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Annual changes of some metabolical parameters in dairy cows in the Mediterranean area

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ABSTRACT

The seasonal rhythms reflect the ability of the endogenous adaptive mechanism to react in advance to the regular environmental changes associated with the seasons. It seems that these biochemical parameters are often influenced by a change in physiological status. The aim of this study was to assess the trend of annual variations of some biochemical parameters in dairy cows. The experimental subjects were ten dairy cows and the start of the experimental period coincided with the last month of pregnancy. These animals were monitored for one year and blood samples were collected every month. From the obtained sera, total bilirubin, blood urea nitrogen (BUN), creatinine, total cholesterol, non-esterified fatty acids (NEFA), tryglicerides, β-hydroxybutyrate, total proteins, calcium and phosphorus were assessed by means of an automated spectrophotometer. All the results were expressed as mean ± standard deviation (SD). Data were normally distributed ($P<0.05$, Kolmogorov-Smirnov test). One method of repeated measurement, the Analysis of Variance (ANOVA), was applied to determine the effect of time during the experimental period (12 months) and P values of <0.05 were considered statistically significant. Using cosinor-rhythmometry, three rhythmic parameters were determined: mesor (mean level), amplitude (half the range of oscillation) and acrophase (Φ, time of peak). A significant effect of time ($P<0.0001$) was shown for all the parameters, except for NEFA. After application of cosinor rhythmometry, only total bilirubin, creatinine, triglycerides and β-hydroxybutyrate showed a seasonal rhythm. In conclusion we can affirm that our results supplement the current information available about the circannual changes in the metabolic activity of these animals and help us to evaluate the adaptation of these animals to environmental changes, together with the metabolic effect of calving and lactation.

Key words: metabolism, dairy cows, annual rhythm, blood parameters, pregnancy, lactation

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Introduction

Most long-lived species, including ruminants, exhibit seasonal cycles of physiological functions in order to cope with seasonal fluctuations in climate and food availability (DUARTE et al., 2010; PATKOWSKI et al., 2006). These seasonal rhythms are reflected in the ability of the endogenous adaptive mechanism to react in advance to regular environmental changes associated with the seasons (PICCIONE et al., 2009). It is well known that in mammals, seasonal timekeeping depends on the generation of a signal reflecting day-length (FELSKA-BLASZCZYK, 2005; PALA and SAVAS, 2005; HAZLERIGG et al., 2004).

In sheep and hamsters, melatonin is indicated as being responsible for mediating control of seasonal changes by gonadotropin secretion through pituitary gland activity, and acts within the pars tuberalis (LINCOLN et al., 2006). A study conducted on rabbits demonstrated that there is a temporal pattern for some biological variables, which is very helpful for identification of the elements, which could provide adaptation indicators in animals reared using different systems (PICCIONE et al., 2002).

In ruminants, photoperiod-dependent changes in the duration of nocturnal melatonin secretion by the pineal gland synchronize circannual rhythms to the time of year (LINCOLN et al., 2006).

Different studies have investigated the more frequent pathologies and their relationship with some physiological stages and performance in calves (EL SHERIF and ASSAD, 2001; BABU et al., 2009). Various factors, such as the housing environment, demands for higher productivity, high herd densities and individual susceptibility, can predispose cows to several disorders (NISHIMORI et al., 2006). As observed in goats, sensitivity to several diseases appears to be higher during the winter season (AL BUSAIDI et al., 2008). Such investigations demonstrate how animal welfare is increasingly of interest, especially regarding biochemical response, which fluctuations are shown to reflect the physiological conditions in an animal and results in an important diagnostic tool to assess animal health (ALBERGHINA et al., 2010; ANTUNOVIC et al., 2002). Biochemical determination of serum constituents can provide valuable information relating to nutrition, sex, age and physiological status of the animal (OSMAN et al., 2003). Moreover, it seems that these biochemical parameters are often influenced by a change in physiological status, and, as also observed in goats and sheep (PICCIONE et al., 2009; PICCIONE et al., 2012), it seems that there is a close relationship between blood parameters and the season.

On the basis of these considerations, the aim of this study was to assess the trends in annual variations in some biochemical parameters in dairy cows.

