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Energetic chronometabolism in New Zealand white rabbits

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ABSTRACT

The temporal organization of some physiological parameters in rabbits by evaluating the circatrigintan rhythms of some haematochemical and haematological parameters, and of rectal temperature, in New Zealand white rabbits was studied. For 30 days before the study the animals all followed the same pattern of daily activity with the natural photoperiod for that season, and were fed on hay *ad libitum*. Subsequently, the same animals followed another pattern of daily activity for a further 30-day period with a natural photoperiod and were fed on commercial pellets. At the end of each experimental period, blood samples were taken and rectal temperature was measured every 5 days for 30 days. Spectrophotometry in UV was used to calculate the concentration in each sample of: glucose, NEFA, triglycerides, total cholesterol, total proteins, uric acid, urea, albumin, creatinine, calcium, phosphorus and magnesium. The following values were measured on samples rendered unclottable: haematocrit, haemoglobin, RBC, MCV, WBC and VES. A periodic statistical model was used to elaborate the data, on average values of the two sets of samples. Intra-group variance was not significant. Glucose, triglycerides, RBC, Hb, PCV and rectal temperature showed a circatrigintan rhythm in both the experimental periods, but with different acrophases; creatinine, magnesium and phosphorus showed periodicity only when the subjects were fed on hay, while total proteins, urea, albumin and calcium were periodic only when they were fed with commercial pellets. The results obtained showed that the type of ration synchronizes the circatrigintan rhythm of some haematochemical and haematological parameters in the rabbit.

Key words: biorhythm, chronometabolism, rabbit, haematochemical parameters, haematological parameters, rectal temperature

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Introduction

In recent years many studies have shed light on the neurophysiological mechanisms which regulate biological rhythms and on the influence of the photoperiod on various physiological parameters, such as the sleeping/waking and exercise/rest rhythms. The knowledge gained has made it possible to identify a typical spectrum of frequency for different physiological functions, which can be used to evaluate how various domestic species adapt to their environment and, therefore, to improve production by employing optimum rearing techniques (KILGOUR, 1978).

Using Halberg's criteria, we defined the temporal pattern of several haematochemical, haematological and physiological parameters in various domestic species in various experimental conditions (PICCIONE et al., 2001a; 2001b and 2001c; PICCIONE et al., 2002a and 2002b). In particular in the rabbit, the object of the present study, circadian rhythms for some haematochemical parameters and for rectal temperature, have been shown (ROSI et al., 1981; PICCIONE et al., 1995; JILGE et al., 2000; JILGE et al., 2001). We also know that there are circadian variations in plasmatic leptin, with the highest value at the end of the ingestion phase in rabbits fed at different times (ROSI and CAPALBO, 1999). Moreover, the knowledge that, typically, nocturnal feeding is prevalent (PRUD'HON et al., 1972), made it possible to identify feeding peaks (FINZI and VERITÀ, 1975; 1976), influenced both by the energy content of the ration and by the photoperiod (REYNE et al., 1978) which act as synchronizers, to modify both the acrophases and the amplitude of the feeding curve. These same factors also influence the rhythm of other physiological functions connected with feeding, such as intestinal motility, digestion and excretion of caecotrophs (JILGE et al., 1987; JILGE, 1992). Given the foregoing, both in man and in rats (SENSI, 1974; PERET et al., 1972; HAUS et al., 1972; HALBERG et al., 1972) there is a temporal pattern for some biological variables even with low-caloric rations, and observation of a possible temporal pattern for various feeding-connected physiological parameters could be very helpful for identification of elements which could provide adaptation indicators in animals reared using different systems. On the basis of this knowledge and as a continuation of our research, we studied the circatrigintan rhythm of some haematochemical and haematological

parameters and of the rectal temperature in female New Zealand White rabbits, in various feeding regimes.

