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Seasonal variations of the serum proteins in sheep and goats (Short Communication)

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Abstract

The aim of this study was to assess the seasonal trend of the serum protein content of sheep and goats. The tested animals were six female goats (Maltese breed) and six female sheep (Valle del Belice breed). All animals were clinically healthy and not pregnant or lactating before or during the study. On blood samples, collected through an external jugular venipuncture every 30 days for 12 months, electrophoresis was performed using a semiautomated AGE system and then electrophoretic curves with the relative protein concentrations were analyzed. One way for repeated measure analysis of variance (ANOVA) was applied to determine the effect of time and by means of cosinor rythmometry, mesor (mean level), amplitude (half the range of oscillation) and acrophase (Φ, time of peak) were determined. The results showed a seasonal rhythm on Albumin and Alumin/Globulin ratio for sheep and goats, with different acrophases, winter for goats and spring for sheep. A seasonal rhythm was shown also in Alpha 2 globulins by sheep and in Beta globulins by goats. The difference in the acrophase can be attributed to a different production pattern of melatonin in goat, so the liver production of albumin is major during the winter that has a longer scotophase.

Keywords: seasonal trend, serum protein content, sheep, goats, electrophoresis

Zusammenfassung

Jahreszeitliche Schwankungen der Serumproteine bei Schafen und Ziegen (Kurzmitteilung)

Ziel dieser Studie war es, die jahreszeitlichen Schwankungen des Serumproteins von Schafen und Ziegen zu bewerten. Die Versuchstiere - sechs weibliche Malteser Ziegen und sechs weibliche Valle del Belice Schafe - waren vor und während der Studie klinisch gesund sowie nicht tragend oder säugend. Über einen Zeitraum von 12 Monaten wurden alle 30 Tage Blutproben durch Punktion der Vena jugularis externa entnommen. Mit Hilfe eines halbautomatischen AGE-System wurde eine Elektrophorese durchgeführt und die elektrophoretischen Kurven der relativen Proteinkonzentration untersucht. Eine Varianzanalyse (ANOVA) wurde durchgeführt, um die Wirkung von Zeit und - mittels Cosinor-Rhythmometrie - Mesor (mittlere Stufe), Amplitude (Hälfte des Schwingungsbereichs) und Acrophase (F, Zeit des Spitzenwerts) zu bestimmen. Die Ergebnisse zeigten jahreszeitliche Schwankungen beim Albumin und Alumin/Globulin-Verhältnis für Schafe und Ziegen, mit verschiedenen Acrophasen, Winter für Ziegen

und Frühling für Schafe. Jahreszeitliche Schwankungen gab es auch für Alpha 2-Globulin bei Schafen und Beta-Globulin bei Ziegen. Der Unterschied in der Acrophase kann auf die unterschiedliche Melatoninproduktion bei Ziegen zurückgeführt werden, weil die Produktion von Albumin in der Leber aufgrund der längeren Dunkelphase im Winter größer ist.

Schlüsselwörter: jahreszeitliche Veränderungen, Serum Eiweißgehalt, Schafe, Ziegen, Elektrophorese

Introduction

Most long-lived species exhibit seasonal cycles of physiological functions to cope with seasonal fluctuations in climate and food availability (Patkowski *et al.* 2006, Duarte *et al.* 2010). The light initiates a cascade of physiological events mediating the input and interpretation of day length to the output of specific hormones that ultimately determine whether animals prepare to develop, reproduce, hibernate, enter dormancy, or migrate (Schneider & Rehbock 2003, Bradshaw *et al.* 2010). These seasonal rhythms reflect the endogenous adaptive mechanism to react in advance to the 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 that reflects daylenght (Felska-Blaszczyk & Brzozowski, Hazlerigg *et al.* 2004, Pala & Savas 2005). Limited information is provided about the seasonal rhythms of small ruminants (Alila-Johansson *et al.* 2001, Alila-Johansson *et al.* 2004, Piccione *et al.* 2007, Piccione *et al.* 2009a). Growing interest is rising about the serum proteins, which concentration reflects the physiological conditions of an animal and results as an important diagnostic tool to assess animal health (Castillo *et al.* 1997, Castillo *et al.* 1999, Antunovic *et al.* 2004, Alberghina *et al.* 2010). Different studies investigated the more frequent pathologies and their relationship with the serum protein variations (Yarim *et al.* 2009, Braun *et al.* 2010). In ruminants, the serum protein contents, which differ between sheep and goat, were assessed as necessary to study metabolic disorders (Alberghina *et al.* 2010, Waziri *et al.* 2010). In fact, as observed in goats, sensitivity to several diseases appears to be higher during the winter season (Al-Busaidi *et al.* 2008). On the basis of these considerations, it was opportune to assess the trend of seasonal variations of serum proteins in sheep and goats.

