Daily rhythm and seasonal variation of serum pancreatic alpha amilase in Holstein bovine maintained under natural environmental condition in Southern Hemisphere

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SUMMARY

In this study the effect of daily rhythm, season, gender and productive status on serum pancreatic alpha amylase was evaluated in clinically healthy Holstein cattle from Southern Hemisphere. A total of 18 animals were enrolled in the study and equally divided in three groups according to their gender and lactation class: Group A (dairy cows at first lactation class); Group B (dairy cows at third lactation class); Group C (bulls). Groups A and B were in the same productive throughout the investigation (dry period in winter, early lactation in spring, mild lactation in summer, end lactation in autumn). From each animal blood samples were collected every 3 hours over 24-hour period, every three months (winter, spring, summer, autumn) and on the serum obtained the concentration of serum alpha amylase has been assed. The application of two-way analysis of variance (ANOVA) showed a significant effect of season and group (gender and productive status) on alpha amylase. Cosinor rhythmometry showed a diurnal daily rhythmicity of alpha amylase in autumn, winter and spring in group A, in summer and autumn in group B and in all seasons in group C. This study suggests that seasonal and physiological conditions must be taken into consideration for the correct interpretation of serum chemistry in bovine in order to establish an accurate interpretation of laboratory data, critical in diagnosis, prognosis and treatment of diseases.

KEY WORDS

Alpha amylase; bovine; daily rhythm; seasonal variation.

INTRODUCTION

Different hematochemical parameters are affected by daily, circadian, and annual rhythm and, in particularly, when the daily rhythm has a large enough amplitude, it may modificate the clinical interpretation[1,2]. Animals have become adapted to the season of their environment by responding to the changes in the lengths of daylight and night in preparation for the climatic changes associated with the different seasons[3]. In particularly, in mammals, light signals detected from the photosensitive retinal ganglion cells serve entrain circadian rhythms to the daylight cycle that affect most physiological and biochemical processes[4]. As reported for other ungulates and mammals, bovine were expected to adjust their physiological responses on daily and seasonal basis, in accordance with the seasonal variations in the photoperiod and environmental conditions. Daily oscillation in the levels of physiological parameters has been described in a variety of animal species for a multiple of variables, including locomotor activity, body temperature, heart rate, blood pressure, hormonal secretion, and urinary excretion[5-7]. Increasing the productivity or efficiency of farm animals necessarily involves changes in metabolism. In particular, several studies have been carried out in cows in order to evaluate the rhythmicity of the serum urea and ammonia concentration[8], arterial blood gas[9,10], blood electrolytes[8], body temperature, peripheral concentrations of insulin, nitrogen[11], acute phase proteins, redox states[12,13], metabolic parameters including non-esterified fatty acids (NEFA) and total cholesterol[14], as well as of the total locomotor activity[15].

Alpha amylase catalyses the hydrolyses of alpha 1-4 glicosidic bonds present in starch, glycogen and other related carbohydrate. Serum alpha amylase concentration is normally low and fairly constant, and it is found to increase in different physiological and pathological conditions. It has been demonstrated that exocrine pancreas not only have 24-h variations of morphometric and functional parameters, but also it show a circadian modification of the pancreatic enzyme[16]. Nutrients play the most important role for regulation of secretory and motor activities at gastrointestinal level, whereas the influence of environmental factors such as circadian rhythm can be studied if the subjects are constantly fed or constantly fasting.

On the basis mentioned cases and considering that the environmental conditions are major physiological stressors which could affect the bovine's biological system, the aim of this study was to evaluate and describe the daily and seasonal rhythm of the serum concentration of pancreatic alpha amylase in Holstein bovines under different environmental condition. Moreover, the possible difference in the daily and seasonal rhythmicity of this serum parameter between bulls and dairy cow was investigated.