Materials and methods

We used 5 non-pregnant female NZW rabbits, 4 months old, average weight 3200 ± 100 g. The animals were clinically healthy and were kept in individual cages in a naturally lit box. For 30 days before the study all animals followed the same pattern of daily activity characterized by natural photoperiod and were fed on hay *ad libitum*. Blood samples were then taken by venipuncture of the marginal vein of the ear, every 5 days at the same time for 30 days. Before taking each sample rectal temperature was measured by a rectal probe connected to a digital thermometer with a printer (Phase A, September). Immediately after taking the samples, spectrophotometry in UV was used to establish the concentration in each sample of: glucose, NEFA, triglycerides, total cholesterol, total proteins, uric acid, urea, albumin, creatinine, calcium, phosphorus and magnesium. The following values were measured on unclotted blood samples: haematocrit, haemoglobin, RBC, MCV, WBC, and VES. Subsequently, the same animals followed a further 30-day pattern of daily activity characterized by natural photoperiod and were fed on commercial pellets *ad libitum* (Phase B, November). At the end of this period blood samples were again taken and rectal temperature measured, following the same protocol as the first experiment. During both tests thermometric and hygrometric values in the box were recorded. Environmental temperature and relative humidity showed slight variations but which did not affect thermogenesis and thermoregulation in the animals. For the three different experimental conditions statistical elaboration of the data was based on the average values obtained at the various time points (5 days equidistant), since the intragroup variance was not significant. For each parameter we applied a trigonometrical statistical model to the average values of each temporal series, in order to describe the periodic phenomenon analytically, by identifying its main characterizing parameters: Mesor (M) (Midline Estimating Statistic of Rhythm) - which represents the mean level of rhythm between the highest and lowest value of the function originated to

approximate the rhythm itself, corresponding to the arithmetic average only when the data are equidistant and cover the whole number of cycles with fiducial limits at 95%, the Amplitude (A) that corresponds to half the total variation of an arithmetic function and the Acrophase (ϕ) which indicates the distance (expressed in degrees or time units) from a conventional point of reference to the highest point (apex) of that function with the fiducial limits at 95%. Additionally, the single Cosinor method was applied to the obtained results based on the principle that a series of data deriving from multiple measurements is elaborated by a cosine curve through the least squares method; if the hypothesis of a void amplitude is rejected, such rhythm is considered significant. The function computed to approximate the time series is:

$$y(t_i) = A_0 \cos (\omega t_i + \varphi) + e_i \quad i = 1, 2, 3, \ldots n$$

Where $y(t_i)$ = the single observation at time t_i

n = number of observations

A_0 = mesor or mean level

A = amplitude or difference between maximum of function and mean level

φ = acrophase or interval in degrees from time 0^{00} to maximum of cosine function adopted to approximate rhythm to data

ω = angular velocity = $\frac{360}{\tau}$ where τ is the period fixed a priori

e_i = errors associated with measurement and committed for unknown and imponderable reasons

For those phenomena which are rhythmic and which are studied under conditions synchronous with the 24 h period it is possible to estimate the parameters A_0, A and φ by the least-squares method. By applying a trigonometrical statistical model it is also possible to test the statistical significance of an amplitude (i.e.: $A = 0$ vs $A \neq 0$) assuming that the errors have normal distribution.

Results

Application of the periodic model and the statistical analysis of the "Cosinor" enabled us to define the periodic parameters and their acrophases

Table 1. Specification of myomorphus mammals examined by renoculture and microscopic agglutination acording to the trapping area with corresponding results

(expressed in days), with the relative fiducial limits at 95%. These were as follows: Phase A (September): glucose, on 19 September (17-21 September); triglycerides, on 19 September (18-20 September); creatinine, on 22 September (18-26 September); magnesium, on 23 September (16-30 September); phosphorus, on 21 September (16-26 September); RBC, on 8 September (5-11 September); haemoglobin, on 6 September (1-11 September); PCV, on 5 September (3-7 September); rectal temperature, on 12 September (8-16 September). In Phase B (November): glucose, on