Materials and methods

The experimental subjects were six female goats (Maltese breed, aged 3±1 years, mean weight 45±3 kg) and six female sheep (Valle del Belice breed, aged 4±1 years, mean weight 68±6 kg). All subjects were clinically healthy and not pregnant or lactating before or during the study. They were kept free from internal and external parasites. 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, appetite, faecal consistency and haematological profile. Animals were housed in Sicily, Italy (Latitude 38° 6′ N, Longitude 13° 20′ E, Altitude 50 m above sea level) into two different pens for species under natural photoperiod and environmental conditions (Figure 1). 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, UK). All the animals had free access to water and to good-quality alfalfa hay. Concentrate (oats 23 %, corn 36 %, barley 38 % and mineral and vitamin supplement 3 %) was provided once daily (200 g per animal per day). Protocols of animal husbandry and experimentation followed applicable regulation 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 total protein concentrations were determined by the biuret method using an automated analyzer (Konelab 20, Dasit, Helsinki, Finland). The protein standard was albumin (5.00 g/dL; Dasit Milano, Italy). Electrophoresis was performed using a semiautomated AGE system (Helena Laboratories, Helena Biosciences, Gatheshade, UK) according to the procedures described by the manufacturer. For each serum sample, 10 µL were applied to numbered sample wells containing Agarose Gel previously prepared. Each gel could accommodate up to 24 samples. Films were electrophoresed for 28 min at 450 V. After electrophoresis, films were simultaneously fixed using an automated system (SAS2, Helena Biosciences), stained in blue stain acid solution (Coomassie Blue Brilliant R250, Helena Biosciences) for 10 min, and then dried at 37 °C. After destaining in acetic acid and drying completely for 15 min films were scanned on a densitometer (Ez-Scan, Helena Biosciences), electrophoretic curves plus related quantitative specific protein concentrations for each sample were displayed. Relative protein concentrations within each fraction were determined as the optical absorbance percentage, and absolute concentrations (g/dL) were calculated using the total protein concentration.

All the results were expressed as mean±standard deviation (SD). Data were normally distributed ($P<0.05$, Kolmogorov-Smirnov test). One way for repeated measure 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 2004). Using single cosinor rhythmometry procedure (Nelson *et al.* 1979), three rhythmic parameters were determined: Mesor (middle value of the fitted cosine representing a rhythm-adjusted mean), expressed in the same conventional unit of the relative parameter, with the confidence interval (C.I.) at 95 %, Amplitude (the distance from the Mesor to the minimum and maximum of the fitted cosine function), expressed in the same unit as the relative Mesor, and Acrophase (Φ. Time of peak value in fitted cosine function), expressed in months with C.I. at 95 %. For each parameter, the mean level of each rhythm was computed as the arithmetic mean of all values in the data set (12 data points). The amplitude of a rhythm was calculated as half the range of oscillation, which on its turn was computed as the difference between peak and trough. The acrophase of a rhythm was determined by an iterative curve-fitting procedure based on the single Cosinor procedure. For each variable for each animal, a cosine wave was fitted to the data points according to the function:

$$Y_t = M + A\cos(\theta_t + \varphi) \quad (1)$$

where Y_t denotes each data pointing the time series, M is the mean level of the rhythm, A is the amplitude, θ_t is the trigonometric angle (in degrees) corresponding to time t, and φ is the angle displacement for the acrophase. The value of φ was determined by iteration: the true value was considered to be the one that produced the smallest sum of squares of the deviations between iterated cosine functions and the raw data.

