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Complete List of Authors:	MIGLIO, ARIANNA; UNIVERSITA' DEGLI STUDI DI PERUGIA, Facoltà Medicina Veterinaria Perugia, Patologia, Diagnostica e Clinica veterinaria Riondato, Fulvio; University of Torino, Department of Veterinary Science Mangili, Vittorio; University of Perugia, Department of Veterinary Medicine Moncada, Claudia; Unità Sanitaria Locale n°2, Maresca, Carmen; Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Scoccia, Eleonora; Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Tersa, Antoni; Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche,
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Arianna Miglio, Fulvio Riondato[1], Vittorio Mangili, Claudia Moncada[2], Carmen Moresca[3], Eleonora Scoccia[3], Antoni Tersa Maria[2]

Department of Veterinary Medicine, University of Perugia - 06124 - Perugia, Italy

[1] Department of Veterinary Sciences, University of Torino - Torino, Italy

[2] Unità Sanitaria Locale n°2 - Perugia, Italy

[3] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche - Perugia, Italy

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For Correspondance:

Arianna Miglio

Department of Veterinary Medicine, University of Perugia - 06124 Perugia, Italy. e-mail: miglioarianna@libero.it, phone:+390755857610, Fax +390755857606

Abstract

Background: Serum protein electrophoresis (SPE) provides useful information in ruminants. Reference intervals (RIs), however, are dissimilar to those of other species. There have been no reports of SPE RIs for dairy sheep using agarose gel electrophoresis (AGE).

Objective: To evaluate the serum concentration of total protein (TP) and protein fractions determined by AGE in dairy ewes, in order to establish RIs and to assess potential differences between Sarda (S) and Lacune (L) sheep breeds.

Methods: Blood samples were collected from 175 healthy, mid-lactating ewes (119 Lacaune and 56 Sarda ewes), ranging from 2 to 6 years of age. SPE was assessed using a semi-automated AGE system. Measurements of variability and 99% confidence interval were calculated for TP and each protein fraction. Data from S and L sheep were compared.

Results: Significant differences were found between S and L breeds and separate RIs were calculated for TP (7.67-8.24 g/dL; 7.19-7.44 g/dL), albumin (3.40-3.79 g/dL; 3.78-3.98 g/dL), α_1-globulin (0.33-0.38 g/dL; 0.31-0.35 g/dL), α_2-globulin (0.79-0.87 g/dL; 0.74-0.79 g/dL), β_1-globulin (0.24-0.29 g/dL; 0.16-0.20 g/dL), β_2-globulin (0.53-0.73 g/dL; 0.34-0.39 g/dL), γ_1-globulin (1.64-2.18 g/dL; 1.20-1.36 g/dL) and γ_2-globulin (0.33-0.43 g/dL; 0.46-0.56 g/dL) concentrations and for the Albumin/Globulin ratio (A/G:0.77-1.05; 1.11-1.23), respectively. S showed higher values for TP, α_2-, β_1-, β_2- and γ_1-globulins, whereas L had higher values for Albumin, γ_2-globulin and A/G.

Conclusions: AGE gave excellent resolution and accurate results. Seven protein fractions were standardised and RIs for an Italian (Sarda) and a French (Lacaune) dairy sheep breed are reported as a diagnostic aid for clinicians.

Introduction

Overall body protein status is usually assessed by means of the levels of total proteins (TP) in serum, and serum protein electrophoresis (SPE) is the current reference standard for establishing quantitative distribution and fractionation of blood proteins in clinical biochemistry.[1]

Serum proteins have multiple functions and include two major protein fractions: albumin and globulins. Albumin is the main protein of mammal serum constituting 35-50% of the animal TP. It is the most osmotically active protein and maintains the oncotic pressure of the blood. It also serves as carrier for molecules of low water solubility (lipid-soluble hormones, bile salts, unconjugated bilirubin, free fatty acids, calcium, ions and some drugs). [2] Globulins are a heterogeneous group of proteins, which include antibodies (mostly IgG, IgM), inflammatory molecules (alfa1-antitripsin, alfa2-macroglobulin, ceruloplasmin, haptoglobin and amyloid A), hemostatic and fibrinolytic factors (Antithrombin III). They are also carriers of lipids, vitamins, and hormones. [1,3] SPE is commonly used to assess the potential changes in the production and consumption of albumin and globulins. [1,2]