Table 1. Mesor (M), with fiducial limits (F. L.) at 95%, amplitude (A) and acrophase (ϕ), expressed in days, with fiducial limits (F. L.) at 95%, of the periodic parameters during phase A (September)

Parameter	M	F. L. (95%)	A	ϕ	F. L. (95%)
Glucose	7.88	(7.81 - 7.95)	0.33	19	(17 - 21)
Triglycerides	0.92	(0.91 - 0.93)	0.01	19	(18 - 20)
Creatinine	120.30	(117.12 - 123.47)	11.40	22	(18 - 26)
Magnesium	0.91	(0.89 - 0.93)	0.04	23	(16 - 30)
Phosphorus	1.63	(1.60 - 1.66)	0.08	21	(16 - 26)
Rbc	6.69	(6.66 - 6.73)	0.17	8	(5 - 11)
Haemoglobin	14.31	(14.22 - 14.39)	0.27	6	(1 - 11)
Pcv	43.41	(43.39 - 43.43)	0.11	5	(3 - 7)
Temperature	39.06	(39.03 - 39.09)	0.15	12	(8 - 16)

Table 2. Mesor (M), with fiducial limits (F. L.) at 95%, amplitude (A) and acrophase (ϕ), expressed in days, with fiducial limits (F. L.) at 95%, of the periodic parameters during phase B (November)

Parameter	M	F. L. (95%)	A	ϕ	F. L. (95%)
Glucose	7.90	(7.88 - 7.92)	0.10	21	(19 - 23)
Triglycerides	1.01	(0.99 - 1.03)	0.05	19	(13 - 25)
Total protein	8.40	(8.33 - 8.48)	0.18	18	(11 - 25)
Urea	4.02	(4.00 - 4.05)	0.06	24	(18 - 30)
Albumin	33.28	(33.21 - 33.35)	0.33	6	(4 - 8)
Calcium	3.76	(3.71 - 3.82)	0.14	23	(16 - 30)
Rbc	6.45	(6.42 - 6.47)	0.22	20	(19 - 21)
Haemoglobin	13.72	(13.67 - 13.78)	0.28	23	(21 - 25)
Pcv	39.55	(39.41 - 39.69)	0.47	22	(18 - 26)
Temperature	38.98	(38.91 - 39.05)	0.18	14	(7 - 21)

21 November (19-23 November); triglycerides, on 19 November (13-25 November); total proteins, on 18 November (11-25 November); urea, on 24 November (18-30 November); albumin, on 6 November (4-8 November); calcium, on 23 November (16-30 November); RBC, on 20 November (19-21 November); haemoglobin, on 23 November (21-25 November); PCV, on 22 November (18-26 November); rectal temperature, on 14 November (7-21 November). Tables 1 and 2 show the Mesor (expressed in the conventional unit of the relative parameter), with the fiducial limit at 95%; amplitude (expressed in the same unit as the relative Mesor) and the

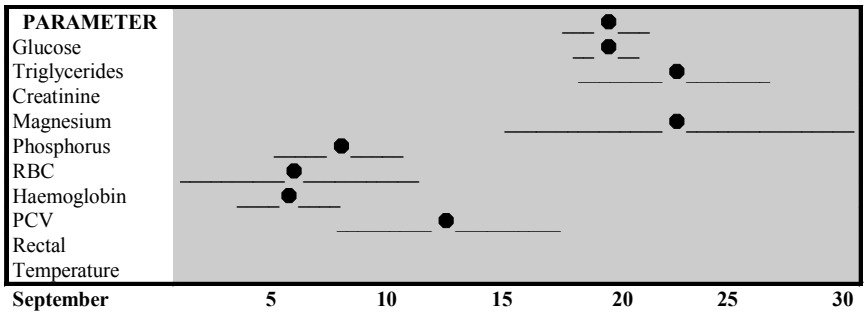

Fig. 1. Map of the acrophases of haematochemical, haematological parameters and rectal temperature showing circatrigintan periodicity during phase A (September)