Table 1
Mesor (M), with 95 % confidence interval (CI), amplitude (A) and acrophase (Φ), expressed in months, with 95 % CI of Albumin, Alpha 2 globulin, Beta globulin and Alb/Glob ratio, that showed a seasonal periodicity during 12 months of study, with the F and *P*-value resulted from ANOVA application.

Parameters	Mesor	CI 95 %	A	Φ	CI 95 %	$F_{(11;5)}$	*P*<
Sheep							
Albumin	36.30	(34.70-37.80)	0.30	2 Jul	(23 Apr-13 Sep)	2.28	0.02
Alpha 2 glob.	8.40	(7.60-9.20)	0.16	12 Mar	(21 May-31 Dec)	5.13	0.0001
Alb/Glob ratio	12.80	(11.80-13.80)	0.19	11 Sep	(29 Jun-20 Nov)	5.25	0.0001
Goat							
Albumin	34.60	(33.20-35.90)	0.28	12 Dec	(7 Oct-15 Feb)	1.97	0.04
Beta glob.	12.50	(11.40-13.50)	0.23	12 Aug	(9 Jun -9 Apr)	4.01	0.0001
Alb/Glob ratio	9.00	(8.40-9.50)	0.15	11 Dec	(1 Nov-22 Jan)	4.05	0.0001

Results

The application of ANOVA showed a significant effect of time during the experimental period on all the variables in sheep, and it showed a significant effect of time on albumin, alpha 2 and beta globulin and albumin/globulin ratio (alb/glob) in goats.

The application of the periodic model and statistical analysis of the cosinor procedure demonstrated seasonal rhythms of Albumin and Alb/Glob both in sheep and goats (Table 1). Alpha 2 globulins showed a seasonal rhythmicity in sheep, whereas beta globulins showed seasonal rhythmicity in goats (Figure 1).

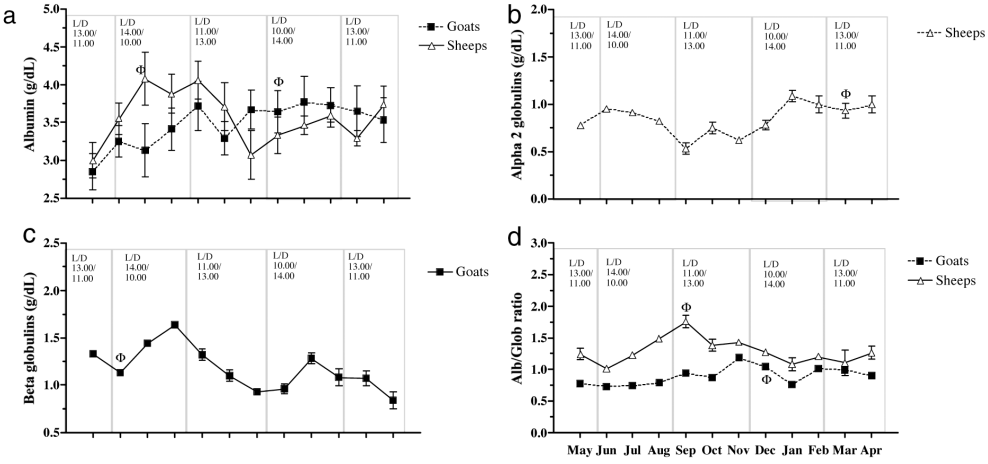

Figure 1
Mean (±SD) serum concentration of Albumin (a), Alpha 2 globulins (b) Beta globulins (c) and Alb/Glob ratio (d), expressed in their conventional units of measurement, obtained in six sheep and six goats, with the acrophase (Φ), during 12 months of study, with the mean light/dark cycle duration (L/D, expressed in hours).

