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Plasmatic protein values in captive adult Iberian red deer stags (*Cervus elaphus hispanicus*)

Research Article

Enrique Gaspar-López[1,2], Jose A. Estevez[2,3,4], Tomás Landete-Castillejos[2,3,4], Francisco Ceacero[2,4], Laureano Gallego[2,3,4], Andrés J. García[2,3,4,*]

[1]*Venadogen S.L.*
02006 Albacete, Spain

[2]*Cynegetic and Cattle Resources, Institute for Regional Development,*
University of Castilla-La Mancha,
02071 Albacete, Spain

[3]*Animal Science Technology Applied to Wildlife Management Research Group,*
Institute for Research into Hunting Resources Sec. Albacete,
02071 Albacete, Spain

[4]*Agricultural Technology and Science and Genetics Department,*
School of Advanced Agricultural Engineering, University of Castilla-La Mancha,
02071 Albacete, Spain

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Abstract: The aim of this study was to assess the time trend of plasmatic proteins in red deer stags. Blood samples were taken monthly from 17 male red deer for 22 months. Total plasmatic determination and protein electrophoresis were performed. Plasmatic proteins showed minimum values during spring and summer and a maximum at the peak of the mating period. Total globulins, β and γ, followed a pattern similar to that observed for total proteins, whereas α1 and α2 globulins showed no seasonal variations. Albumin showed higher values in early spring and summer and lower values at the beginning of autumn, coinciding with the mating season. These seasonal changes in plasmatic proteins should be taken into account when assessing blood protein analysis results.

Keywords: *Albumin • Globulins • Electrophoresis • Proteinogram • Iberian red deer*

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1. Introduction

Blood parameters can be useful for monitoring the health status of deer herds [1], diagnosing subclinical diseases [2] and studying animal physiology [3]. Plasma proteins consist of hundreds of proteins with a wide range of functions and structures that, based on protein electrophoresis, are divided into albumin and globulins. Globulins are divided into α-, β- and γ-globulins [4]. Most plasmatic proteins are produced by the liver, whereas γ-globulins are produced primarily in lymphoid organs [3].

Previous studies [5,6] have found variations in the protein values of cervids because of variations in diet. In other species, such as dogs [7], seasonal variations of total proteins and some fractions of the proteinogram have been described. Similarly, Jeon *et al*. [8] report significant differences in some protein values during antler growth in sika deer (*Cervus nippon*), despite all blood parameters being regulated within a relatively narrow range [4]. The differences reported by Jeon *et al*. [8] were found in a study that only comprised the first 50 days after antler casting, but deer undergo large physiological variations all year round [9]. Accordingly, the aim of this article was to describe the seasonal changes in plasmatic proteins under similar food conditions all year.

2. Experimental Procedures

The current study was performed on 17 male Iberian red deer (*Cervus elaphus hispanicus)* aged six years at the beginning of the study period, and lasted for 22 months. Stags were born and kept all their life at the

* *E-mail: andresjose.garcia@uclm.es*

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Experimental Farm of Castilla-La Mancha University, Spain (38º57'10"N, 1º47'00"W, 690 m altitude). Animals were kept in a 10,000 m^2 fenced enclosure on an irrigated pasture including tall fescue (*Festuca arundinacea,* 50%), cocksfoot (*Dactylis glomerata*, 30%), lucerne (*Medicago sativa*, 15%) and white clover (*Trifolium repens*, 5%). Deer were fed *ad libitum* on barley straw and meal from barley, alfalfa, oat and sugar beets (16% crude protein, 11% humidity, whole meal composition described by Landete-Castillejos *et al*. [10]). The natural photoperiod oscillated between nine (December) and 15 (June) hours of light/day [11].

Blood samples were obtained monthly from the right jugular vein while the animal was standing in a small handling box (2 m × 2 m × 0.9 m), and collected into vacuum tubes containing an anticoagulant (lithium heparin). Sampling was performed at the same time of day (between one and three hours after dawn) to avoid possible variations because of the circadian rhythm. None of the animals showed clinical signs of disease at the moment of extraction and no sedation was required to perform sample collection. Blood was centrifuged at 1,500 *g* for 15 min with a maximum delay of 1 h after extraction, following the indications by Gómez *et al*. [12]. In accordance with these authors, the resulting plasma was decanted directly, deposited into labelled tubes and frozen at –20ºC until analysis.

Total plasmatic protein concentration was determined by the Biuret method using a Vernon photometer. To differentiate protein fractions, serum protein electrophoresis was performed on cellulose acetate membranes in a high resolution buffer at 220 V for 40 min. Membranes were then stained and cleared before reading in a photo densitometer Digiscan Atom 434 (Biotron Scientific Instruments, Spain).

