

Short Communication

Circulating levels of 25-hydroxyvitamin D and testosterone during the rutting and non-rutting periods in Moroccan dromedary camels (*Camelus dromedarius*)

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Abstract: The camel is largely known to be a seasonal breeder, thus, in the male camel, the breeding activity maximizes during the rutting period (winter and spring seasons) but ceases completely during the non-rutting period (summer and autumn). Plasma vitamin D3 concentrations showed significant seasonal variations; the purpose of this study was to investigate a possible role of vitamin D in the sexual activity in Moroccan dromedary camels by evaluating the variation in plasma vitamin D, calcium, and phosphorus concentrations in relation to those of testosterone during these two periods. Blood samples were collected from 14 adult male camels aged 5-8 years, slaughtered during March (n=7) and September (n=7) at the Tit-Mellil Municipality slaughterhouse. All animals were clinically healthy and blood samples were taken at 06 h AM into heparinized tubes. In the work reported here, our results showed that plasma levels of 25-hydroxyvitamin D were significantly higher and those of testosterone were significantly lower in the non-rutting animals when compared to the rutting ones ($p<0.005$). While, the plasma levels of calcium and inorganic phosphorus showed no seasonal variation. Based on the values obtained in this investigation, vitamin D does not appear to contribute directly or indirectly to camel steroidogenesis. Further studies, from the one hand of an eventual relationship between T and other parameters such as thyroid hormones and corticoids, and from the another one of testing the vitamin D action on rutting behavior are needed in camels.

Key words: Calcium, dromedary camels, 25hydroxyvitamin D, Phosphorus, Rutting.

مستويات فيتامين "د" والتستوسترون في الدم خلال فترتي النشاط والخمود الجنسيين للبعير المغربية ذات السنم الواحد

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المخلص: يعتبر الجمل من بين الحيوانات التي تتوالد في فترة معينة في السنة، حيث يبلغ نشاطه الجنسي ذروته خلال فصل الشتاء والربيع، بينما يكون ذلك النشاط كاملا خلال فصلي الصيف والخريف. تهدف هذه الدراسة إلى البحث عن مدى أهمية فيتامين "د" في الوظيفة الجنسية للبعير المغربية. تم تقييم التغيرات على مستويات فيتامين "د" وهرمون التستوسترون والكالسيوم والفوسفور خلال هاتين الفترتين، وذلك في بلازما دم البعير. أخذت عينات الدم من مجموعتين تتكون كل واحدة منهما من سبعة ذكور عمر 5-8 أعوام، أثناء عملية الذبح بالمجازر البلدية لطيط مليل بالمغرب، حيث أن المجموعة الأولى ذبحت في مارس والثانية في شتنبر. بينت النتائج التي تم تحليلها إحصائيا أن خلال فترة النشاط الجنسي (الشتاء) كان مستوى التستوسترون عاليا ومستوى الفيتامين "د" منخفضا، وكل هذا بمستوى معنوية مقارنة مع فترة الخمود الجنسي (الخريف). يستنتج من ذلك أن فيتامين "د" لا يبدو له دور في تركيب وفرز هرمون التستوسترون لدى الجمل. نأمل في دراسة لاحقة في البحث عن علاقة بين التستوسترون وهرمونات أخرى، وكذلك في تأثير فيتامين "د" على السلوك الجنسي للجمل.

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Introduction

The male Moroccan camel is described as a seasonal breeder with a marked peak in sexual activity (the rut) during the breeding season and its sexual activity coincides with that of the female and both respond to the same environmental conditions (Sghiri, 1988; Sghiri and Driancourt, 1999). The breeding season of the Moroccan camels begins in November and ends in April, with decreasing then increasing daylight, while the non-breeding season is in summer which is strongly related to the length increase of the photoperiod (Sghiri, 1988). Furthermore, it is largely known that encouragement of rapid growth during the pubertal period by the good nutritional and environmental conditions can assist early sexual development and breeding maturity in dromedary camels (Marai et al., 2009).

