Seasonal variation in the concentrations of vitamin D and macroelements in the adipose tissue of the hump, blood serum, and muscle and liver tissues in the Arabian camel

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Abstract

The objective of the present study was to analyse total vitamin D and key macroelements in the serum, meat, liver and the hump of the Arabian camel (*Camelus dromedarius*) during the summer and winter seasons. Twelve male camels were randomly divided into 2 groups of 6 animals each: Group I was slaughtered in mid-winter and Group II was slaughtered in the following summer season. Blood samples were collected before slaughter while other samples were collected postmortem. Slaughter was performed according to the traditional halal practice without stunning. Samples of muscle (*Triceps barchii*), liver and adipose tissue from the hump were taken. The total vitamin D in the serum was significantly ($P<0.05$) higher during the summer season compared with the winter season. However, the total vitamin D in all tissues was similar ($P>0.05$) in the summer and winter seasons, with the hump as the richest tissue for vitamin D storage.

Keywords: adipose tissue, camel, Morocco, season, vitamin D, minerals.

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Introduction

Vitamin D3, the cholecalciferol, comes from UV-induced skin synthesis and foods of animal origin, while vitamin D2, the ergocalciferol, is of fungal and vegetable origin. Once in blood circulation, vitamin D is converted to 25-hydroxycholecalciferol (25-OH-D) in the liver. The active hormone form 1,25-dihydroxycholecalciferol is produced in the kidney by hydroxylation of 25-OH-D (Holick, 2009). Vitamin D could be involved in the prevention of many pathologies, such as myopathy, immune deficiency, infections, inflammations, cardiovascular diseases, type II diabetes, cancer and bone diseases (Holick, 2009; Spiro and Buttriss, 2014). In the dromedary camel, the metabolism, storage and functional roles of vitamin D

are unclear, due to the lack of information on the tissular distribution of vitamin D and its metabolites. Many research studies have analysed vitamin D in camel blood and meat (El khasmi et *al*., 2010; 2013; Bargaâ *et al*., 2015; Tabite *et al*., 2019; El khasmi and Faye, 2019), and suggested that this species could be a significant source of vitamin D for populations in desert regions. In addition, according to Heaney *et al.* (2009), vitamin D is mainly stored in adipose tissue. However, to our knowledge, no work has measured vitamin D in the hump adipose tissue or evaluated its variation between summer and winter seasons in the dromedary camel. Thus, the objective of the present study was to analyse total vitamin D amounts in the hump, liver, meat and serum, and macroelements including Ca, P, Mg, Na and K levels in the hump, meat and serum in summer and winter seasons in the Arabian camels (*Camelus dromedarius*).

Materials and Methods

Study area

The study was carried out at the municipal slaughterhouse of Casablanca, Morocco. The annual averages of ambient temperature, relative humidity, UV index and wind speed recorded during the day were, respectively, 12–21°C, 40–80%, 3–5 and 10–25 km/h in the winter season, and 24–26 °C, 63–80%, 9.5-11 and 9-21 km/h in the summer season. The corresponding temperature humidity index (THI) for winter and summer were 17.55 and 27.98, respectively. The THI was calculated according to Marai et al. (2001), using the following equation: THI = T°C- [(0.31-0.031xRH) (T°C-14.4)], whereas T°C is the max dry bulb temperature and RH is the relative humidity (%). There is no heat stress when THI is below 27.8.

The animals and blood and tissue sampling

This study was conducted on 12 male Arabian camels (*Camelus dromedarius*), in good health, aged 3 to 8 years and weighing 210 to 330 kg. They were maintained under similar conditions and fed with some barley-based concentrate and straw. Prior to transportation to the abattoir, the camels were deprived of water and food for 12 hours and were examined by a veterinarian to ensure they were clinically healthy. They were divided into 2 groups of 6 animals: Group I was slaughtered in winter (February) and Group II was slaughtered in summer (July). These animals were exposed before slaughter to road transport for 2 hours.

The blood was collected just before slaughter, around 7 a.m., from a puncture to the right jugular vein and in dry tubes. Three hours after the *halal* slaughter without stunning, tissue samples (50 g) were taken from the right muscle (*Triceps barchii*), liver and hump. All samples were transported for 10 min in a cooler at 4 °C to the Laboratory of Physiopathology and Molecular Genetics, at the Ben M'Sick Faculty of Sciences. Tissues were washed with a 0.9% saline solution and were placed in sterile polyethylene bags, and the blood was centrifuged at 750 g for 15 minutes to distribute the serum into aliquots. Tissue bags and serum aliquots were

stored at -80 °C until the subsequent analyses, which were performed in duplicate using reagents of analytical grade.

Mineral analysis

In the serum, meat and hump, levels of Ca, Pi and Mg were measured using a spectrophotometric (JENWAY 6320D Spectrophotometer, Model 6320D) procedure from commercially available kits (CHRONOLAB, Switzerland) according to the manufacturer's procedures. Tissular mineral content was determined after complete digestion in a muffle furnace at 200 °C. A mixture of concentrated HNO_3 and 30% H_2O_2 was used for the complete digestion. Na and K were determined by flame photometry.