Materials and methods

The experimental subjects were ten dairy cows (Bruna Italiana breed, multiparous, mean weight 450 ± 30 kg) and the start of the experimental period coincided with the last month of pregnancy. All subjects were clinically healthy, and free from internal and external parasites before and during the study. Their health status was evaluated every month, before the blood sampling, based on behaviour, rectal temperature, heart rate, respiratory profile, cough, nasal discharge, ocular discharge and haematological profile. The animals were housed in Sicily, Italy (Latitude 38° 8' N, Longitude 15° 11' E, Altitude 50m above sea level) in a sheltered pen exposed to natural photoperiod and environmental conditions (Fig. 1). The natural photoperiod in this region varies from L/D (light dark ratio) 15/9 at the summer solstice to L/D 10/14 at the winter solstice. The area is characterized by an annual rainfall of 51.5 mm (range: 5-98), generally occurring in autumn and winter. Mean annual maximum and minimum temperatures are 33 °C in August and 10 °C between January and February, respectively, with relative humidity between 69 and 73% through the year. Ambient temperature and relative humidity were continuously recorded, during all the experimental period, with a data logger (Gemini, Chichester, West Sussex, U.K.). The temperature Humidity Index (THI) was calculated successively using the following equation:

$THI_{[°C]} = t_{bs} - (0.55 - 0.55\ \varphi/100)\ (t_{bs} - 14.4)$

Where t_{bs} = dry-bulb temperature (°C) and φ = Relative Humidity (%) (Fig. 1).

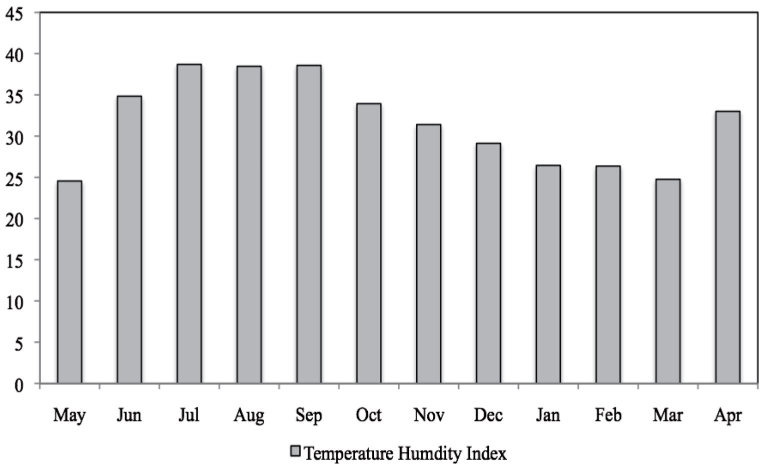
All the animals were kept in a stanchion barn, with free access to water and good-quality alfalfa hay. Concentrate (oats 23%, corn 36%, barley 38% and mineral and vitamin supplement 3%) was provided once daily. Protocols of animal husbandry and experimentation followed the regulations applicable in Italy.

Blood samples were collected through an external jugular venipuncture, using vacutainer tubes (Terumo Corporation, Japan) with no additive, every 30 days at the same hour (09:00) for one year (12 months).

The sera, obtained by means of centrifugation at 3000 g for 15 minutes, were divided into two aliquots and the subsequent analyses were performed twice. So, total bilirubin, BUN, creatinine, total cholesterol, NEFA, tryglicerides, β-hydroxybutyrate, total proteins, calcium and phosphorus were assessed by means of an automated spectrophotometer (Slim, SEAC, Firenze).

All the results were expressed as mean ± standard deviation (SD). Data were normally distributed (P<0.05, Kolmogorov-Smirnov test). One method for repeated measurement, the Analysis of Variance (ANOVA), was applied to determine the effect of time during the experimental period (12 months) and P values <0.05 were considered statistically significant. Data were analyzed using the software STATISTICA 7.0 (StatSoft Inc., USA). Using cosinor rhythmometry (NELSON et al., 1979), three rhythmic parameters were determined: mesor (mean level), amplitude (half the range of oscillation) and acrophase (Φ, time of peak).

a.



b.

