

ORIGINAL ARTICLE

Serum concentration of calcium, phosphate and 1,25-dihydroxyvitamin D₃ in goats (*Capra hircus*): daily rhythms

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Summary

The goal of the present study was to investigate the daily rhythms of calcium, phosphate and 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂ D₃) in the blood serum of goats. Blood samples from six Girgentana breed goats were collected via an intravenous cannula inserted into the jugular vein at four-hour intervals over a 48-hour period (starting at 08:00 hours on day 1 and finishing at 04:00 on day 2). The serum concentration of calcium and phosphate was measured by photometric test and of 1,25-(OH)₂ D₃ by HPLC. All parameters were expressed as mean \pm SEM. The one-way repeated measures analysis of variance (ANOVA) was used to determine significant differences. ANOVA showed a highly significant effect of time in all the parameters studied. The application of the periodic model and the statistical analysis of the Cosinor enabled us to define the periodic parameters and their acrophases (expressed in hours) during the 2 days of monitoring: all the studied parameters exhibited diurnal acrophases, which were within 12:44 and 17:28 hours. The results obtained led us to reveal the existence of a daily rhythm for the parameters considered and their temporal physiological values are useful for their implications in the formulation of therapeutic and nutritional protocols in the goat.

Keywords: daily rhythm – calcium phosphate – 1,25-dihydroxyvitamin D₃ – *Capra hircus*

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INTRODUCTION

Many metabolic aspects are assessed in different productive and physiological conditions based on their rhythmic variations. The knowledge of rhythmicity as an intrinsic component of living matter led us to redefine some important physiological concepts such as homeostasis and to study physiological rhythms and their modifications from a diagnostic viewpoint, in order to show diseases not evident clinically. Previous research has documented the existence of circadian rhythms

in a multitude of variables in different species, such as liver function in sheep (Piccione et al. 2003) and body temperature in horse (Piccione et al. 2002a). Recently the rhythmicity of some haematological parameters in different species has been reported (Berger 2006).

Some studies have been carried out in the goat, on the daily rhythms of body and auricle temperature and liver functions in subjects maintained under various schedules of lighting and feeding and under different environmental conditions (Piccione et al. 2002b, 2003, 2005). The presence of circadian rhythms in the blood concentrations of calcium and phosphate has been well documented in humans (Markowitz et al. 1985, Portale et al. 1987, Rejnmark et al. 2002), while data available on the daily pattern of serum minerals are scarce in domestic animals. In normal vital processes, minerals play an essential role; calcium and phosphate are the most present minerals (between 70% and 95% in the body) and vitamin D plays a key role in regulating their homeostasis. Calcium and phosphate are the two major constituent minerals of the ruminant body. The skeleton contains 99% of the total calcium and 78% of the total phosphate; only a residual 1% of the total calcium exists in the soft tissues and the extracellular fluid. This calcium plays an important role in neurotransmittance and other metabolic activities. It is well known that the major absorption site of calcium and phosphate in the gastrointestinal tract of ruminants is the small intestine, as it is in monogastric animals. All ruminants are able to regulate with strict precision the concentration of calcium in plasma, under the influence of three hormones: the parathyroid hormone, calcitonin and 1,25-dihydroxyvitamin D₃. The concentrations of phosphate in plasma may also be controlled by these three hormones. Some authors have shown an opposing pattern for these two minerals; a late morning peak in calcium levels and a nocturnal peak in phosphate levels (Markowitz et al. 1981). The physiological significance of these metabolites and their daily pattern is explained by their involvement in the different productive stages of livestock (i.e. pregnancy, and lactation). Because of the intimate relationship between calcium, phosphate and 1,25-dihydroxyvitamin D₃ and their implication from a clinical point of view, it seemed interesting to investigate the existence of a daily pattern for calcium, phosphate and 1,25-(OH)₂ D₃ in the goat, in order to better understand the homeostasis of minerals involved in the productive performance of this species.