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MATERIALS AND METHODS

Animals

Eighteen clinically healthy Holstein bovines were selected from a high production dairy farm in Villa Trinidad (Santa Fe, Argentina, longitude 26°13' and latitude 58°17'). Their health status was evaluated based on rectal temperature, heart rate, respiratory profile, appetite, faecal consistency, and hematological profile. No subject showed any sign of diseases during the study. Animals were divided into three equal groups according to their gender and lactation classes: Group A (n=6) were dairy cows at first lactation class, aged 2 years old with a mean body weight of 500 ± 21 kg; Group B (n=6) were dairy cows at third lactation class, aged 4 years old with a mean body weight of 550 ± 20 kg; Group C (n=6) were bulls, aged 2 years old with a mean body weight of 520 ± 19 kg. The cows of the Groups A and B had the same gestational and productive period. Specifically, the cows were in dry period in winter, early lactation in spring, mild lactation in summer, end lactation in autumn. During lactation period dairy cows were milked twice a day at the same hour of the day (05:00 h and 15:00 h). All animals were raised under the uniform pasture conditions, and they were subject to natural variation in the light-dark cycle. Animals were under extensive management system allowing them to move in outdoors grazing area with pastures planted with alfalfa and water was available *ad libitum*. The protocol of this study was reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU.

Blood sampling

From each animal blood samples were collected through jugular intravenous catheters (FEP G18*45mm) secured in place with suture (Vicryl, Ethicon, Somerville, NJ). Samples were collected every 3h over 24h period. Blood sampling was performed at the same hour of the day starting at 8:00 of day 1 and ending at 08:00 of day 2 in January (winter season), April (spring season), July (summer season) and October (autumn season). The blood samples were centrifuged at 3.500 rpm for 10 min and the obtained sera stored at -20°C until analyzed. On serum samples the concentration of alpha amylase was evaluated by means of an automated analyzer (Metrolab 2100, Laboratory Rodriguez Corswant SRL, Argentina) using commercially available kits.

At the same times of blood collection, thermal and hygrometric records were carried out for the whole study period by means of a data logger with a high reading accuracy and resolution (Model Tinytag Ultra 2, Gemini Data Logger, West Sussex, United Kingdom). Temperature-humidity index (THI), used as indicator of thermal comfort for cattle, was calculated using the U.S. Weather Bureau's Temperature Humidity Index Formula for bovine specie[17]:

THI[°C] = T° ambient + (0.36 * point of steam condensation) + 41.5.

Statistical analysis

Two-way analysis of variance (ANOVA) was applied on the serum alpha amylase recorded at the same time of day (08:00) to assess significant effect of the experimental conditions (season, gender and productive status) on the tested parameter. When significant differences were found Bonferroni's post hoc comparison was applied. P values <0.05 were considered statistically significant.

Using cosinor rhythmometry[18,19] 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[6,19]. Robustness greater than 60% is above noise level and indicates statistically significant rhythmicity. The cosinor procedure uses an *F* test to evaluate whether the amplitude of a cosine wave fitted to the data is significantly greater than zero[6,19]. Amplitude not significantly greater than zero implies absence of rhythmicity. All results were expressed as mean ± standard deviation (SD). P value <0.05 was considered statistically significant.

RESULTS

The environmental temperature, relative humidity and THI recorded during the experimental period (winter, spring, summer and autumn) are shown in Figure 1.

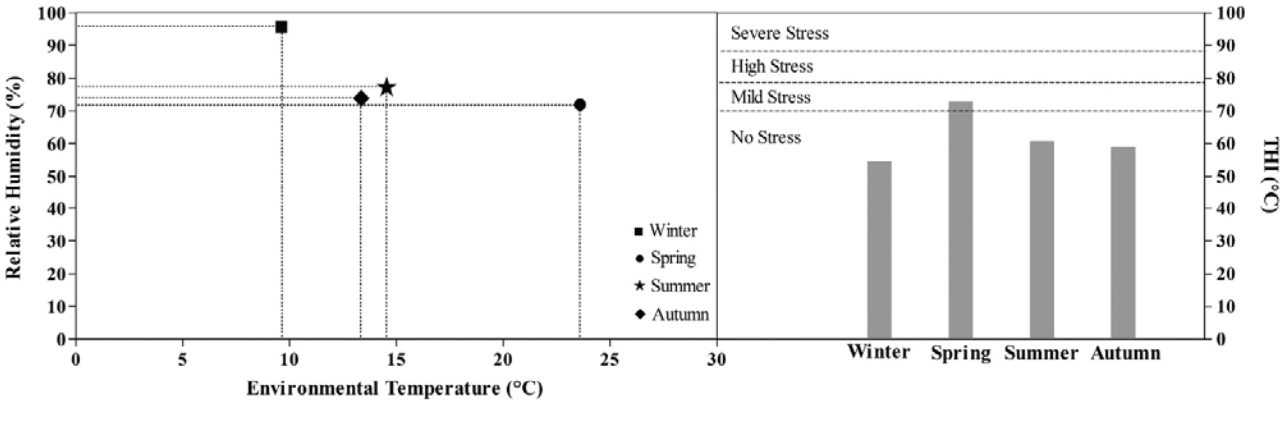

Figure 1 - Means values of environmental temperature, relative humidity and temperature-humidity index (THI) recorded during the different seasons (winter, spring, summer and autumn).