It is largely accepted that the calcium signal plays an important role in the control of the secretory process of some adenohypophyseal hormones such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) which respond to the administration of calciotropic substances (Zofkova et al., 1989) and steroid hormones (Nett et al., 2002) by a marked change. In addition, other investigations have suggested that vitamin D which plays a major role in phosphocalcic metabolism, and the vitamin D receptor (VDR) is critical for the maintenance of male reproduction and fertility (ANON, 1965; Corbett et al., 2006; Menegaz et al., 2009). So, several animal studies have reported that vitamin D supplementation may have implications for managing reproductive performance and male infertility (Hignett, and Hignett., 1953; Kwiecinski et al., 1989; Zofkova et al., 1989; Barrera et al., 2007; Hirai et al., 2009; Menegaz et al., 2009). Thus, in the present study we have investigated the seasonal changes (i.e., during the rutting and non rutting periods) of plasma levels of 25-hydroxyvitamin D (25-OH-D), calcium (Ca) and phosphorus – inorganic- (Pi) in relation to those of testosterone (T) in Moroccan dromedary camels.

Materials and Methods

To investigate the physiological changes of plasma T, 25-OH-D, Ca and Pi concentrations during the rutting and non rutting periods, 14 male camels aged 5 to 8 years and weighted 430 ± 50 kg were used. All animals were clinically healthy, feed deprived overnight and were slaughtered during March (n=7) and September (n=7) at 06 h AM at the Tit-Mellil Municipality slaughterhouse (Province of Mediouna, Morocco) according to traditional local procedures. Blood samples were taken from each camel into heparinized tubes. Plasma was separated by centrifugation at 750 g for 15 min, pipetted into different aliquots and then stored at -20°C until analysis. In plasma, Ca levels were measured using an atomic absorption spectrophotometer (Perkin-Elmer 400) and Pi levels were measured by colorimetry (Kit Phosphorus-UV, SPINREACT, S.A.- Ctra. Santa Coloma, 7 – E-17176 SANT ESTEVE DE BAS – (Girona) SPAIN). The plasma 25-OH-D and T concentrations were analyzed by radioimmunoassay (RIA) in medical and biological application laboratory (National Center of Energy of Nuclear Science and Technology in Maamoura, Morocco) by using commercially available coated RIA tubes for human 25-OH-D and T. These human kits proved efficient in previous experiments in dromedary camels (Al-Qarawi and ElMougy, 2008, El Khasmi et al., 2009), and were purchased from BioSource (Europe S.A., Nivelles, Belgium) and DIASource (Immunoassays S.A., Nivelles, Belgium) respectively. The 25-OH-D assay had a sensitivity of 0.1 ng/mL and its intra- and inter-assay coefficients of variation were at a precision of 6.6% and 7.7%, respectively. The T assay had a sensitivity of 0.08 ng/mL with intra- and inter-assay coefficients of variation of 8.8% and 9.8% respectively.

The data were expressed in SI units and analyzed by the Mann-Whitney *U* test for comparison between groups. All values were expressed as mean and standard error (SE), and $P < 0.05$ was seen as statistically significant.

Results and Discussion

As shown in Figure 1, we found no significant difference in plasma Ca and Pi levels (mmol/L) in rutting and non rutting camels (2.4 ± 0.2 vs 2.5 ± 0.1 and 2.2 ± 0.1 vs 2.0 ± 0.2 respectively). Mean plasma T levels (ng/mL) from non-rutting (sexually inactive) camels were significantly lower ($P < 0.05$) than those measured in rutting (sexually active) camels (8.23 ± 2.11 vs 2.13 ± 1.08 , respectively) (Figure 2). However, during the non-rutting period, plasma levels of 25-OH-D (ng/mL) were significantly higher ($P < 0.05$) than those measured during the rutting period (420 ± 90 vs 250 ± 60 , respectively) and this increase is any time associated with any significant change in plasma levels of Ca and Pi (Figure 3).

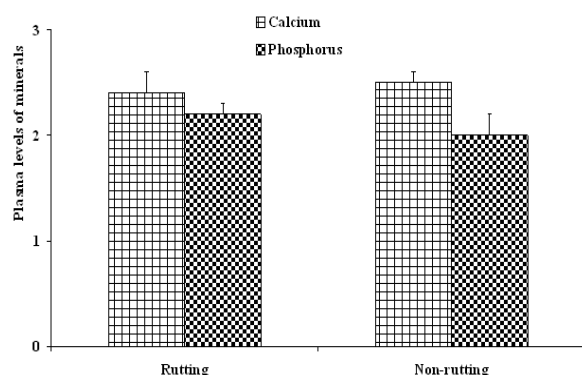


Figure 1. Plasma levels of calcium and phosphorus (mmol/L) in male camel in relation to physiological status.
Values are SEM.