MATERIALS AND METHODS

Six females Girgentana breed goats, 20 months old, mean body weight 44.0 ± 1.0 kg, clinically healthy,

not pregnant and not lactating, were used. The animals were housed in separate indoor stalls under a natural photoperiod (sunrise at 06:30 and sunset at 19:00) and natural indoor temperature in Sicily, Italy (latitude 38° 6' N, longitude 13° 20' E, altitude 50 m). According to standard farming practice, the animals had free access to water and to good quality alfalfa hay (90.0% DM, 15.8 CP % DM, 50.4 NDF % DM, 31.6 ADF % DM, 5.8 lignin % DM, 2.2 EE % DM). Concentrate (oats 23%, corn 36%, barley 38%, and mineral and vitamin supplement 3%) was provided once daily at 07:00 hours (200 g per animal per day).

30 days before the experiment, all the subjects underwent the same daily activity pattern. After this preconditioning period, blood samples were collected via an intravenous cannula inserted into the jugular vein, at 4-hour intervals over a 48-hour period (starting at 08:00 hours on day 1 and finishing at 04:00 on day 2). Blood samples were transferred into Vacutainer tubes containing no additive. The tubes were clotted at room temperature for 1 hour and subsequently centrifuged at 3000 rpm for 10 minutes. The resulting serum was stored at -80 °C until analysis. The concentration of calcium and phosphate in each sample serum was assessed by means of a UV spectrophotometric test, and a serum concentration of 1,25-(OH)₂ D₃ was measured by high performance liquid chromatography (HPLC), using UV spectrophotometry.

Statistical analysis

All the results were expressed as mean \pm SEM. Data were normally distributed (Kolmogorov-Smirnov test, significance level $2\alpha=0.05$) and the one-way repeated measures analysis of variance (ANOVA) was used to determine significant differences ($2\alpha=0.05$). Bonferroni's Multiple Comparison test was applied for post hoc comparison. The data were analyzed using STATISTICA 5.5 software (StatSoft Inc., USA).

In addition, we applied a trigonometric statistical model to the average values of each time series, so as to describe the periodic phenomenon analytically, by individuating the main rhythmic parameters according to the single cosinor procedure (Nelson et al. 1979): mesor (Midline Estimating Statistic of Rhythm), expressed in the same conventional unit of the relative parameter, with the confidence interval (C.I.) at 95%, amplitude (A), expressed in the same unit as the relative mesor, and acrophase (Φ), expressed in hours with C.I at 95%.

For each parameter, the mean level of each rhythm was computed as the arithmetic mean of all values in the data set (12 data points). The amplitude of a rhythm was calculated as half the range of oscillation, which in its turn was computed as the difference between peak and trough. The

acrophase of a rhythm was determined by an iterative curve-fitting procedure based on the single cosinor procedure. For each variable for each animal, a cosine wave was fitted to the data points according to the function $Y_t = M + A \cos(\theta_t + \phi)$, where Y_t denotes each data point in the time series, M is the mean level of the rhythm, A is the amplitude, θ_t is the trigonometric angle (in degrees) corresponding to time t , and ϕ is the angle displacement for the acrophase.

The value of ϕ was determined by iteration: the true value of ϕ was considered to be the one that produced the smallest sum of squares of the deviations between iterated cosine functions and the raw data.

RESULTS

The results obtained during the experimental period indicate the existence of a daily rhythm of serum calcium, phosphate and 1,25 dihydroxyvitamin D₃, as shown in Fig.1. ANOVA showed a significant effect of time on all the parameters studied in the serum of goats in either day, as follows: for calcium, $F_{(11,55)} = 8.06$, $p < 0.0001$, for phosphate, $F_{(11,55)} = 14.13$, $p < 0.0001$, for 1,25-(OH)₂-D₃, $F_{(11,55)} = 4.09$, $p < 0.0002$. Application of the periodic model and the statistical analysis of the Cosinor procedure throughout the time series studied in two days, allowed us to ascertain the periodic pattern of calcium, phosphate and 1,25-(OH)₂-D₃ (Table 1).

Table 1: Mesor (M), with confidence interval (C. I.) at 95%, amplitude (A) and acrophase (Φ), expressed in hours, with C. I. at 95%, of calcium, phosphate and 1,25-(OH)₂ D₃, that showed a circadian periodicity during the two days of study

	MESOR	C. I. 95%	A	Φ	C. I. 95%
<i>First day</i>					
Calcium	2.47	(2.46 – 2.49)	5.78	12:44	(10:24 – 15:04)
Phosphate	2.34	(2.22 – 2.47)	0.37	17:28	(13:44 – 21:12)
1,25-(OH) ₂ D ₃	25.08	(24.22 – 25.94)	3.94	12:52	(09:24 – 16:20)
<i>Second day</i>					
Calcium	2.49	(2.48 – 2.51)	9.29	12:56	(11:16 – 14:32)
Phosphate	2.26	(2.20 – 2.32)	0.32	17:00	(15:16 – 18:48)
1,25-(OH) ₂ D ₃	25.62	(22.83 – 28.42)	7.26	12:52	(10:48 – 14:56)