Discussion

A seasonal rhythm was found for the serum proteins in albumin and alb/glob both for sheep and goats with acrophases between July and September for sheep and December for goats. These changes are connected to the changes of light and temperature throughout the year (Alila-Johansson *et al.* 2004). In fact, the hypothalamic pacemaker, directly innervated from retina, results as the principal factor involved in determining circadian rhythms (Moore Ede *et al.* 1986). In normal conditions, albumin variations are affected by numerous factors such as heat haemoconcentration that can take to an increase of its value (Braun *et al.* 2010). In this case, the opposite trend shown for the two small ruminant species is attributable only to a difference, found by other authors, in melatonin pattern. The nocturnal production of melatonin is higher in sheep than in goats (Todini *et al.* 2010). As already demonstrated by other studies in mouse and goat liver, there is a circadian clock in this organ that is not strictly dependent from feeding (Oishi *et al.* 2003, Piccione *et al.* 2008). This peacemaker regulates transcriptional proteins process also, and, in particular, makes this organ like an autonomous peacemaker but its relationship with the effect of melatonin in its regulation is not still clear. On the basis of the present results, it is conceivable that albumin production from liver is strictly dependent on this circadian clock and, maybe, on melatonin release (Alila-Johansson 2001, Piccione *et al.* 2008). This theory is widely demonstrated in the human species, in which transcription factors, like »albumin site D-binding proteins« and others, are present in peripheral tissues and participate as »cell-clock« in controlling regulation of other proteins (Allaman-Pillet *et al.* 2004).

The goat is a diurnal animal with a nocturnal acrophase of melatonin so, the longer scotoperiod (winter) corresponds with the major amount of melatonin production (Refinetti 1999). In sheep, circulating melatonin concentrations seems instead to be independent from photoperiod length and this is due to withdrawal of inhibitory effect of light on melatonin production by the pineal gland in this species (Andresson *et al.* 2005, Molik *et al.* 2006).

The same explanation could be given for the albumin/globulin ratio, because this ratio is strictly dependent from the albumin serum content and so demonstrated the same seasonal pattern in the studied species.

Relatively to the other protein fractions, alpha 2 globulins for sheep and beta globulins for goats, which showed a seasonal trend, these were different from those of albumin and alb/glob ratio and had both the acrophase during the spring-summer season. It is conceivable that these two fractions have a different liver production despite the other fractions and the major serum release corresponds with the hot season, as these are widely within the range of the relative species and that a dehydration is excluded.

This study shed light on the effective seasonal characteristic of the most common small ruminant species, and indicates that an important difference exists between goat and sheep, just dependent on the seasonal »clock«. Further investigations should be conduct to evaluate how the serum proteins synthesis is subjected to change in other small ruminants species and if these changes interest in some manner other liver products.

References

Alberghina D, Casella S, Vazzana I, Ferrantelli V, Giannetto C, Piccione G (2010) Analysis of serum proteins in clinically healthy goats (Capra hircus) using agarose gel electrophoresis. Vet Clin Pathol 39, 317-321

Al Busaidi R Johnson EH Mahgoub O (2008) Seasonal variations of phagocytic response, immunoglobulin G (IgG) and plasma cortisol levels in Dhofari goats. Small Rum Res 79, 118-123

Alila-Johansson A, Eriksson L, Soveri T, Laakso ML (2001) Seasonal variation in endogenous serum melatonin profiles in goats: a difference between spring and fall? J Biol Rhythms 16, 254-263

Alila-Johansson A, Eriksson L, Soveri T, Laakso ML (2004) Daily and annual variations of free fatty acid, glycerol and leptin plasma concentrations in goats (Capra hircus) under different photoperiods. Comp Biochem Physiol A 138, 119-131

Allaman-Pillet N, Roduit R, Oberson A, Abdelli S, Ruiz J (2004) Circadian regulation of islet genes involved in insulin production and secretion. Mol Cell Endocrinol 226, 59-66

Andersson H, Johnston JD, Messager S, Hazlerigg D, Lincoln G (2005) Photoperiod regulates clock gene rhythms in the ovine liver. Gen Comp Endocrinol 142, 357-363

Antunovic Z, Speranda M, Steiner Z (2004) The influence of age and the reproductive status to the blood indicators of the ewes. Arch Tierz 47, 265-273

Bradshaw WE, Holzapfel CM (2010) Light, time, and the physiology of biotic response to rapid climate change in animals. Annu Rev Physiol 72, 147-166