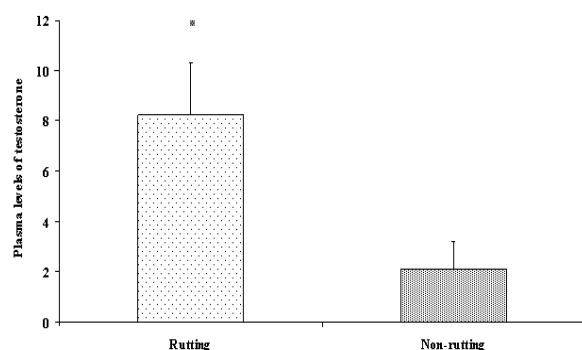


Figure 2. Plasma levels of testosterone (ng/mL) in male camel in relation to physiological status.
Values are SEM; * values of rutting camel are significantly different from those measured in non-rutting camel, $P < 0.05$.

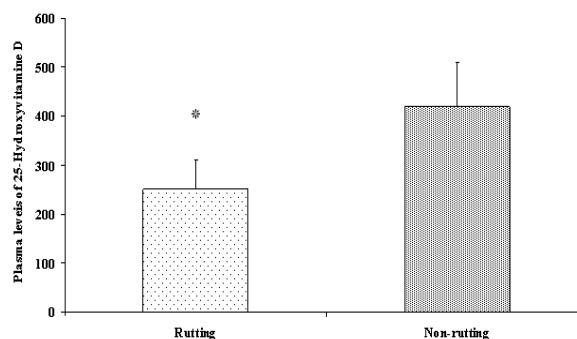


Figure 3. Plasma levels of 25-hydroxyvitamin D (ng/mL) in male camel in relation to physiological status.

Values are SEM; * values of rutting camel are significantly different from those measured in non-rutting camel, $P < 0.05$.

Mineral status

Regarding Ca status our results were not in accordance with those of Zia-Ur-Rahman et al., (2007) showing higher Ca level in serum (11.6 ± 1.2 vs 8.5 ± 1.4 mg/dl) and testis (980 ± 70 vs 770 ± 39.5 μ g/g) respectively during the rutting and non rutting period in male *Camelus dromedarius*. These findings may imply that these minerals are required for the vitality of testis to regulate the process of spermatogenesis.

Testosterone status

In dromedary camels, the full reproductive potential of the male camel is reached at 5-6 years (Novoa, 1970). According to Sharma and Vyas (1981), although display of general behaviour in male dromedary has been reported as early as 2 years of age, field observation suggest that puberty and fertility ability are reach until 3 to 5 years. Champak Bhakat et al. (2005) found that early sexual behaviour/rut in adult mature male camel can be aroused by giving a regular exposure of 20 to 30 minutes in front of adult female camel at least for 2 weeks during the onset of winter season. In addition, it has been reported that at 6 years old the male dromedary became fully function as stud (Gombe and Oduo-Okello, 1977), and it's first ejaculum may contains higher concentrations of spermatozoa (Al-Qarawi et al., 2001).

The post-racing plasma levels of T reported by Abdel Hafid and Wasfi (2001) in mature

male racing camels during the rutting season, was low compared to those found in our rutting animals. Camels, both male and female, are seasonal breeders (Yasin and Wahid, 1957), mating during the rainy, or cold season (Yagil and Etzion, 1980). The seasonality in the male is evidenced by changes in sexual behaviour, morphology and function of the genital organs (Tingari et al., 1984). Seasonal changes in the plasma T observed in our camels have also been reported by other studies in the same species (Agarwal, 1996; Al-Qarawi and ElMougy, 2008) with higher levels in the breeding season as compared to non-breeding season. Higher level of circulating T from late December to end of March was also reported by Yagil and Etzion (1980). These higher T levels may be due to increase synthesis and release of T either by an increase sensitivity of Leydig cells to luteinizing hormone (LH) and or an enhance secretion of LH from the pituitary gland (Azouz et al., 1992). Lower levels of T were found in non-rutting camels used in this work (Figure 2). In fact, Tibary and Anouassi (1997) reported that T level in non-rutting animals were similar to those observed in prepubertal males while that of mature studs during breeding season were higher.