All the parameters studied showed diurnal acrophases, as follows: for calcium, at 12:44 (10:24 – 15:04) during day 1 and at 12:56 (11:16 – 14:32) during day 2; for phosphate, at 17:28 (13:44 – 21:12) during day 1 and at 17:00 (15:16 – 18:48) during day 2; for 1,25-(OH)₂-D₃, at 12:52 (09:24 – 16:20) during day 1 and at 12:52 (10:48 – 14:56) during day 2.

DISCUSSION

In our study, calcium, phosphate and 1,25-(OH)₂ D₃ values were within the physiological range for the goats (Kaneko 1989). In the analysis of the results obtained, all the studied parameters showed diurnal peaks, between 12:44 and 17:28, which are consistent with previous studies on dogs (Piccione et al. 2004a) and on horses (Piccione et al. 2004b, Piccione et al. 2004c).

As described in previous studies, calcium and phosphate are parameters whose rhythmicity shows different periods (from circadian to circannual) in almost all domestic animals (Piccione et al. 2004a). Two major sources may contribute to the changes in the blood concentrations of the minerals observed over time: newly absorbed mineral from the intestine, and redistribution from endogenous stores. Both are dependent on local factors, such as a functioning intestinal tract or responsive bone, and on other influences such as dietary intake of calcium and phosphate as well as the mineral-regulating hormones (Markowitz 1994). The existence of diurnal acrophases for calcium, phosphate and 1,25-(OH)₂ D₃ is not in agreement with values recorded in men, which show an opposite pattern for the two minerals, with a late morning peak in calcium levels and a nocturnal peak in phosphate levels (Markowitz et al. 1981).

