Journal of
APPLIED BIOMEDICINE

J. Appl. Biomed.
3: 123–128, 2005
ISSN 1214-0287

ORIGINAL ARTICLE

Nycthemeral change of some haematological parameters in horses

Giuseppe Piccione[1], Francesco Fazio[1], Elisabetta Giudice[2], Fortunata Grasso[1], Massimo Morgante[3]

[1]Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni Animali, Sezione di Fisiologia Veterinaria, Università degli Studi di Messina, Italy
[2]Dipartimento di Scienze Mediche Veterinarie, Università degli Studi di Messina, Italy
[3]Dipartimento di Scienze Cliniche Veterinarie, Università degli Studi di Padova, Italy

Received 28th May 2005.
Revised 9th June 2005.
Published online 29th June 2005.

Summary
Many physiological processes of domestic animals exhibit daily rhythmicity. The goal of the present study was to investigate, in horses, the existence of the daily rhythms of some haematological parameters: red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT). Blood samples from 6 Thoroughbred mares were collected at 4-hour intervals for 48 hours (starting at 08:00 hours on day 1 and finishing at 04:00 on day 2) via an intravenous cannula inserted into the jugular vein. ANOVA showed a highly significant effect of time on haemoglobin and on haematocrit, in all the horses, on either day, with p values < 0.05. Cosinor analysis identified the periodic parameters and their acrophases (expressed in hours) during the 2 days of monitoring. RBC, Hb, Hct and MCV showed nycthemeral variations included between 17.44 and 22.28, which could suggest the hypothetical influence of various exogenous factors.

Keywords: circadian rhythm – haematological parameters – horse

INTRODUCTION

A variety of biological variables oscillate in organisms, including behaviour, physiological systems and biochemical factors. If any event within a biological system recurs at approximately regular intervals, we talk about a biological rhythm. The predominant rhythms in nature are daily rhythms, for example those in body temperature, in rest and activity and many other physiological functions. The rhythms are not merely passive responses to external stimuli, e. g. the daily alternation of light and darkness, but they persist also in the non periodic environment. Thus, daily biological rhythms are innate and endogenously controlled by self-contained circadian clocks. The term "circadian" implies that under constant external conditions, without time cues, the rhythms

✉ Giuseppe Piccione, Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni animali, Sezione di Fisiologia Veterinaria, Facoltà di Medicina Veterinaria, Università degli Studi di Messina, 98168 Messina, Italy
☎ +39903503584; Fax: +39903503975.
💻 Giuseppe.Piccione@unime.it

occur with a period of approximately but not precisely 24 h. Such self-sustained oscillations with a period of about one day are called “circadian rhythms” (Piccione and Caola 2002).

In nature, circadian rhythms usually do not occur freely, as they are entrained to the 24 h day by external cycles. Environmental factors are capable of determining the timing of circadian rhythms and could act as synchronizers which do not create rhythms, but determine their placement in time. The light-dark cycle is the most important and universal entraining agent, but other external factors may serve as synchronizers, such as the daily temperature cycle, the timing of meals, social cues, etc. Several studies aimed at locating the anatomical responsibility for the generation of these rhythms, have revealed the existence of “pacemaker tissues” in the central nervous system of animals, whose signals direct circadian output responses in peripheral tissue areas (Takahashi 1995). Many studies have investigated the temporal organization of some haematological parameters in man, where the circadian rhythms of the formed elements in the circulating blood are of a complex nature and the number of circulating formed elements in the peripheral blood shows circadian rhythms in all cell lines (Haus and Touitou 1994, Berger 2004).

The recognition of a multifrequency time structure in the number and functions of the circulating corpuscular elements in the peripheral blood is essential for the scientific and clinical exploration of haematological parameters in the horse. Given the lack of experimental studies on the rhythmic pattern of haematological parameters in domestic animals based on the organism’s physiological status, we have investigated the existence of circadian rhythmicity in some haematological parameters [red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT)] in the horse under natural environmental conditions.

