)	8	Fillet, rump, mince[a], striploin, tail butt, tail	Wild	Queensland, New South Wales

[a]Mince/diced are a mixture of various cuts

[b]Body trim is the subcutaneous outer layer of flesh

544 **Table 2.** Moisture, fat, vitamin D_3, 25-hydroxyvitamin D_3, vitamin D_2, and 25-hydroxyvitamin D_2 in raw Australian game products[a]
545

Product	Primary samples *n*	Moisture (g/100 g)	Fat (g/100 g)	Vitamin D_3[b] (µg/100 g)	25(OH)D_3[b] (µg/100 g)	Vitamin D_2[b] (µg/100 g)	25(OH)D_2[b] (µg/100 g)	Total vitamin D[c] (µg/100 g)
Camel (*Camelus dromedarius)*								
Meat (fillet, mince[d], diced[d], eye fillet, rump, knuckle)	6	76.0 ± 1.1 (74.8 - 77.3)	1.5 ± 0.7 (0.6 - 2.6)	ND	1.07 ± 0.73 (0.40 - 2.46)	ND	ND-Tr	1.07-5.33
Hump	4	11.3 ± 5.5 (5.7-17.8)	79.8 ± 7.3 (73.4 - 90.2)	Tr	2.13 ± 2.04 (0.95 - 5.17)	ND	Tr	2.19-10.95
Crocodile (*Crocodylus porosus)*								
Lower-fat cuts (tail fillet, mince[d], striploin, sweet cut (cheek))	4	77.2 ± 0.5 (76.8 - 77.8)	2.3 ± 1.6 (0.5 - 4.2)	0.48 ± 0.07 (0.39 - 0.65)	Tr	ND	ND	0.48
Body trim[e]	4	63.3 ± 5.7 (59.8 - 71.9)	17.2 ± 4.8 (10.3 - 21.1)	1.62 ± 0.63 (0.83 - 2.20)	0.11 ± 0.01 (0.10 - 0.13)	ND	ND	1.73-2.16
Emu (*Dromaius novaehollandiae)*								
Meat (fan fillet, flat fillet, mince[d])	6	74.1 ± 1.2 (72.2 - 75.7)	1.7 ± 1.0 (0.5 - 3.2)	0.88 ± 0.18 (0.70 – 1.11)	Tr	0.13 ± 0.03 (Tr – 0.16)	ND	1.01
Heart	4	69.7 ± 1.2 (68.7 - 71.3)	13.0 ± 2.8 (9.5 - 16.4)	4.05 ± 0.71 (3.35 - 5.00)	0.14 ± 0.05 (0.10 - 0.20)	0.16 ± 0.07 (0.10 - 0.24)	ND	4.34-4.90
Eggs	8	69.2	16.0	2.95	0.20	0.20	ND	3.35-4.15
Oil	6	0.3	100.0	14.50	0.15	1.30	ND	15.95-16.55
Kangaroo (*Macropus giganteus* and *Macropus rufus*)								
Meat (fillet, rump, mince[d], striploin, tail butt, tail)	8	75.5 ± 1.6 (73.1 - 78.1)	0.4 ± 0.6 (0.0 - 1.9)	ND	ND	Tr	Tr	-

546 25(OH)D_3, 25-hydroxyvitamin D_3; 25(OH)D_2, 25-hydroxyvitamin D_2; ND, not detected (<LOD); Tr, trace, >LOD<LOQ
547 [a]Concentrations ≥ LOQ are presented as mean ± SD (range). Exceptions are emu eggs and emu oil for which composite samples were analysed and the values presented are the mean
548 of duplicate analyses.

[b]Limit of quantitation (LOQ) = 0.10 µg/100g, except camel hump and emu oil for which the LOQ = 0.25 µg/100g; Limit of detection (LOD) = 0.01-0.06 µg/100g

[c]Total vitamin D calculated as the sum of vitamers. Where a range is presented: Lower bound = sum of all vitamers assuming equal bioactivity; Upper bound = (vitamin D_3 + vitamin D_2)+ (5*(25(OH)D_3 + 25(OH)D_2)).

[d]Mince/diced are a mixture of various cuts

[e]Body trim is the subcutaneous outer layer of flesh

554 **Table 3.** Vitamin D_3, 25-hydroxyvitamin D_3, vitamin D_2, and 25-hydroxyvitamin D_2 per 100 g fat in raw Australian game meats[a]
555

Sample	Fat (g/100 g ± SD)	Vitamin D_3 (µg/100 g fat)	25(OH)D_3 (µg/100 g fat)	Vitamin D_2 (µg/100 g fat)	25(OH)D_2 (µg/100 g fat)
Camel (*Camelus dromedarius)*					
Meat (fillet, mince[b], diced[b], eye fillet, rump, knuckle)	1.5	NA	73.5	NA	2.1
Hump	79.8	NA	2.7	NA	NA
Crocodile (*Crocodylus porosus)*					
Lower-fat cuts (tail fillet, mince[†], striploin, sweet cut (cheek))	2.3	21.0	NA	NA	NA
Body trim[c]	17.2	9.45	0.63	NA	NA
Emu (*Dromaius novaehollandiae)*					
Meat (fan fillet, flat fillet, mince[b])	1.7	52.9	NA	7.8	NA
Heart	13.0	31.1	1.1	1.2	NA
Eggs	16.0	18.4	1.2	1.2	NA
Oil	100.0	14.5	0.2	1.3	NA
Kangaroo (*Macropus giganteus* and *Macropus rufus*)	0.4	NA	NA	NA	NA
Meat (fillet, rump, mince[b], striploin, tail butt, tail)					

556 25(OH)D_3, 25-hydroxyvitamin D_3; 25(OH)D_2, 25-hydroxyvitamin D_2; ND, not detected; NA, not applicable: the vitamer was not detected in the sample or the amount detected was
557 too low to be quantitated (limit of detection = 0.01-0.06 µg/100g)
558 [a]Values of D-vitamer per 100 g fat calculated as ((D vitamer per 100 g sample /fat per 100 g sample) x 100)
559 [b]mince/diced are a mixture of various cuts
560 [c]Body trim is the subcutaneous outer layer of flesh