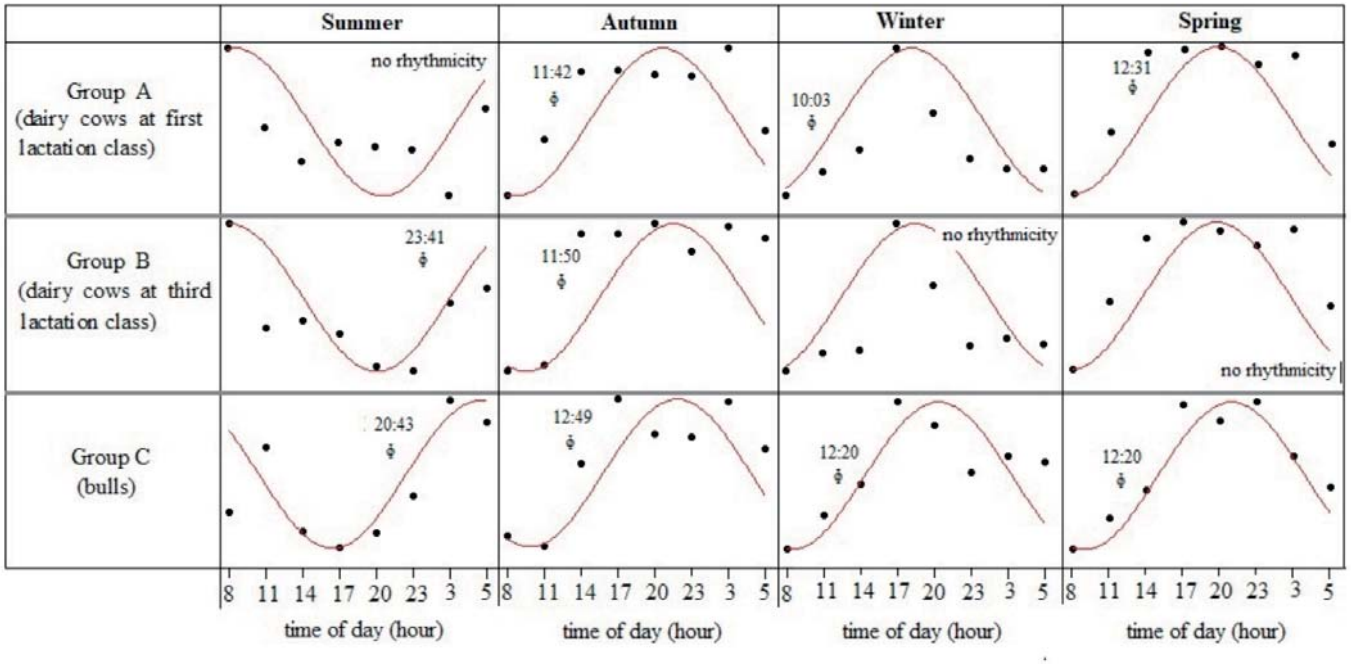

Figure 2 - Acrophase of alpha amylase daily rhythm observed in the three investigated groups (Group A - dairy cows at first lactation class; Group B - dairy cows at third lactation class; Group C - bulls) during the four seasons.

The application of two-way ANOVA showed a significant effect of season ($F_{(3,60)} = 54.25$; $p < 0.0001$), gender and productive status ($F_{(2,60)} = 13.18$; $p < 0.0001$) on alpha amylase. In particular, Bonferroni's post hoc comparison showed statistically higher alpha amylase value during summer season than spring, winter and autumn in groups A and B. In group B the statistically lower alpha amylase value was observed in spring respect to summer, winter and autumn. In group C a statistically lower alpha amylase value was found in spring than winter. In all groups, the alpha amylase value was higher in winter than in autumn. During the summer season, group C showed a statistically lower alpha amylase value than the other two groups. During spring season, group A showed a statistically higher alpha amylase value than the other two groups (Table 1).