MATERIALS AND METHODS

Six thoroughbred mares, 8 years old, were housed in individual stalls under a natural spring photoperiod (sunrise at 06:06, sunset at 18:50) with an ambient temperature of 18 – 21°C and relative humidity of 50 – 60%. Food (hay and concentrate) was provided three times daily, with water *ad libitum*. Blood samples were collected at four-hour intervals over a 48 hour period (starting at 08:00 on day 1 and finishing at 04:00 on day 2) via an intravenous cannula inserted into the jugular vein. Blood samples were transferred into tubes containing EDTA, to evaluate, by means of a double-capillary automatic cell counter (Hemat 8 SEAC – Italy) red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT). The Hct was determined by the haematocrit method.

Statistical analysis

All the results were expressed as mean ± SD. A one – way repeated measures analysis of variance (ANOVA) was used to determine significant differences. P values <0.05 were considered statistically significant. Data were analyzed using the software STATISTICA 5.5 (StatSoft Inc., USA). For each measurement we applied a trigonometrical statistical model to the average values of each time-series measurement so as to analytically describe the periodic phenomenon. The procedure used the cosine function: $f(t) = M + A \cos(\omega t + \phi)$ as a model for biological rhythms, where $f(t)$ was the value at time t of the function defined by parameters M (mesor = the value about which oscillation occurred), A (amplitude = half the difference between the highest and lowest values), ω (angular frequency = degrees/unit time, with 360° representing a complete cycle) and ϕ (acrophase = timing of higher point, in degrees). The cycle duration (and hence ω) was given on the basis of either prior knowledge or a reasonable assumption.

In a time-series of data to be analysed, consisting of $j = 1, 2, \ldots, n$ and the measurements $y_1, y_2, \ldots, y_n$ taken at times $t_1, t_2, \ldots, t_n$, as long as there are at least 4 different time points, the t_j in a given series need not be all different, thus allowing for the replication of measurements at a given time point. The interval between the time points does not need to be fixed, but should be reasonably uniform throughout the cycle (Nelson 1979).

The single Cosinor procedure, as described by Nelson, was applied to the results obtained. This method is used for data that can be described by the fitting of a mathematical model. It represents the crest time of the cosine curve best fitting the data and it may be expressed in (negative) degrees as the lag from the acrophase reference (360° = 1 period) or in calendar time units (hours for the circadian rhythm).

RESULTS

The results obtained during the experimental period indicate the existence of a nycthemeral rhythm of haematological parameters in the horse, as shown in Figs. 1−4.

ANOVA showed a highly significant effect of time, in all the horses, on either day, for some haematological parameters studied, as follows: Hb, $F_{(11,44)} = 2.49$, $p<0.01$; Hct, $F_{(11,44)} = 2.32$, $p<0.02$. The application of the periodic model and the statistical analysis of the "Cosinor" enable us to define the periodic parameters and their acrophases (expressed in hours) during the 2 days of monitoring. RBC, Hb, Hct and MCV showed nocturnal acrophases as follows: RBC, at 19.12 (day 1) and at 18.44 (day 2); Hb, at 18.48 (day 1) and at 17.44 (day 2); Hct, at 20.24 (day 1) and at 20.48 (day 2); MCV, at 21.12 (day 1) and at 22.28 (day 2). WBC and platelets did not show any rhythmic pattern in our study.

DISCUSSION

From the analysis of the results obtained, RBC, Hb, Hct and MCV showed a circadian rhythm with nocturnal acrophases. This is not in agreement with data from other studies on rats (Berger 1983), which did not reveal any circadian rhythmicity for RBC counts. This could underline the importance of exogenous factors, primarily light/dark cycle, on the rhythmic pattern of the haematological parameters, and could suggest that their circadian pattern is under light/dark control.

