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Short communication

Daily rhythms of acute phase proteins in cattle under different natural environmental conditions

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ABSTRACT

This study was conducted to assess the pattern of daily rhythms of acute phase proteins (APPs), including haptoglobin (Hp) and serum amyloid A (SAA), and the pattern of daily rhythms of fibrinogen (Fb), white blood cell counts (WBC) and packed cell volume (PCV) in Holstein Friesian and Modicana dairy cattle. Blood samples were collected every 3 h over a 48 h period from six Holstein Friesian and six Modicana dairy cattle during two different periods: January and July.

Hp was higher in Modicana cattle than Holstein Friesian cattle in both periods, and it was lower in July than January in both breeds. All parameters, except PCV, showed daily rhythmicity in both breeds, during the two periods. In Holstein Friesian cattle, Hp and WBC acrophases were postponed by about 2 h during July with respect to January and with respect to Modicana cattle in the same period. A high correlation between individual values of WBC and of APPs was observed. The exact mechanism that causes this variation is not clear. More studies are necessary to understand the amount of these variations and to provide diagnostic and prognostic information useful for the animal welfare control.

1. Introduction

Genetic selection and environmental pressure may predispose farm animals to many unfavorable consequences, ranging from discomfort to death. For monitoring adverse environmental and/or management stressors, thus enabling better animal welfare practice, a potential role is played by acute phase proteins (APPs) (Murata, 2007; Piñeiro et al., 2007).

In cattle, measurement of APPs, such as haptoglobin (Hp) and serum amyloid A (SAA), often in combination, is widely used to detect inflammatory conditions (Chan et al., 2010; Petersen et al., 2004) and as marker of exposure to complex stress (Lomborg et al., 2008). For the lack of a standardization of their assessment, they are not widely used in bovine practice yet, but only in experimental studies (Petersen et al., 2004). Due to a relatively short half of life in serum and high response in diseased animals, APPs serum response constituted a valid measure of a systemic response to an initiating stimulus at the time of blood sampling (Petersen et al., 2004). In cattle, after inflammatory stimuli, SAA increases around 2–8 fold during an acute phase reaction and seems to react faster than Hp (Jain et al., 2011).

Dominguez-Rodriguez and Abreu-Gonzalez (2009) demonstrated that APPs showed diurnal variations and the amplitude of this variation was greater than the seasonal variation. Other findings have suggested that some, but not all, APPs daily variations may be influenced

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by the environmental light intensity (Kanikowska et al., 2005). Diurnal variation may be an important source of heterogeneity or bias, and standardization for sampling time may be important in population based studies, as well as in using this variable as biomarkers that can provide an objective measure of welfare status, with precise indicators of stress or no-stress. Single samples from individuals are of little value for monitoring changes in biochemical variables due to pathologic conditions; a series of measurements should be taken over a period of time, or samples should be collected at precise times for results to be meaningful (Piccione et al., 2012). In bovine species, 12 of the 25 variables studied showed daily rhythmicity (Giannetto and Piccione, 2009).

Aside from the physiological and economic efficiency, the determination of whether an animal is in an optimal environment or stressed is of current interest in most developed nations due to a rapid proliferation of animal welfare laws (Johnson, 1987). It is known that the optimum environmental temperature for bovine milk production is between 16 and 35 °C, but it is also important to consider the perceived thermal conditions, that are influenced by other environmental parameters, such as relative humidity and wind (Jacobsen, 1998).

On this basis, the aim of this study was to evaluate the serum concentration of positive APPs, such as Hp and SAA, Fb and WBC in two different cow breeds in order to show their daily fluctuation and if these values could be influenced by environmental conditions.