A daily rhythmicity of alpha amylase was observed in autumn, winter and spring in group A, whereas the alpha amylase concentration obtained by cow belonging to group B showed a daily rhythmicity in summer and autumn. In group C daily rhythmicity of alpha amylase was observed in all seasons. The acrophase was always diurnal (Figure 2), and a percentage of robustness upper 60% was recorded (Figure 3).

DISCUSSION

Our findings could be useful data for the evaluation of the animals' adaptive mechanisms to environmental modifications throughout the year. Ambient temperature between 5 and 25°C is considered the ideal ambient temperature for cow[20]. In the current study the ambient temperature and THI were within the critical zone during all seasons, except during the spring season, where these were upper that.

In physiological conditions, pancreatic exocrine secretion is closely correlated to intestinal motility; both are regulated by a complex interplay of hormonal and neural mediators to achieve optimal digestion and absorption of food and to maintain the physiological intraluminal milieu in the inter-digestive state. Moreover, morphological and functional parameters of the pancreas as well as intestinal motility are modulated by circadian rhythm and/or affected by the wake-sleep cycle[16].

In our study, all groups were kept in pasture and were fed *ad libitum*. Alpha amylase showed a daily rhythmicity influenced by gender and productive conditions. In particular, bulls were the only group in which daily rhythmicity of alpha amylase was observed in all seasons. Moreover, in bulls the acrophase of daily rhythm of alpha amylase was recorded in the middle of the photophase during autumn, winter and spring. Noteworthy, a nocturnal acrophase in summer season, precisely three hours after the sunset, was observed in bulls. This result needs a deeper investigation in order to clarify the reason of the mostly nocturnal activity found in bulls. Different daily fluctuations of alpha amylase were recorded in dairy cows, with an influence of age and stage of lactation. In particular, in dairy cows at the first lactation class a disruption of alpha amylase

Table 1 - Mean ± standard deviation of serum alpha amylase expressed in U/L recorded in the three experimental groups (Group A, dairy cows at first lactation class; Group B, dairy cows at third lactation class; Group C, bulls) at the same time of day (8:00) during the four seasons.

	Autumn	Winter	Spring	Summer
Group A	33.58±7.00	75.43±11.38	63.65±8.88	148.24±57.55
Group B	41.09±4.73	77.46±19.91	7.70±1.72	142.33±33.53
Group C	38.58±5.37	74.56±18.37	27.01±4.78	51.64±13.91