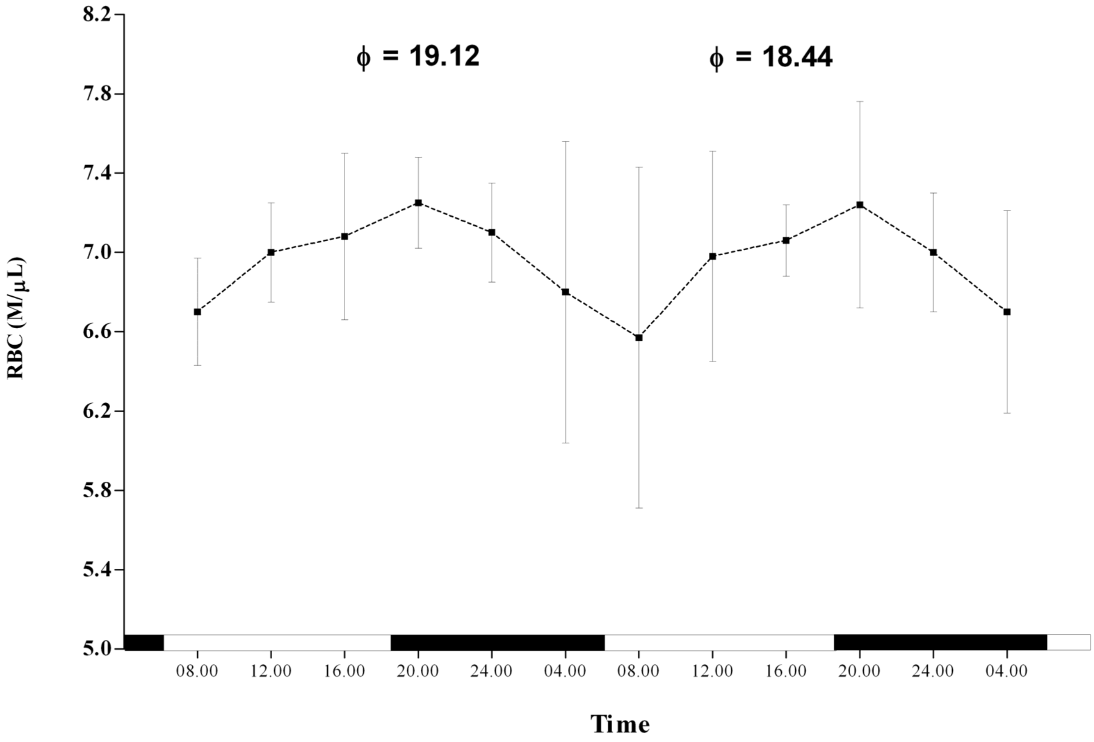
In the study of haematological parameters in the peripheral blood, rhythmic events have been described in the horse in several ranges of frequencies, from hours to years (Gill et al. 1978, Gill et al. 1985, Gill and Kompanowska-Jezierska 1986, Gill and Rastawicka 1986, Komosa et al. 1990, Piccione et al. 2001, Yashiki et al. 1995).