Descriptive analysis for mean and standard error of the mean (S.E.M.) was performed using the statistical software SPSS 15.0.

Handling procedures and sampling frequency were designed to reduce the stress and health risks for subjects, according to European and Spanish laws and current guidelines for ethical use of animals in research [13]. All experimental animal procedures were conducted under the approval of the University of Castilla-La Mancha Animal Ethics Committee (Albacete, Spain).

3. Results

The time trend for total protein, albumin and globulin concentration is shown in Figure 1. Seasonal changes were observed for total proteins, with minimum values in spring (77.4±2.2 g/L in April) and maximum values in early autumn (97.3±5.5 g/L in September). The globulin pattern was similar to that observed for total protein, ranging from 48.1±2.2 g/L (April) to 71.9±6.5 g/L (September). Albumin concentration was higher in early spring (31.0±0.6 g/L in March) and summer (30.6±0.9 g/L in August) and lower in autumn (25.5±1.3 g/L in September).

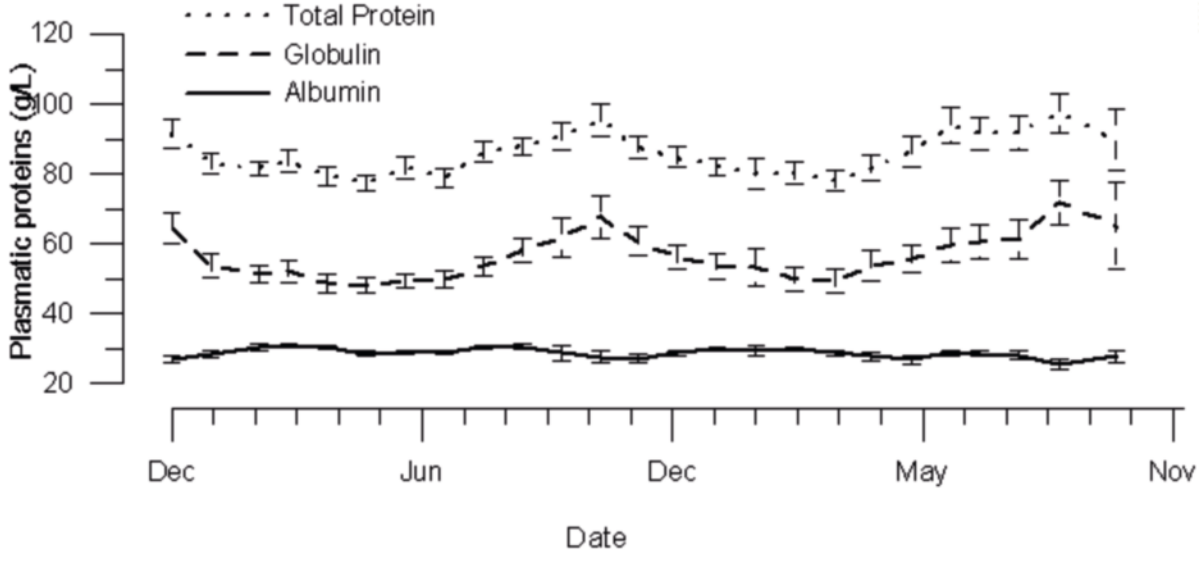

Figure 1. Total protein, globulin and albumin quantity time trend (from top to bottom in both graph and legend; mean ± S.E.M.) in 17 red deer stags (*Cervus elaphus hispanicus*).

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Variations in globulin fractions showed the same pattern as total globulins (Figure 2), especially in the case of γ- and β-globulins, whose values oscillated between 15.7±1.0 g/L (April) and 29.6±3.8 g/L (September) for γ-globulins and 23.2±1.2 g/L (April) and 33.2±2.7 g/L (September) for β-globulins. In contrast, α-globulins remained almost constant all year with a value of approximately 3.0 and 7.5 g/L for α1 and α2 globulins, respectively.

4. Discussion

The range of total proteins is similar to that observed by Marco *et al*. [14], but higher than that reported by Haigh and Hudson [15], who found a range from 51 and 74 g/L. Previous studies have not found seasonal variations in other ruminants such as sheep [16] or white-tailed deer (*Odocoileus virginianus*; [17]). In contrast, DelGuidice *et al.* [18] described seasonal variations in total protein that are similar to the pattern observed in our study. According to these authors, those changes were related to changes in diet, as in other studies [5,19,20]. According to our results, the minimum plasmatic protein values occurred in spring and summer while antler growth was taking place [9]. This decrease in protein values during antler growth was observed by Jeon *et al*. [21] when analysing only the first 50 days after antler casting, and might be a result of the expenses generated by this process [22].