Vitamin D status

The two major natural sources of vitamin D to ruminants result from photochemical conversion of 7-dehydrocholesterol to vitamin D₃ in the skin or from plants as a result of photochemical conversion of ergosterol to vitamin D₂. Vitamins D₃ and D₂ also can be supplemented in the ruminant diet by commercially available crystalline forms (Wolter, 1988). Once vitamin D is in the liver, it is converted to 25-OH-D (De Luca, 1981). This metabolite is the major circulating form of vitamin D under normal conditions (Horst and Littledike, 1982). The 25-OH-D is converted to several polar metabolites. However, of all the vitamin D₂ and D₃ metabolites known, only the function of 1,25-dihydroxyvitamin D (produced predominantly in the kidney) has been established.

It has been reported in camels, that circulating levels of vitamin D are 10 to 15 times higher than those of sheep and cattle,

without any significant change in calcemia or phosphatemia (Shany et al., 1978; El Khasmi et al., 2005). However, in this work, the lower circulating levels of 25-OH-D observed in our camels during the rutting period (Figure 3) suggest that this hormone doesn't appear to contribute directly or indirectly to the psychological-neurological-endocrinology of camel reproduction. In fact, interaction of photoperiod (intensity, duration, wavelength) with other factors (ambient temperature, nutrition, housing, latitude) and hormonal status (thyroid and corticosterone levels (Zia-Ur-Rahman et al., 2007) are able to influence the reproductive performance of several mammalian species (Wilson, 1984; Arthur, 1992). For example, melatonin has been proposed as the pineal hormone and its timed administration replicates the effect of day length on seasonal breeding in these animals, like the hamster (Cutty et al., 1981), the sheep (Zaraazga et al., 1998) and the camel (Al-Qarawi and Elmougy 2008). However, although photoperiodic variations have a strong influence, yet there is some evidence suggesting that the suprachiasmatic nucleus is an important structure regulating circadian and seasonal rhythms of most biological functions and may be sensitive to changes in ambient temperature (Pando and Sassone-Corsi, 2001). The higher circulating levels of 25-OH-D observed in our dromedary camels during the non-rutting period (summer) (Figure 3) may be explained by the increasing daylight. In fact, (Hymoller et al., 2009) reported in cows and cattle that vitamin D status is determined by season rather than supplementation. However, in camelids (Fernandez-Buca, 1993; Sumar, 1996) and ruminants (Brown, 1994), the sexual activity which is essentially androgen induced phenomenon, is largely influenced by plane of nutrition. In the camel, activity of the Leydig cells of camel, becomes maximal during the rutting season (Yagil and Etzion, 1980; Tingari et al., 1984; Agarwal and Khanna, 1990), and is less active in the non-breeding season with a resulting reduction in steroidogenic activity by the testes (El-Wishy, 1988).

Vitamin D and reproductive function

Several studies investigating the effect of the vitamin D on reproductive function of mammals, are conflicting. Audet et al. (2009) reported that vitamin D supplementation enhanced the ejaculate volume but had no effect on sperm production or quality in boars. In human species, the calcitriol [1,25(OH)₂-vitamin D₃] has specific receptors which predominates on the head/nucleus of the sperm and mid-piece (Corbett et al., 2006), and appears to be a modulator of placental steroidogenesis (Barrera et al., 2007) and of FSH secretion (Zofkova et al., 1989). In experimental cryptorchid mouse, Hirai et al. (2009) noted that 1,25-dihydroxyvitamin D contributes to spermatogenesis by up-regulating certain specific genes in Sertoli's cells, and suggested that vitamin D supplementation may have implications for managing male infertility. Furthermore, in Vitamin D-deficient male rats with incomplete spermatogenesis and degenerative testicular changes, Menegaz et al. (2009) suggested that calcitriol may play a critical role for the maintenance of normal reproduction via a genomic mechanism that can be triggered by PKA, as well as to a rapid response involving Ca²⁺/K⁺ channels on the plasma membrane.

Conclusion

From the above study it may be concluded that in Moroccan dromedary camel, the rutting period shows lower circulating levels of 25-OH-D and higher levels of T. During this period, vitamin D does not seem to contribute directly or indirectly to steroidogenesis activity. However, further studies from the one hand of an eventual relationship between T and other parameters such as thyroid hormones and corticoids, and from the another one of testing the vitamin D action on rutting behavior are needed in camels.

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