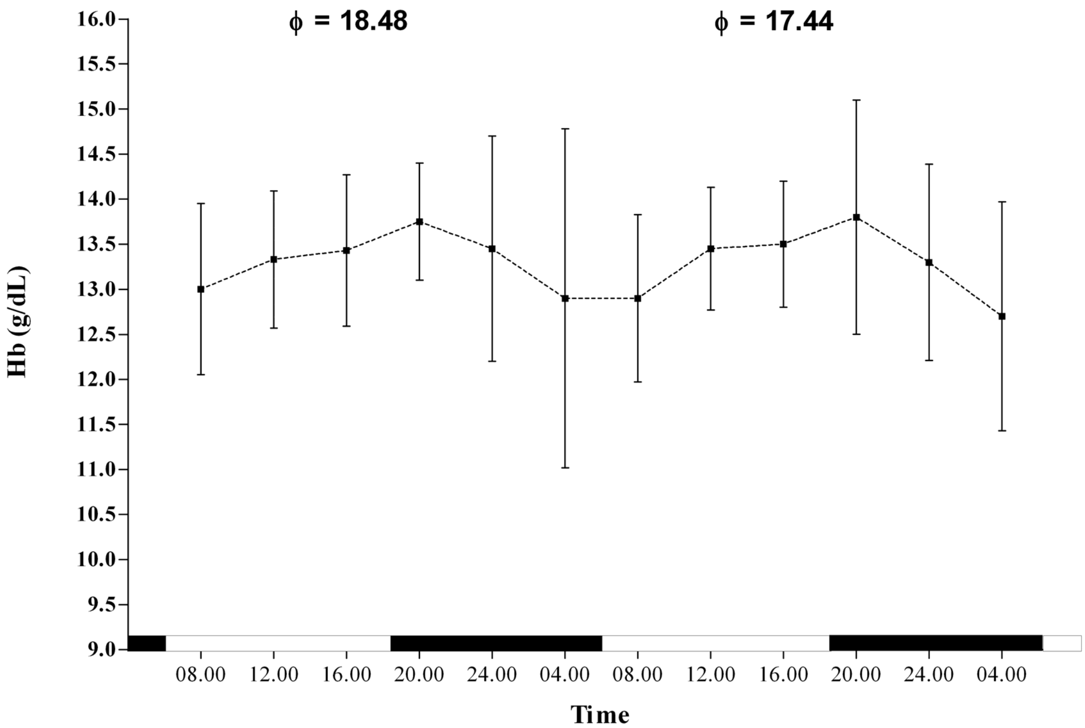
Several factors could be involved in the definition of the rhythmicity of these parameters; studies on Arabian horses revealed a higher value of red blood cells and a lower value of white blood cells in young and regularly trained horses, compared to older reproductive horses of the same breed, that could suggest the probable effect of adaptation to physical effort. However, previous studies carried out on Thoroughbred and Arabian horses showed the existence of a rhythmic pattern for some haematological parameters which were not influenced by endogenous or exogenous factors (Gill et al. 1978, Gill and Kownacka 1979).

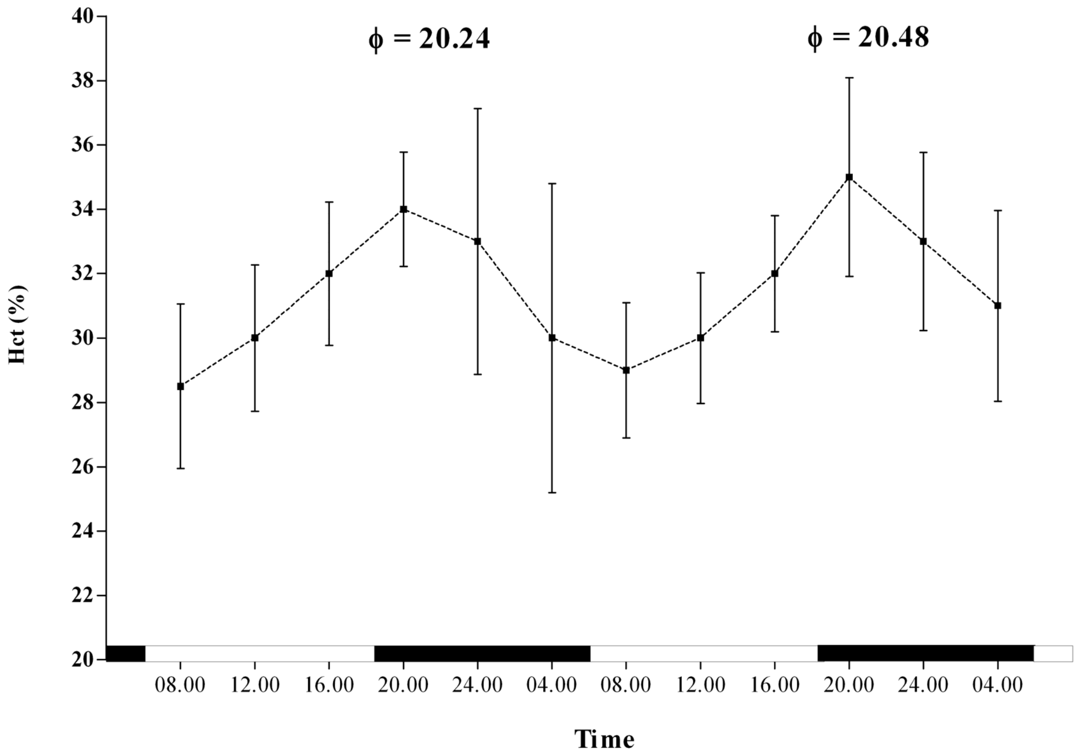
Previous studies on the rhythmic pattern of haematological parameters showed an opposite trend in the diurnal variation between the WBC on one hand, and RBC, PCV and Hb on the other hand, in different species (Durotoye et al. 2000; Pocock et al. 1989).