Extraction and analysis of total vitamin D

Two g of muscle and one g of liver were removed after thawing, finely chopped, then homogenized in a phosphate buffer solution (PBS) at 5% for the muscle and 10% w/w for the liver. Exact masses were recorded to determine the tissue mass fraction in the homogenates. Tissue homogenates were then extracted with 2.5 mL of acetonitrile diluted with distilled water (10v/4v) for 3h, during which time the mixtures were vigorously shaken at 30 min intervals to facilitate extraction. The extracts obtained were centrifuged for 5 min at 4000 rpm at 4°C and the supernatants were aliquoted and then stored at -80°C until subsequent analysis of the total vitamin D.

Concerning the hump adipose tissue preparation, the extraction procedure was adapted from that of Folch *et al*. (1957) and Lipkie *et al.* (2013). The unsaponifiable fraction was isolated by saponification of 2 g of fat with 5 mL of 50% alcoholic potassium hydroxide and extraction with diethyl ether and petroleum ether (1:1), vortexing vigorously for 5 minutes. The samples were centrifuged to separate the 2 organic and aqueous layers. The analysis of vitamin D in the tissues and serum was carried out using Biosource radioimmunoassay kits (Biosource Europe SA., Belgium; product KIP1961) (El khasmi *et al*., 2010; 2013; Tabite *et al*., 2019), at the National Centre for Energy, Sciences and Nuclear Techniques in Maâmoura, Morocco. We evaluated specificity, selectivity, linearity, sensitivity, accuracy, precision, stability, and dilution tests. The concentration range covered by the test kit standards was 1.5 to 172 ng/mL, and the range encountered for meat extracts in this study was 1.6 to 50 ng/mL. The minimum detection limit for vitamin D was 0.6 ng/mL.

Statistical analysis

All values were expressed as mean ± standard error of the mean (SEM). Data were analysed using the analysis of variance (ANOVA) of the general linear models procedure of the Statistical Analysis System software (SAS, 2005). $P<0.05$ was considered the level of significance.

Results

Serum, meat, liver and hump levels of total vitamin D

In the camel, the serum levels of total vitamin D (ng/mL) were significantly (P<0.05) higher in the summer season than in the winter season (32.16±5.11 vs 21.39±4.21) (Figure 1). However, the total vitamin D in the meat, liver and hump showed no significant variations between the summer and winter seasons (Figure 1). The total vitamin D contents (ng/g) in the liver and the hump were significantly higher in the summer and winter seasons compared to the meat (respectively, 68.9±6.38 vs 45.95±9.06, P<0.05 and 92.4±11.79 vs 45.95±9.06, P<0.005 in the summer season, and 56.14±6.27 vs 35.23±8.12, P<0.05 and 84.37±10.61 vs 35.23±8.12, P<0.005 in the winter season), but the hump was found to be the richest tissue in total vitamin D (Figure 1).

Serum, meat and hump levels of macroelements

All macroelements levels analysed in the serum, meat and hump showed no significant variation between the summer and the winter seasons (Table 1).

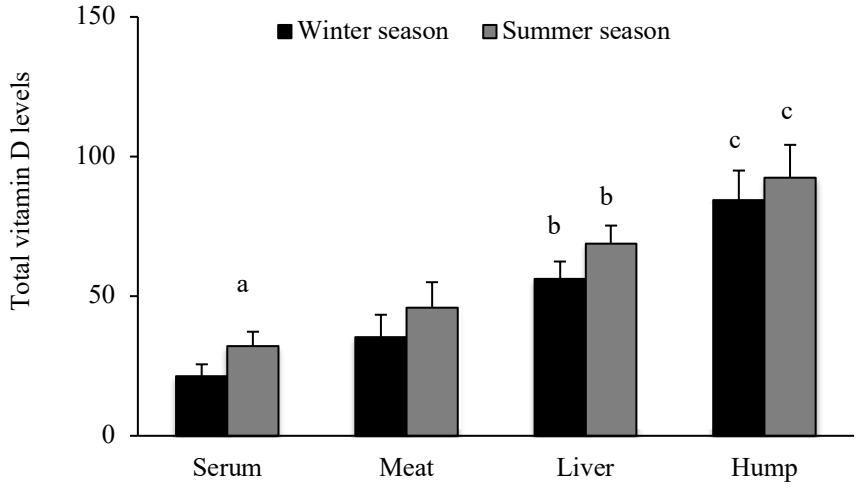

Figure 1. Total vitamin D in serum (ng/mL), meat, liver and hump (ng/g) in summer and winter seasons in dromedary camels. (M±SEM. [a]: P <0.05: differences between summer and winter seasons values, [b]: P <0.05, [c]: P <0.005: differences between liver or hump, and meat in the same season).