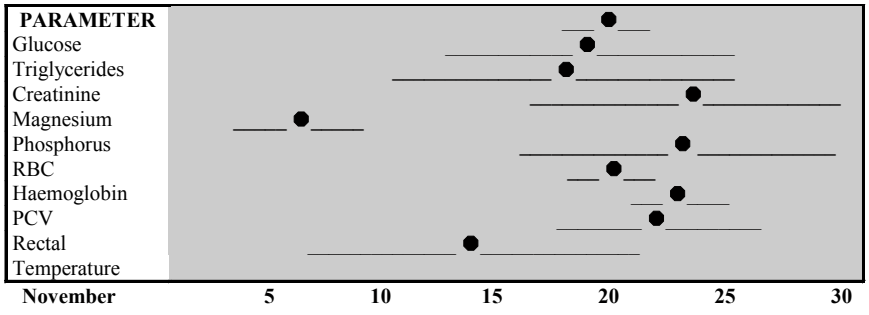

Fig. 2. Map of the acrophases of haematochemical, haematological parameters and rectal temperature showing circatrigintan periodicity during phase B (November)

acrophase (expressed in days), with the fiducial limit at 95%, of those serum parameters which showed periodicity during Phases A and B. Fig. 1 and 2 show their maps of the acrophases.

Discussion

Analysis of the results obtained shows that some of the parameters studied (glucose, triglycerides, RBC, haemoglobin, PCV and rectal temperature), showed circatrigintan periodicity in both (Phases A and B) experimental periods, but with different acrophases; creatinine, magnesium and phosphorus were periodic only when the animals were fed on hay, while total protein, urea, albumin and calcium showed periodicity when the animals were fed on commercial pellets. A review of the specific literature indicated that there are few chronophysiological studies on the rabbit which have used rigorous statistical methods to evaluate biological periodicity. Therefore, we are able to make comparisons only with those parameters that were periodic in our previous studies, or in the studies of other authors which used the same mathematical model to define the temporal pattern. Glucose, which was periodic in both tests, with the acrophase on 19 September for the animals fed on hay, and on 21 November for the animals fed on commercial pellets, had a circadian rhythm with the acrophase at 03.03 pm in NZW rabbits, while in Californian rabbits it had circadian rhythm with the acrophase at 03.44 pm in males and at 04.00 am in females. Triglycerides showed a circatrigintan rhythm in both phases, with the acrophases on 19 September, for animals fed on hay, and on 19 November for animals fed on pellets. Californian female rabbits also showed a circadian rhythm, with the acrophase at 01.24 pm (PICCIONE et al., 1995). Total proteins showed a circatrigintan periodicity, with the acrophase on 18 November in subjects fed on pellets; they also showed a circadian periodicity with the acrophase at 02.11 am, in NZW rabbits, at 03.56 pm in Californian male rabbits, and at 11.56 am in Californian females (PICCIONE et al., 1995). Phosphorus, which had a circatrigintan rhythm, with the acrophase on 11 September in animals fed on hay, also showed a circadian rhythm, with the acrophase at 04.16 pm in NZW rabbits. Urea, which had a circatrigintan progress with the acrophase on 24 November, also showed a circadian