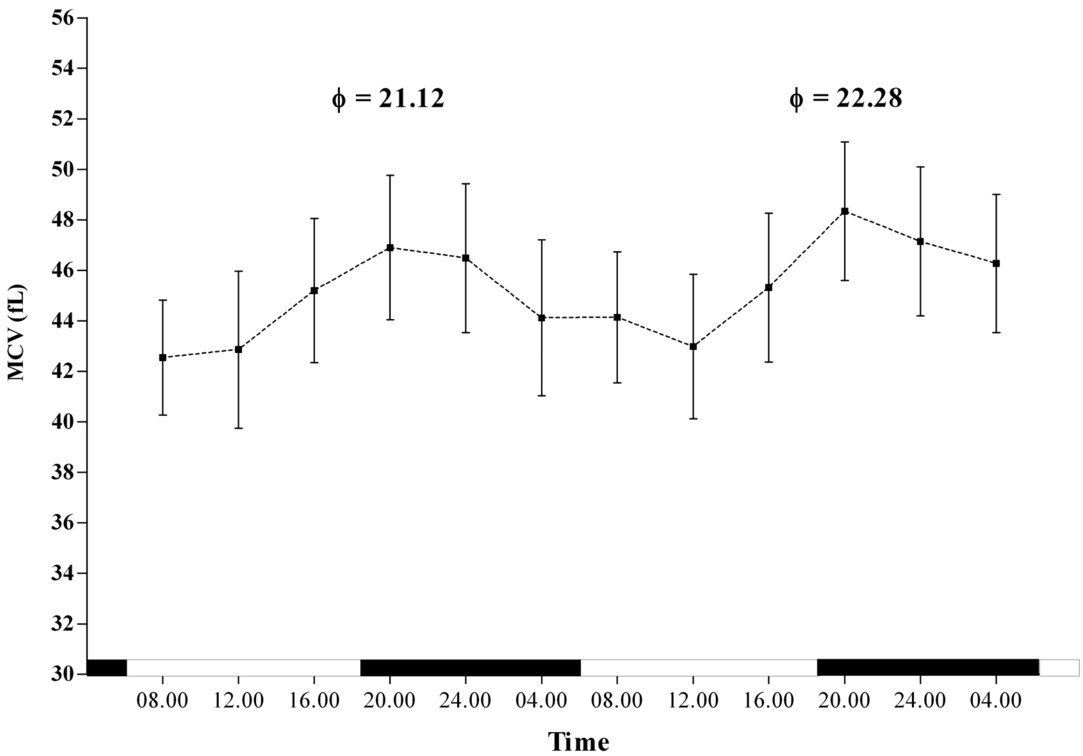
In horses fed on varying diets, some studies showed a circadian pattern with nocturnal acrophases for WBC, Hb and PCV (Gatta et al. 1992, Greppi et al. 1996). The literature shows: variable behaviour for the WBC, which was related to the mares being not pregnant or lactating a diurnal circadian periodicity with the acrophase at 10.00, only in the autumn-winter period in not-pregnant mares; no rhythmicity revealed during pregnancy, but a diurnal rhythmicity, with the acrophase at 19.00 observed a month post-partum (Gill et al. 1994). An increase in WBC in the early afternoon, after morning physical exercise, has also been reported. This increase, which particularly concerned the neutrophils, might result from different factors such as: 1) the drawing of leukocytes from different organs (bone marrow, spleen, lungs, etc.) into the bloodstream due to an increase in circulating blood volume; 2) a shift of leukocytes from the marginal poll in response to plasma catecholamine increase and, as a contributory factor, 3) the stimulation of neutrophil production by increase such as interleukin 1, but in these studies a mathematical periodic model was used for data processing (Yashiki et al. 1995).