During summer, plasmatic total protein increased, reaching a peak in late summer and early autumn, similar to that observed by DelGiudice *et al*. [18] in adult female white-tailed deer. This peak coincides with the start of the rutting season and the peak in mating [23]. After the mating season, plasmatic protein decreased to minimum values in the following spring, as was observed by DelGiudice *et al*. [18]. According to these authors, this pattern corresponds to variations in diet. In contrast, stags used in this study had free access to a similar diet all year round. Based on this fact, the winter decrease in plasmatic protein could be related to a decrease in voluntary food intake [24] rather than to food availability.

According to our electrophoresis results, variation in total protein was mainly due to globulins, which showed a similar pattern to that observed for total proteins, while albumin followed a different pattern. This presence of protein fractions is different from that described by Waid and Warren [25] in adult female white-tailed deer, in which the pattern for total protein and albumin is similar, but globulins do not show a consistent seasonal trend. However, previous studies [26] have pointed out that the decrease in albumin synthesis might be a response to the increase in globulin synthesis, a statement that agrees with out results.

Albumin patterns during spring and summer show that it reaches high levels at the beginning and the end of this period and lower ones at the end of spring and early summer. This pattern could be because of antler

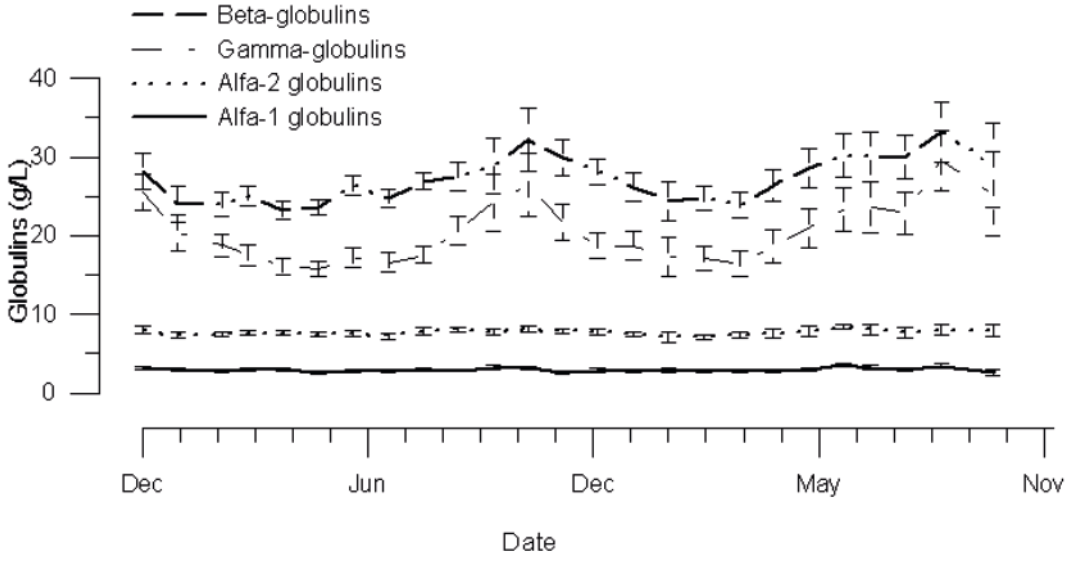

Figure 2. Globulin fractions (α1, α2, β- and γ-globulins) time trend (from top to bottom in both graph and legend; mean ± S.E.M.) in 17 red deer stags (*Cervus elaphus hispanicus*).

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growth, which occurs immediately after the cast of the old antler set in early spring and ends at antler cleaning in summer [9,21]. Accordingly, lower albumin values are observed when antler growth is faster [27] and might reflect the effort expended in this process because antlers are an enriched-protein bone that grows in a short period of time [28].

Globulin fractions showed a similar pattern for γ- and β-globulins, whereas α-globulins remained almost constant all year round. These oscillations are especially relevant when talking about γ-globulins, because high values are understood as a sign of chronic infection or inflammation [3]. The peak of γ-globulins at mating coincides with the increases in leukocytes and platelets observed in a previous unpublished study and may be related to the higher chance of being injured because of the intense aggression and fights between stags during the rut [29].

According to this study, there are seasonal changes in plasmatic proteins that could push their values outside the reference ranges. Obviously, they cannot be considered pathological and should be further studied and taken into account before drawing conclusions from blood analyses.

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