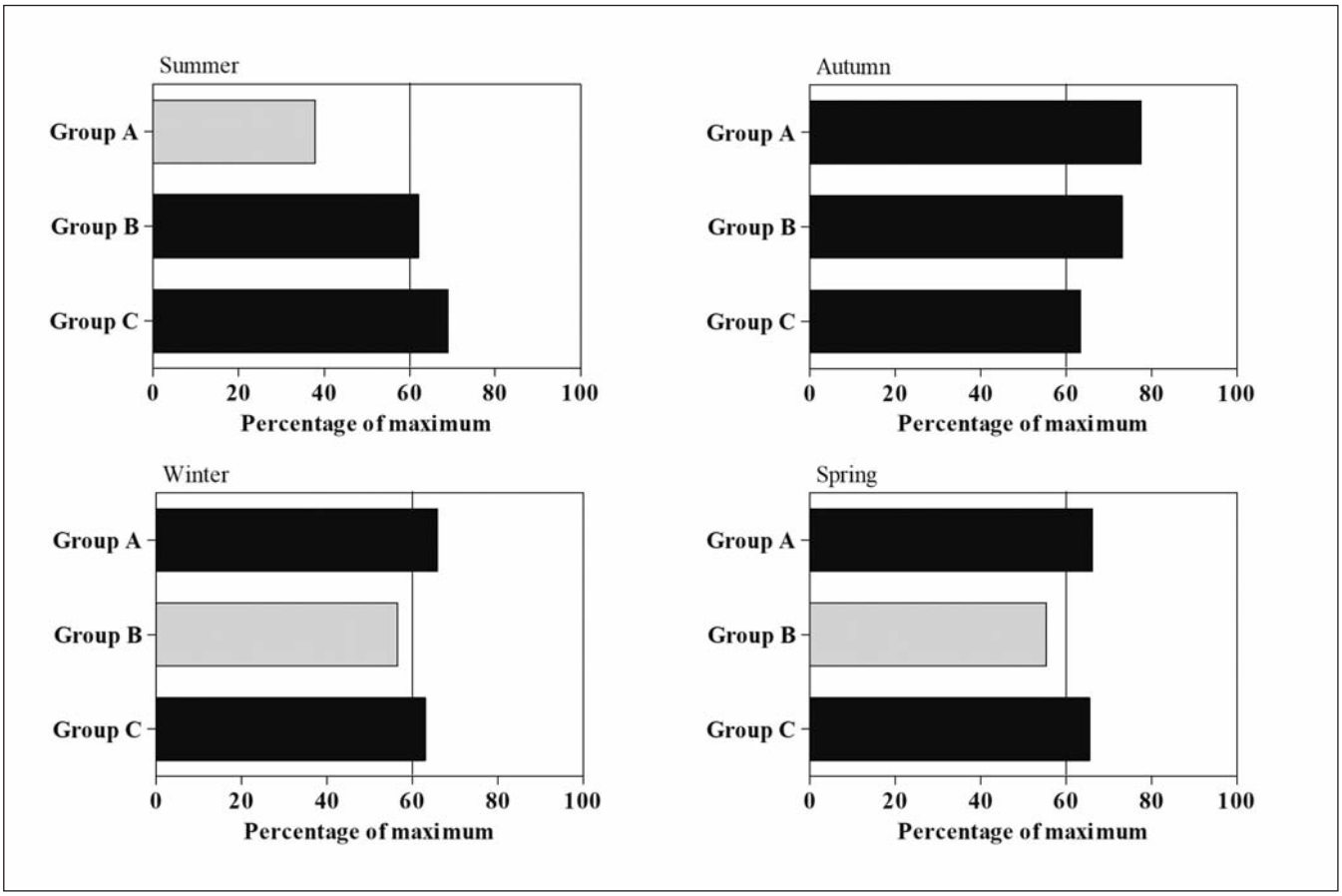

Figure 3 - Robustness of alpha amylase daily rhythm observed in the three investigated groups (Group A - dairy cows at first lactation class; Group B - dairy cows at third lactation class; Group C - bulls) during the four seasons. Vertical line indicates the noise level of 60% index of statistically significant rhythmicity.

daily rhythmicity was observed during summer season (mild lactation).

In the other three phase of lactation, acrophase was observed at five hours after sunrise.

In the dairy cows at the third lactation class, the disruption of alpha amylase daily rhythmicity was observed both during dry period and early lactation. Also, in this group, a nocturnal acrophase was recorded in the mild of lactation, five hours after sunset.

The only period in which the acrophase of alpha amylase daily rhythm was similar to the other groups was at the end of lactation in autumn.

CONCLUSION

According to the findings gathered in the present survey it can be concluded that various factors such as environmental conditions and productive levels influence the serum pancreatic alpha amylase concentration in Holstein cattle. This study provides new insights to plan properly the blood sampling schedule to assess the concentration of this metabolite according to the environmental conditions, sex and reproductive state of the animal. The knowledge of the temporal pattern of alpha amylase in relation to productive performance gives useful information about the time and the effect of administration of exogenous alpha amylase in cows. Also, this knowledge contributes to clarify the influence of the effect of some drugs on the alpha amylase secretion and activity.

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Author Contributors

Conceptualization, R.D.C., G.P., and M.C.S.; Methodology, C.G., F.A., and E.G.; Software, C.G.; Formal analysis, F.A., and C.G.; Investigation, G.P., and R.D.C.; Resources, M.C.S., and E.G.; Data curation, C.G., and F.A.; Writing original draft preparation, G.P.; Writing-review and editing, G.P., and C.G.; Visualization, R.D.C., M.C.S., and E.G.; Supervision, G.P.; Project administration, G.P. All authors have read and agreed to the published version of the manuscript.

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Ethical approval

No approval from Ethics Committee was required. No invasive medical procedures were executed to perform the study. The study was performed with the consent of
the animals' owner during the routinary clinical activity. Animal care and procedures are in accordance with the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal experiments (National law: D.L. 26/2014).

Data Availability Statement

The data will be available by sending an email to the corresponding author.