Studies on man showed diurnal rhythms of several haematological parameters (RBC, Hb, Hct) in clinically healthy subjects, with diurnal acrophases (Haus 1994), while some authors who have investigated the diurnal rhythm of some haematological parameters in the rat showed an opposite pattern of RBC and WBC, with a gradual increase of RBC from 12.00 to 24.00 (acrophase at 21.00) and a progressive decline of WBC during the same period (Sanni et al. 2000). A nocturnal peak of RBC in the rat could be justified by the nocturnal nature of this species. On the other hand, the occurrence of a nocturnal acrophase of the haematological parameters from our study is in agreement with previous studies which have revealed, in the horse, a nocturnal peak of body temperature (Piccione et al. 2002). This parameter starts its daily ascent at the time of lights on, as other small diurnal mammals (Refinetti 1999). The nocturnal acrophase occurring both in haematological parameters and body temperature led us to hypothesize the influence of the same synchronizers on the pattern of these parameters. Thus, on the basis of the results obtained and the literature available it can be asserted that the circadian pattern of some haematological parameters could be affected by several factors both external and internal. Further investigations are essential to better understanding of the influence of both exogenous and endogenous stimuli on the horse.


ϕ = 19.12
ϕ = 18.44
RBC (M/μL)
8.2
7.8
7.4
7.0
6.6
6.2
5.8
5.4
5.0
08.00 12.00 16.00 20.00 24.00 04.00 08.00 12.00 16.00 20.00 24.00 04.00
Time


ϕ = 18.48
ϕ = 17.44
Hb (g/dL)
16.0
15.5
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
08.00 12.00 16.00 20.00 24.00 04.00 08.00 12.00 16.00 20.00 24.00 04.00
Time





Figs. 1 — 4. **Daily rhythm of red blood cells (RBC), haemoglobin (Hb), haematocrit (Hct) and mean corpuscular volume (MCV) in the horse.** Each time point represents the mean value ± SD. Φ represents the acrophase. Black and white stripes at the bottom of the graphic represents dark and light duration of the natural photoperiod.

REFERENCES

Berger J.: Seasonal influences on circadian rhythms in the blood picture of SPF rats housed under artificial illumination. Folia Haematol. 110:55–70, 1983.

Berger J.: Chronohaematology. J. Appl. Biomed. 2:179–185, 2004.

Durotoye L.A., Fadairo M.O., Avwemorue A.K.: Diurnal variation in blood parameters in the chicken in the hot tropical climate. Afr. J. Biomed. Res. 3:143–147, 2000.

Gatta D., Greppi G.F., Casini L. et al.: Feeding schedule and daily fluctuations of hematological parameters in horse. Gesellschaft für Ernahrungsphysiologie. Gottingen, 1992, p. 86.