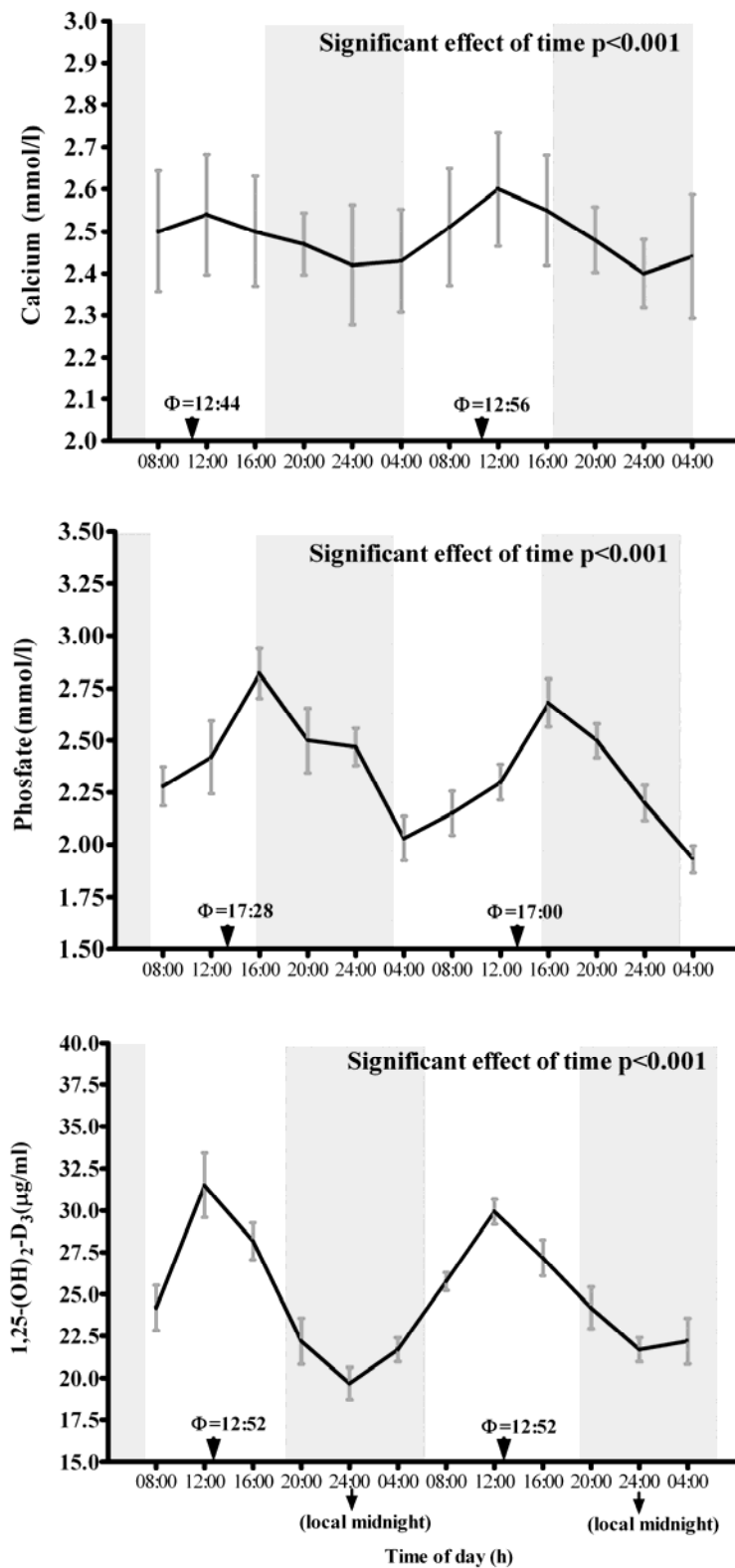


Fig. 1. **Daily rhythms of calcium, phosphate and 1,25-(OH)₂ D₃ in goats.** Each point represent the mean (±SEM) (n = 6) of calcium, phosphate and 1,25-(OH)₂ D₃. Grey bars indicate the dark phase of the 48 hr light and dark duration of the natural photoperiod. Arrowheads indicate the acrophases.

The important role of food intake on the diurnal variation of phosphate in humans compared to other factors such as physical activity or prolonged bed rest is confirmed by other authors (Rejnmark et al. 2002).

The different pattern in the goat compared to man could be ascribed to the different digestive processes of these two species: the continuous passage of food could influence the absorption of the two minerals in the gastrointestinal tract of ruminants, regulating their rhythmicity. Our study reveals a circadian rhythmicity of 1,25-(OH)₂ D₃, which represents the active form of vitamin D, with acrophases always during the first hours of the afternoon. 1,25-(OH)₂ D₃ plays an important role in maintaining cellular and neural functions that involve fluxes of calcium ions through cellular membranes, while its implication in the calcium homeostasis, well known in man, is uncertain in domestic animals (Breidenbach et al. 1998). Small diurnal fluctuations in serum 1,25-(OH)₂ D₃ concentrations were described in humans (Halloran et al. 1985; Markowitz et al. 1985), but no correlation with calcium and phosphate was found. In domestic animals, a few studies have been carried out on daily rhythms of circulating vitamins (Piccione et al. 2004b, 2004c). Further investigations could explain the influence of the numerous factors (hormones, diet, serum mineral concentrations) on daily fluctuations of vitamin D in all its forms. We can conclude that the existence of a daily pattern in serum concentrations of calcium, phosphate and 1,25-(OH)₂ D₃, with diurnal acrophases, could suggest the influence of exogenous or endogenous synchronizers on the studied parameters. As for calcium and phosphate, the variables which have to be considered from a temporal aspect are urinary excretion rhythmicity, hormonal control, and bone remoulding processes along with the intestinal absorption rhythms. Further investigations involving experimental manipulation of ration quality and feeding time are needed for optimizing the utility of these substances according to their temporal pattern. As for 1,25-(OH)₂ D₃ it would be interesting to better define what factors affect its renal productions and its diurnal variations. For all the studied parameters, the onsets of the acrophases were between 12:00 and 17:00 (after meals); for this reason the feeding time seems to be an external synchronizer. This assumption will be verified in further studies using different meal times, and food with different components, and taking into account the complex digestive system of ruminants. The existence of a rhythmicity for calcium, phosphate and 1,25-(OH)₂ D₃, added to the knowledge of their metabolism regulation and of their temporal physiological values could have clinical implications, for example, in choosing the time of day at which the

administration of these minerals and of vitamin D would have its greatest effects.

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