2. Material and methods

Six non-pregnant and non-lactating (at least 45 days from the start of the study) Holstein Friesian dairy cattle (4 years old, mean body weight 620 ± 40 kg, BCS 3.39 ± 0.18) and six non-pregnant and non-lactating Modicana dairy cattle (4 years old, mean body weight 500 ± 35 kg, BCS 3.40 ± 0.23) were used. Body Condition Score (BCS) was measured on a 5 point scale as reported by Edmonson et al., 1989. Before the start of the study, cattle health status was evaluated based on behavior, rectal temperature, heart rate, respiratory rate, cough, nasal and ocular discharge. All subjects underwent a complete hematology and plasma biochemistry profile. Only animals with all tested parameters within the physiological range were used. All animals were kept in a stanchion barn and were housed in the same farm located in Ragusa (Sicily, Italy, latitude 36°55′45″N, longitude 14°43′4″E) under natural photoperiod and environmental conditions. The animals were fed hay (*Triticosecale* 40%, barley 40% and oats 20%, cut at the early flowering stage) ad libitum and had free access to water. General animal care was carried out by professional staff not associated with the research team.

Thermal and hygrometric records were carried out for the whole study by means of a data logger with a high reading accuracy and resolution (Model Tinytag Ultra 2, Gemini Data Logger, West Sussex, United Kingdom) placed inside the stanchion-barn. Thermal hygrometric index was calculated using the U.S. Weather Bureau's Temperature Humidity Index Formula for bovine specie (Potter and Jacobsen, 2000):

$$\mathrm{THI}_{(^\circ\mathrm{C})} = \mathrm{T}^\circ_{\text{environmental}} + (0.36 \times \text{point of steam condensation}) + 41.5$$

Blood samples were collected every 3 h over a 48 h period, starting at 8:00 on day 1 and finishing at 8:00 on day 3 during two different periods: period 1 (January 2010) sunrise 06:40, sunset 17:40; period 2 (July 2010) sunrise 05:20, sunset 20:50. Blood was collected from the coccigeal vein of each subject using vacutainer tubes (Terumo, Japan) without anticoagulant, with sodium citrate 3.8% (one part of citrate and nine part of blood) and with K_3-EDTA. All samples were stored at 4 °C before being analyzed.

On all sera, obtained after centrifugation at $3000g \times 15$ min, Hp (g/L) and SAA (μg/mL) concentrations were determined by using an ELISA obtained from Tridelta Development Ltd. (Dublin, Ireland), according to the manufacturer's instructions. On plasma, Fb (g/L) was determined by means of an automatic coagulometer (Clot 2, SEAC, Florence, Italy) according to the manufacturer instructions and to a standard protocol to exclude differences that result from irregular test procedures. WBC (10^9/L) and PCV (%) were tested on whole blood by means of the multiparametric automatic analyzer for hematology (HecoVet, SEAC, Florence, Italy). All samples were analyzed in duplicate within 2 h from collection. Samples exhibited parallel displacement to the standard curve. The overall intra-assay coefficient of variation has been calculated to be $< 5\%$.

3. Statistical analysis

Data were normally distributed (Kolmogorov–Smirnov test). A mixed repeated measures model was used to determine a statistical significant effect of time of day, period and breed at the significant level $2\text{alpha} = 0.05$.

Using cosinor rhythmometry four rhythmic parameters were determined: mesor (mean level), amplitude (half the range of oscillation), acrophase (time of peak) and robustness (strength of rhythmicity). Rhythm robustness (stationarity of a rhythm) was computed as the quotient of the variance associated with sinusoidal rhythmicity and the total variance of the time series. Robustness greater than 40% is above noise level and indicates statistically significant rhythmicity. On each rhythmic parameter two-way ANOVA was applied to determine statistical effect of breed and period.

On individual values of WBC, and of APPs studied and Fb during the two different experimental periods, a linear regression model ($y = a + bx$) was applied in order to determine the correlation between the studied parameters in the two different breeds, the correlation coefficient (r) was determined.

4. Results

Fig. 1 shows the ambient temperature, relative humidity and THI observed during the 48 h of monitoring in the two experimental periods.

The application of mixed repeated measures model showed a statistical significant effect of time of day on all studied parameters, except on PCV. These changes during