Gill J., Szwarocka-Priebe T., Krupska U., Peplowska Z.: Seasonal changes in hematological indices, protein and glycoprotein levels and in activity of some enzymes in Arabian horses. Bull. Acad. Polon. Sci. Ser. Sci. Biol. 26:719–723, 1978.

Gill J., Kownacka M.: Seasonal changes in erythrocyte, haemoglobin and leukocyte indexes in pregnant mares of thoroughbred horses. Bull. Acad. Polon. Sci. Ser. Sci. Biol. 27:143–148, 1979.

Gill J., Kompanowska-Jezierska E., Jakubow K., Kott A., Szumska D.: Seasonal changes in the white blood cell system, lysozyme activity and cortisol level in Arabian brood mares and their foals. Comp. Biochem. Physiol. 81A:511–523, 1985.

Gill J., Kompanowska-Jezierska E.: Seasonal changes in the red blood cell indices in Arabian brood mares and their foals. Comp. Biochem. Physiol. 83A:643–651, 1986.

Gill J., Rastawicka M.: Diurnal changes in the hematological indices in the blood of racing Arabian horse. Polskie Archiwum Weteryjne 26:169–179, 1986.

Gill J., Flisinska-Bojanowska A., Grzelkowska K.: Diurnal and seasonal changes in the WBC number, neutrophil percentage and lysozyme activity in the blood of barren, pregnant and lactating mares. Advan. Agr. Sci. 3:15–23, 1994.

Greppi G.F., Casini L., Gatta D. et al.: Daily fluctuations of haematology and blood biochemistry in horses fed varying levels of protein. Equine Vet. J. 28:350–353, 1996.

Haus E.: Chronobiology of circulating blood cells and platelets. In Touitou Y. and Haus E. (eds.): Biological Rhythms in Clinical and Laboratory Medicine. Springer-Verlag, Berlin 1992, pp. 504–526.

Haus E, Touitou Y.: Chronobiology in laboratory medicine. In Touitou Y. and Haus E. (eds.): Biological Rhythms in Clinical and Laboratory Medicine. Springer-Verlag, Berlin 1992, pp. 673–708.

Komosa M., Flisinska-Bojanowska A., Gill J.: Diurnal change in the haemoglobin level, red blood cell number and mean corpuscular haemoglobin in foals during the first 13 week of life in their lactating mother. Comp. Biochem. Physiol. 96A:151–155, 1990.

Nelson W., Tong U., Lee J., Halberg F.: Methods for cosinor rhythmometry. Chronobiologia 6:305–323, 1979.

Piccione G., Assenza A., Fazio F. et al.: Different periodicities of some haematological parameters in exercise-loaded athletic horses and sedentary horses. J. Equine Sci. 12:17–23, 2001.

Piccione G., Caola G.: Biological rhythm in livestock. J. Vet. Sci. 3:145–157, 2002.

Piccione G., Caola G., Refinetti R.: The circadian rhythm of body temperature of the horse. Biol. Rhythm Res. 33:113–119, 2002.

Pocock S.J., Ashby D., Shaper A.G. et al.: Diurnal variations in serum biochemical and haematological measurements. J. Clin. Pathol. 42:172–179, 1989.

Refinetti R.: Relationship between the daily rhythms of locomotor activity and body temperature in eight mammalian species. Am. J. Physiol. 277:R1493–R1500, 1999.

Sanni A.A., Oyedokun O.R., Alaka O.O.: Preliminary observations on diurnal rhythm in the haematological parameters of male African giant rats (*Cricetomys gambianus*, Waterhouse). Afr. J. Biomed. Res. 3:117–120, 2000.

Takahashi J.S.: Molecular neurobiology and genetics of circadian rhythms in mammals. Annu. Rev. Neurosci. 18:531–553, 1995.

Yashiki K., Kusunose R., Takagi S.: Diurnal variations of blood constituents in young Thoroughbred horse. J. Equine Sci. 6:91–97, 1995.