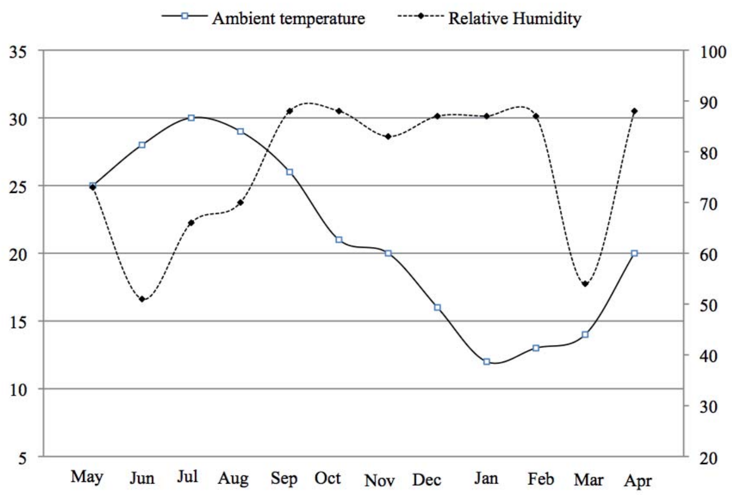


Fig. 1. Trends of: a. Temperature humidity index (THI), b. Ambient temperature and Relative humidity, expressed in their relative units of measurement, recorded during the experimental period.

Results

Table 1. Mean values (± SD) of some haematochemical parameters analyzed in 10 dairy cows, during 12 months, expressed in their relative units of measurement

Month	Bilirubin (mg/dL)	BUN (mg/dL)	Total proteins (g/dL)	Creatinine (mg/dL)	Total cholesterol (mg/dL)	NEFA (mmol/L)	Triglycerides (mg/dL)	β hydroxybutyrate (mmol/L)
May	0.13 ± 0.06	35.00 ± 5.48	8.22 ± 1.07	1.07 ± 0.10	155.60 ± 17.38	0.10 ± 0.00	17.50 ± 4.20	0.59 ± 0.16
Jun	0.11 ± 0.03	33.10 ± 5.53	7.85 ± 0.35	1.33 ± 0.15 [a]	183.10 ± 16.90[a]	0.12 ± 0.04	21.30 ± 3.59	0.68 ± 0.15
Jul	0.28 ± 0.05[a]	24.90 ± 4.79 [a b]	7.77 ± 0.68	1.22 ± 0.10 [a]	146.60 ± 15.53[b]	0.10 ± 0.00	14.80 ± 2.20[b]	0.74 ± 0.11
Aug	0.07 ± 0.01 [a c]	29.20 ± 3.55	8.20 ± 0.41	1.38 ± 0.08 [a c]	156.00 ± 15.26[b]	0.20 ± 0.00	15.40 ± 2.17[b]	0.44 ± 0.12 [bc]
Sep	0.07 ± 0.01 [a c]	36.24 ± 3.65 [cd]	7.55 ± 0.27	0.88 ± 0.06[abcd]	145.75 ± 12.55[b]	0.10 ± 0.00	14.50 ± 3.33[b]	0.54 ± 0.09
Oct	0.21 ± 0.05 [abd]	27.23 ± 4.52 [ae]	8.37 ± 0.34 [e]	0.93 ± 0.07[bcd]	153.78 ± 17.69[b]	0.12 ± 0.04	17.56 ± 1.42	0.40 ± 0.08 [bc]
Nov	0.07 ± 0.02 [acf]	23.80 ± 2.97[abe]	7.54 ± 0.42 [f]	23.80 ± 2.97 [abe]	169.60 ± 12.92	0.10 ± 0.07	11.70 ± 2.83 [abf]	0.52 ± 0.09
Dec	0.08 ± 0.10 [cf]	37.20 ± 3.3 [dgf]	7.36 ± 0.38[adf]	37.20 ± 3.33[cdgf]	170.50 ± 11.94	0.10 ± 0.00	12.20 ± 1.69 [abf]	0.65 ± 0.08 [f]
Jan	0.06 ± 0.01 [acf]	30.60 ± 4.77	7.83 ± 0.31	30.60 ± 4.77	185.90 ± 17.67[acdef]	0.10 ± 0.00	12.50 ± 1.84 [abf]	0.52 ± 0.09
Feb	0.05 ± 0.02 [abcf]	35.20 ± 4.10 [cgf]	7.15 ± 0.54[adf]	35.20 ± 4.10[cgf]	157.10 ± 15.67[bi]	0.10 ± 0.00	8.30 ± 0.67[abcdefi]	0.64 ± 0.37 [f]
Mar	0.06 ± 0.01 [ack]	32.30 ± 5.42 [cg]	7.59 ± 0.34	32.30 ± 5.42 [cg]	157.61 ± 14.72[i]	0.30 ± 0.12	13.30 ± 1.06 [abfk]	0.74 ± 0.06 [df]
April	0.13 ± 0.04 [acekl]	34.60 ± 5.04 [cfg]	7.96 ± 0.39 [k]	34.60 ± 5.04[cfg]	178.60 ± 26.76[ce]	0.14 ± 0.05	17.90 ± 3.78[bghkl]	0.75 ± 0.11 [df]