Braun JP, Trumel C, Bezille P (2010) Clinical biochemistry in sheep: a selected review. Small Rum Res 92, 10-18

Castillo C, Hernandez JR, Lopez M, Miranda M, Garcia-Partida P, Benedito JL (1997) Relationship between venous pH, serum calcium and proteins in the course of anoestrus, pregnancy and lactation in the ewe. Arch Tierz 40, 257-263

Castillo C, Hernandez J, Lopez-Alonso M, Miranda M, Benedito JL (1999) Effect of physiological stage and nutritional management on some serum metabolita concentrations in Assaf ovine breed. Arch Tierz 42, 377-386

Duarte G, Nava-Hernandez MP, Malpaux B, Delgadillo JA (2010) Ovulatory activity of female goats adapted to the subtropics is responsive to photoperiod. Anim Reprod Sci 120, 65-70

Felska-Blaszczyk ML, Brzozowski M (2005) Effect of light intensity on reproduction of Polish, Swedish and Danish chinchillas. Arch Tierz, 48, 494-505

Hazlerigg DG, Andresson H, Johnston JD, Lincoln GA (2004) Molecular characterization of the long day response in the Soay sheep, a seasonal mammal. Curr Biol 14, 334-339

Molik E, Misztal T, Romanowicz K, Wierzchos E (2006) The influence of length day on melatonin and prolactin secretion during lactation in aseasonal sheep. Arch Tierz 49, Special Issue, 359-364

Moore-Ede MC (1986) Physiology of the circadian timing system: predictive versus reactive homeostasis. Am J Physiol Regul Integr Comp Phxsiol 250, 737-752

Nelson W, Tong YL, Lee JK, Halberg F (1979) Methods for cosinor-rhythmometry. Chronobiologia 6, 305-323

Oishi K, Koyomi M, Kadota K, Kikuno R, Nagase T, Atsumi G, Ohkura N, Azama T, Mesaki M, Yukimasa S, Kobayashi H, Iitaka C, Umehara T, Horikoshi M, Kudo T, Shimizu Y, Yano M, Monden M, Machida K, Matsuda J, Horie S, Todo T, Ishida N (2003) Genome-wide exprseeion Analysis of mouse liver reveals CLOCK regulated circadian output genes. J Biol Chem 278, 41519-41527

Pala A, Savas T (2005) Persistency within and between lactations in morning, evening and daily test day milk in dairy goats. Arch Tierz 48, 396-403

Patkowski K, Pieta M, Lipecka C (2006) Effect of maintenace system on the reproduction of sheep as well as the level of some morphological and biochemical blood indicators. Arch Tierz 49, Special Issue, 297-304

Piccione G, Caola G, Refinetti R, (2007) Annual rhythmicity and maturation of physiological parameters in goats. Res Vet Sci 83, 239-243

Piccione G, Fazio F, Caola G, Refinetti R (2008) Daily rhythmicity of glycemia in four species of domestic animals under various feeding regimes. J Physiol Sci 58, 271-275

Piccione G, Giannetto C, Casella S, Caola G (2009) Annual rhythms of some physiological parameters in Ovis aries and Capra hircus. Biol Rhythm Res 40, 455-464

Refinetti R (1999) Relationship between the daily rhythms of locomotor activity and body temperature in eight mammalian species. Am J Physiol 277, 1493-1500

Schneider F, Rehbock F (2003) Induction of fertile cycles in the Blackhead sheep during the anoestrus period. Arch Tierz 46, 47-61

StatSoft (2004) Statistica version 7, StatSoft Inc., Tulsa, OK, USA

Todini L, Terzano GM, Borghese A, Debenedetti A, Malfatti A (2010) Plasma melatonin in domestic female Mediterranean sheep (Comisana breed), and goats (Maltese and Red Syrian). Res Vet Sci 90, 35-39

Waziri MA, Ribadu AY, Sivachelvan N (2010) Changes in the serum proteins, hematological and some serum biochemical profiles in the gestation period in the Sahel goats. Vet Arh 80, 215-224

Yarim GF, Ciftci G (2009) Serum protein pattern in ewe with pregnancy toxemia. Vet Res Commun 33, 431-438

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