rhythm with the acrophase at 06.13 pm, in NZW rabbits, and with the acrophase at 04.04 pm in Californian males (PICCIONE et al., 1995). Albumin, which showed a circatrigintan rhythm with the acrophase on 6 November in subjects fed on pellets, also showed a circadian rhythm, with the acrophase at 12.58 am in NZW rabbits. Calcium showed a circatrigintan progress, with the acrophase on 23 November in animals fed on pellets; it also showed a circadian rhythm, with the acrophase at 08.36 am in NZW rabbits and at 10.36 am in Californian rabbits. Haemoglobin showed a circatrigintan progress with the acrophase on 6 September; it also showed a circadian progress with the acrophase at 01.50 pm in rabbits of the same breed (ROSI et al., 1981) at 08.00 am in hybrid rabbits (FOX and LAIRD, 1970). Rectal temperature showed periodicity in both tests, with the acrophase on 12 September and 14 November, respectively; in our previous studies it also showed a circadian periodicity in Californian rabbits of varying sex and age, with the acrophase at 06.56 pm in males, at 05.36 pm in females and at 06.04 pm in rabbit kittens. In conclusion, we can state that in the study of chronophysiological system of the rabbit, our results contribute to defining the haematochemical and haematological temporal pattern in this species by showing that there is a circatrigintan rhythm influenced by diet. Moreover, some authors have demonstrated a temporal correlation between certain energetic parameters, such as NEFA and triglycerides and leptin; i.e., they have shown the synchronizing effect of feeding times on several haematochemical parameters (ROSI and CAPALBO, 1999). Therefore, in the knowledge that numerous factors such as breed, sex, age and rations are very important variables which influence the main parameters characterizing the periodic function, it is possible to define the temporal organization of the rabbit concerning certain parameters of great physiological interest.

References

FINZI, A., P. VERITÀ (1975): Apparecchio di Carles e Prud'hon per la registrazione continua del consumo di alimento ed acqua. Ann. Fac. Med. Vet. Pisa 28, 180-186.

FINZI, A., P. VERITÀ (1976): Valutazione della appetibilità dei mangimi nei conigli. Atti Convegno S.I.S.Vet. 30, 501-503.

FOX, R. R., C. W. LAIRD (1970): Diurnal variations in rabbits: hematological parameters. Am. J. Physiol. 218, 1609-1612.

HALBERG, F., E. A. JOHNSON, W. NELSON, W. RUNGE, R. SOTHERN (1972): Autorhythmometry - Procedures for physiologic self-measurement and their analysis. Physiol. Theacher 1, 1-2.

HAUS, E., F. HALBERG, L. E. SCHEVING, J. E. PAULY, S. CARDOSO, J. F. W. KÜHL, R. B. SOTHERN, R. N. SHIOTSUKA, D. S. HWANG (1972): Increased tolerance of leukemic mice to arabinosol cytosine with schedule adjusted to circadian system. Science 177, 80-82.

JILGE, B., H. HÖRNICKE, H. STÄHLE (1987): Circadian rhythms of rabbits during restrictive feeding. Am. J. Physiol. 253, 46-54.

JILGE, B. (1993): The ontogeny of circadian rhythms in the rabbit. J. Biol. Rhythms 8 (3), 247-60

JILGE, B., B. KHUNT, W. LANDERER, S. REST (2000): Circadian thermoregulation in suckling rabbit pups. J. Biol. Rhythms 15, 329-35.

JILGE, B., B. KHUNT, W. LANDERER, S. REST (2001): Circadian temperature rhythms in rabbit pups and in their does. Lab. Anim. 35, 364-373.

KILGOUR, R. (1978): The application of animal behaviour and the humane care of farm animals. J. Anim. Sci. 46, 1478-1486.

PERET, J., M. CHANEZ, I. MECAIRE (1972): Consommation de protèines et rhythmes circadien du glycogène hépatique chez le rat. Acad. Sci. (Paris) 274, 1562-1565.

PICCIONE, G., A. M. ASSENZA, G. CAOLA (1995): Organizzazione temporale circadiana di alcuni parametri ematochimici nel coniglio di razza Californiana. Atti Soc. It. Sci. Vet. 49, 285-286.

PICCIONE, G., A. ARCIGLI, A. ASSENZA, G. CAOLA (2001a): Variaciones circadianas de algunos paràmetros fisiològicos en respuesta al entrenaimento en el caballo de competiciòn. Med. Vet. 18, 607-614.

PICCIONE, G., A. ASSENZA, F. FAZIO, E. GIUDICE, G. CAOLA (2001b): Different periodicities of some haematological parameters in exercise-loaded athletic horses and sedentary horses. J. Equine Sci. 12, 17-23.