Significances: [a] vs May, [b] vs June, [c] vs July, [d] vs August, [e] vs September, [f] vs October, [g] vs November, [h] vs December, [i] vs January, [k] vs February, [l] vs March

The results are presented in Tables 1 and 2 as means (± SD), expressed in their relative units of measurements. A significant effect of time ($P<0.0001$) was shown for all the parameters considered, except for NEFA. In particular, all the parameters showed a significant decrease at the end of the experimental period when compared with the beginning (May and June). After application of cosinor rhythmometry, only total bilirubin, creatinine, triglycerides and β-hydroxybutyrate showed a seasonal rhythm. The three rhythmic parameters determined are presented in Table 3.

Table 2. Mean values (±SD) of some serum electrolytes analyzed in 10 dairy cows, during 12 months, expressed in their relative units of measurement

Months	Ca (mg/dL)	P (mg/dL)
May	5.65 ± 0.25	5.85 ± 0.95
Jun	9.67 ± 0.27^{a}	6.32 ± 1.00
Jul	8.94 ± 1.35^{a}	4.31 ± 0.80$^{a\,b}$
Aug	9.61 ± 0.54^{a}	5.51 ± 0.88
Sep	8.08 ± 2.81^{abd}	4.56 ± 0.59^{b}
Oct	10.12 ± 0.37^{ae}	5.63 ± 0.68^{c}
Nov	5.88 ± 0.17^{bcdef}	5.46 ± 0.82
Dec	5.59 ± 0.20^{bcdef}	4.68 ± 0.92^{b}
Jan	5.88 ± 0.17^{bcdef}	6.38 ± 0.59^{ceh}
Feb	9.16 ± 0.42^{agh}	6.25 ± 0.63^{ceh}
Mar	9.66 ± 0.60^{aghie}	4.74 ± 0.65^{bik}
April	9.67 ± 0.32^{aghie}	5.19 ± 1.11

Significances: ^{a} vs May; ^{b} vs June; ^{c} vs July; ^{d} vs August; ^{e} vs September; ^{f} vs October; ^{g} vs November; ^{h} vs December; ^{i} vs January; ^{k} vs February; ^{l} vs March

Table 3. Mesor (M), with 95% confidence interval (CI), amplitude (A) and acrophase (Φ), expressed in months, with 95 % CI and F and P values, resulted from ANOVA application, of the parameters showing a seasonal periodicity during 12 months of study in dairy cows

Parameter	Mesor	CI 95%	A	Φ	CI 95%	F	P<
β-hydroxybutyrate	0.60	0.54 - 0.65	0.12	1 April	(7 June -18 Jan.)	5.02	0.0001
Total bilirubin	0.12	0.08 - 0.15	7.03	14 July	(30 Oct. - 28 April)	4.54	0.0001
Creatinine	1.14	1.07 - 1.21	0.13	12 June	(28 Aug. - 28 Mar)	4.59	0.0001
Triglycerides	14.74	13.06 - 16.42	3.42	16 June	(12 April - 25 Aug.)	5.01	0.0001

Discussion

Our results demonstrated that the metabolic parameters of dairy cows are influenced by a rhythm lasting one year. All the acrophases of the significant parameters were during the late spring-early summer, which corresponds with the peri-partum period/ early lactation. In particular, in our research, an annual change was observed for β-hydroxybutyrate. This is synthesized from absorbed butyrate in the rumen epithelium of ruminants and by the ketogenesis of the liver cells in the conversion of long-chain fatty acids from fat mobilization (LARSEN and NIELSEN, 2005). The β-hydroxybutyrate levels in our study were higher than those observed as reference and showed a significant rhythmicity with the acrophase in early April. We can relate this behaviour to both the feeding and management schedule of the dairy cows during the prepartum period, which was observed by other authors to affect some metabolic components, and to the environmental changes characteristic of early spring that may influence the liver clock genes (LINCOLN et al., 2006; TERAZONO et al., 2003; JANOVICK et al., 2011)