Table 1. Macroelements concentrations in serum, meat and hump in summer and winter seasons in the dromedary camels (M±SEM).

Specimen	Season	Ca	Pi	Mg	Na	K
Serum (mg/100mL)	winter	9.73±0.21	6.12±0.18	2.46±0.21	391.23±23.11	21.71±2.81
	summer	9.82±0.24	5.97±0.17	2.38±0.23	402.43±24.21	23.42±3.22
Meat (mg/100g)	winter	6.4±1.22	196.8±21.5	22.32±3.31	71.32±17.13	367.52±36.65
	summer	5.3±1.20	188.4±22.2	20.6±2.27	68.45±16.77	354.85±33.71
Hump (mg/100g)	winter	1.52±0.21	15.13±4.24	1.11±0.35	40.03±12.64	18.63±6.78
	summer	1.67±0.30	16.45±5.36	1.08±0.27	38.52±11.15	17.16±5.44

Discussion

The estimation of vitamin D intake in populations requires reliable vitamin D values and food composition databases. For these reasons, the results of the present study on the values of vitamin D content in camel meat, liver and hump constitute important information on the natural sources of vitamin D in non-coastal desert regions. The results obtained in this work, and those reported in previous studies (El Khasmi *et al.,* 2013; Tabite *et al.,* 2018; 2019), showed that camel meat could be a source of vitamin D and 25-hydroxyvitamin D (25-OH-D). According to Dunlop *et al.* (2022), in raw meat from the crocodile and emu, and in emu eggs and emu oil, the amount of vitamin D_3 varies between 0.5 and 14.5 µg/100 g, whereas camel meat concentrations of 25-OH-D_3 show a values range of 0.4-5.2 µg/100 g. In the present study, the hump adipose tissue was the richest tissue in terms of total vitamin D during the summer and winter seasons. Vitamin D is mainly stored in adipose tissue, which is purely fat and not associated with water, to be mobilized in the event of a decrease in exogenous and/or endogenous intakes (Heaney *et al.,* 2009). Regarding its distribution in the body, vitamin D3 is mainly stored in fatty tissue (75%), while 25-OH-D has a more ubiquitous distribution (35% in fatty tissue, 20% in muscle, 30% in serum and 15% in other tissues) (Heaney *et al.,* 2009).

Besides the major role of vitamin D in Ca and P homeostasis, vitamin D status affects skeletal muscle function, metabolism and hypertrophic growth as well as muscle fiber composition and size (Ceglia and Harris, 2013), and can influence *postmortem* pH and the water holding capacity (WHC) of meat. In fact, previous studies on beef and pork reported that dietary vitamin D supplementation increased WHC, decreased pH storage and cooking losses (Wilborn *et al.,* 2004) and participated in the antioxidant activity of meat (Duffy *et al.,* 2018). Furthermore, during cold storage of camel meat, Tabite *et al.* (2019) has reported that 25-OH-D levels were negatively correlated with those of malondialdehyde, drip loss and cooking loss, suggesting that vitamin D may be implicated in antioxidant status and the quality of camel meat. In this investigation, circulating total vitamin D was higher in the summer season than in the winter season. Similarly, in camels, levels of 25-OH-D3 in the blood were

significantly higher in summer than in winter, without showing significant seasonal variations in the meat, liver and kidney (El khasmi *et al.,* 2011; Bargaa *et al.,* 2015; Farh *et al*., 2018). In addition, in camels, the concentration of 25-OH-D did not undergo any significant modification during one week of postmortem cold storage of meat (Tabite *et al.,* 2018; 2019), nor in plasma after a 60 km road transport (El Khasmi *et al.,* 2009; 2010). In llamas and alpacas (Smith and Van Saun, 2001) and camels (Mohamed, 2008), circulating levels of vitamin D are not influenced by age, but they vary according to season. These rates were higher during the period from February to July (Mohamed, 2008). According to Shany *et al.* (1978), in the dromedary, the serum levels of 25-OH-D (ng/mL) in summer and winter were respectively 443 ± 96 and 276 ± 13. In summer, the ecosystems of the animal are marked by strong sunlight which could increase the biosynthesis of vitamin D. Variations in circulating concentrations of vitamin D in camelids could also be impacted by the degree of coat colour (Smith and Van Saun, 2001; Mohamed, 2008), month of birth, light intensity (Van Saun *et al*., 1996) and physiological stage (Riad *et al.,* 1994; El Khasmi *et al*., 2000). The mass of the hump varies between 3 and almost 100 kg and can thus represent a significant part of the carcass (8-25%), depending on its weight (Kamili et al., 2006). In addition, Bedouins are known for their dietary habits marked by the consumption of hump fat, and therefore it would be useful to assess their daily intakes of this fatty matter to estimate its contribution to their vitamin D needs.

In conclusion, in the dromedary camel, the liver and the hump were richer in total vitamin D than the meat, and the hump was the richest tissue in total vitamin D, signalling that vitamin D is mainly stored in adipose tissue which could be an important source of vitamin D in arid regions.

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