PICCIONE, G., A. ASSENZA, G. ATTANZIO, F. FAZIO, G. CAOLA (2001c): Cronophysiology of arterial blood pressure and heart rate in athletic horses. Slov. Vet. Res. 38, 243-248.

PICCIONE, G., G. CAOLA, R. REFINETTI (2002a): Maturation of the daily body temperature rhythm in sheep and horse. J. Ther. Biol. 27, 333-336.

PICCIONE, G., G. CAOLA, R. REFINETTI (2002b): The circadian rhythm of body temperature of the horse. Biol. Rhythm Res. 33, 113-119.

PRUD'HON, M., Y. CARLES, J. GOUSSOPOULOUS, P. F. KOEHL (1972): Enregistrement graphique des consommations d'aliments solide et liquide du lapin domestistique nourri ad libitum. Ann. Zoot. 21, 451-460.

REYNE, Y., M. PRUD'HON, J. ANGEVAIN (1978): Influence d'une rèduction de la durèe d'èclairement sur le comportement alimentaire du lapin en engraissement. 2me Journèes rech. Cunicole en France 2, 71-73.

ROSI, F., G. GREPPI, C. NORDIO, M. L. MENCHINI, F. SCHOEN (1981): Osservazioni di ritmi circadiani in parametri ematici di conigli N. Z. W. Zoot. Nutr. Anim. 7, 443-454.

ROSI, F., R. CAPALBO (1999): Leptina ed altri parametri metabolici in conigli N.Z.B.: variazioni circadiane e relazioni con l'ingestione alimentare. Atti Conv. S.I.S. Vet. 53, 57-58.

SENSI, S. (1974): Some aspects of circadian variations of carbohydrate metabolism and related hormones in man. Chronobiol. 1, 396-404.

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SA•ETAK

Istražena je pojavnost određenih fizioloških osobina na osnovi prosudbe mjesečnog ritma nekih biokemijskih i hematoloških pokazatelja i rektalne temperature u novozelandskog bijelog kunića. Trideset dana su životinje bile držane pod jednakim uvjetima trajanja prirodne svjetlosti i hranjene sijenom *ad libitum*. U drugom tridesetdnevnom razdoblju držane su pod drugim načinom dnevnih aktivnosti s prirodnim trajanjem dnevne svjetlosti te hranjene komercijalnom peletiranom hranom. Na kraju svakog pokusnog razdoblja uzimani su uzorci krvi i mjerena temperatura svakog petog dana u tijeku 30 dana. UV-spektrofotometrijom određena je u svakom uzetom uzorku koncentracija glukoze, NEFA, triglicerida, ukupnog kolesterola, ukupnih bjelančevina, mokraćne kiseline, mokraćevine, albumina, kreatinina, kalcija, fosfora i magnezija. Određivan je hematokrit, hemoglobin, broj eritrocita, leukocita i VES. Podaci su obrađeni periodičkim statističkim modelom na razini prosječnih vrijednosti dvije skupine uzoraka. Varijanca između skupina nije bila značajna. Vrijednosti glukoze, triglicerida, hemoglobina, PVC te broj eritrocita i vrijednost rektalne temperature pokazivale su tridesetdnevni ritam u oba pokusna razdoblja, ali s različitim najvišim fazama. Vrijednosti kreatinina, magnezija i fosfora pokazivale su periodičnost samo kad su životinje bile hranjene sijenom dok su vrijednosti ukupnih bjelančevina, mokraćevine, albumina i kalcija pokazivale periodičnost kad su životinje bile hranjene peletiranom komercijalnom hranom. Dobiveni rezultati su pokazali da vrst obroka usklađuje tridesetdnevni ritam određenih biokemijskih i hematoloških pokazatelja u kunića.

Ključne riječi: bioritam, kronometabolizam, kunić, hematološki pokazatelji, biokemijski pokazatelji, rektalna temperatura