A significant periodic rhythmicity was observed for total bilirubin. This parameter seems to be influenced by parturition, because its change is principally due to adaptation by the hepatic function to the new metabolic status (KANEKO et al., 1997). In fact during the delicate period of postpartum and lactation, the cow is often in a state of negative energy balance (BERTONI et al., 2008). Moreover, the acrophase of this parameter was recorded in April, which corresponds to the early changes in the dark/light cycle. As shown by other authors, who investigated annual rhythms in rats, it seems that the liver is a rhythmic organ whose activity is directly related to the environment (STOKKAN et al., 2001). Nevertheless, high ambient temperature and relative humidity have been shown to influence the metabolic functions, especially for dairy cattle (RASOOLI et al., 2004).

Triglycerides and creatinine both showed their acrophase in the middle of June. Triglycerides are another liver-derived parameter, as well as bilirubin, is influenced by the light conditions, but the acrophase in this case is delayed and coincides with the parturition month. For this reason, the higher values of this parameter could be due to mobilization from liver related to the metabolic demands of calving (BUSATO et al., 2002). In contrast, creatinine is a parameter strictly dependent on kidney function and health. It was observed that the melatonin signal has a protective role in kidney and liver function, and, in a study conducted on cows, it was observed that lower values for melatonin serum content were recorded at the summer solstice, which is few days after the acrophases of both liver and kidney parameters (MEKI and HUSSEIN, 2001). In this case the parturition is a stressful event for kidney functionality and the reduced level of creatinine caused a peak in this parameter, even if our values are within the reference range for this species (KANEKO et al., 1997).

Conclusions

In conclusion we can affirm that our results supplement the information currently available on circannual changes in the metabolic activity of these animals. These results are an important method to assess the health of high producing dairy cows and their adaptation to environmental changes, together with the effect on the metabolism of calving and lactation. Further investigations are necessary to supplement knowledge on ruminants' biological rhythms which meet their metabolic requirements.

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SAŽETAK

Ritam promjene godišnjih doba utječe na endogeni adaptacijski mehanizam kako bi on unaprijed reagirao na sezonske promjene u okolišu. Čini se da promjene u fiziološkom stanju često utječu na biokemijske pokazatelje vezane uz taj mehanizam. Svrha je ovog istraživanja procijeniti utjecaj godišnjih promjena na neke biokemijske pokazatelje u mliječnih krava. U pokus je bilo uzeto 10 mliječnih krava, a sam je pokus započeo kad su one bile u posljednjem mjesecu bređosti. Krave su bile promatrane tijekom jedne godine, a krv im je bila uzimana svaki mjesec. Uzeti uzorci seruma bili su automatskim spekrofotometrom pretraženi na ukupni bilirubin, dušik iz ureje u krvi (BUN), kreatinin, ukupni kolesterol, neesterificirane masne kiseline, trigliceride, β-hidroksibutirat, ukupne bjelančevine te kalcij i fosfor. Rezultati su bili izraženi kao srednja vrijednost ± standardna devijacija (SD). Podatci su bili normalno distribuirani ($P<0{,}05$, Kolmogorov-Smirnov test). Za ponovljeno mjerenje bila je primijenjena analiza varijance (ANOVA) kako bi se odredio učinak vremena tijekom pokusnog razdoblja (12 mjeseci) te su P vrijednosti $<0{,}05$ smatrane statistički značajnima. Uporabom Cosinor ritmometrije određena su četiri ritmička pokazatelja: mesor (srednja vrijednost), amplituda (polovica područja oscilacije) i akrofaza (Φ, vršno vrijeme). Značajan učinak vremena ($P<0{,}0001$) dokazan je za sve pokazatelje, osim za neesterificirane masne kiseline. Nakon primjene cosinor ritmometrije samo su ukupni bilirubin, kreatinin, trigliceridi i β-hidroksibutirat pokazivali sezonski ritam. Zaključno se može potvrditi da rezulati predstavljaju doprinos aktualnim informacijama o godišnjim promjenama metaboličke aktivnosti u krava i pomažu vrednovanju njihove prilagodbe okolišnim promjenama kod teljenja i laktacije.

Ključne riječi: metabolizam, mliječne krave, godišnji ritam, krvni pokazatelji, bređost, laktacija