The interpretation of the biochemical constituents of animals is dependent on the availability of reference intervals (RIs) of the parameters as distinct species variations. In some

species (humans, sheep, goat, rabbit, dog and rat), albumin predominates over the globulins, whereas in others (horses and cows), the ratio of albumin and globulins is either nearly equal, or the globulins tend to predominate. As even the electrophoretic mobility of serum proteins differs among species, [5,6] it is essential to determine physiological electrophoretic patterns for each ruminant species. Furthermore, the abnormalities of SPE must be interpreted in the light of the numerous influencing factors not correlated with disease. There is evidence that physiological factors, such as age, body weight, hormones, sexual influences, reproductive phases (pregnancy and lactation), seasonal temperature, nutritional state of the animal (proper and adequate intake of protein materials in the diet) and stress, all affect serum protein levels, especially in ruminant species. [1,7-12]

SPE provides useful information on pathological conditions in ruminants. In sheep, a multitude of diseases, such as liver and kidney dysfunctions, pulmonary diseases, protein-losing enteropathies, local or systemic infections and parasitism, inflammations, ketosis, and fluid loss (dehydration, haemorrhages, massive exudation), can cause shifts in albumin and globulin concentrations. [1,11,13] SPE is, therefore, indicated in ewes with specific or non-specific clinical signs, including poor performance, mastitis, weight loss and diarrhoea. [13] The analysis of the electrophoretic pattern, although not disease specific, can help to evaluate the nature and severity of the pathology and to differentiate between the causes. It also provides means for detecting acute, chronic or sub-clinical disease and monitoring the response to therapy. [14]

The method of protein fractionation on the SPE curve has not been standardised and, depending on the authors and on the support media and technique used, considerable variation is found in the number of protein fractions detected in sheep. Several supporting matrices are available for routine SPE: the most common are acetate cellulose (AC) and agarose gel (AG),

which separate proteins according to the net molecular charge. In recent decades, AC has been replaced by AG, which provides not only greater efficacy and a higher reproducibility of results, but also improved performance and clarity of the electrophoretic pattern. [3,5,15] Agarose gel electrophoresis (AGE) is capable of resolving proteins of serum into 6 to 15 fractions depending on the species. [1]

AC was used in the majority of the reports dealing with ovine serum in physiological [1,4,16] and pathological [11,14] conditions, but only a very few studies used AG. [6,17,18] The latter, in particular, were conducted many years ago on a small number of sheep of different ages, breeds and physiological conditions.

SPE relative and absolute RIs from ewes using AGE under standardized conditions have never been reported so far. Moreover, the differences in SPE RIs among breeds have been described in dogs, [15] but no published data exists for ewes.

In order to maximise the diagnostic value of AGE in sheep, it is essential for clinical laboratories to have access to well-established relative and absolute RIs. The aim of the present study was to investigate the concentration of serum total protein and protein fractions by means of AGE in dairy lactating ewes, in order to establish both relative and absolute RIs and identify possible variations related to breeds. The availability of specific RIs would allow the pathologic modifications of the electrophoretogram to be recognised more easily and could improve the management strategies to help address the nutritional needs of ewes during the lactation phase. RIs were established using the criteria published by the International Federation of Clinical Chemistry and Laboratory Medicine. [19-21]

The study was conducted on two sheep breeds: an Italian breed (Sarda) and a French breed (Lacaune), selected specially for their milk production. The Sarda sheep is an insular

(Sardinian), Italian breed, developed by crossing local lowland sheep with North African sheep. It has become one of the most common and most productive dairy breed despite the harsh environmental conditions. [22] The Lacaune ewe is the most commonly used dairy sheep in France and other countries, due to its high performance and high resistance to mastitis. [23, 24]

Materials and Methods

Blood samples (10 mL) were collected from 175 clinically healthy dairy sheep ranging from 2 to 8 years old (mean 4.4 ± 2.3 SD). 119 Lacaune (L) and 56 Sarda (S), multiparous, mid-lactating sheep were included in the study.

The animals were reared in 3 farms located in the province of Perugia, Region of Umbria (Central Italy) and kept in open-sided barns. All housing and care complied with the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 86/609/EEC (European Economic Community).

The study was carried out in 2012, on 8 different days of a 1-month, experimental period (week 10 to 15 post-partum). All the animals were fed with on mixed hay supplemented with cereal grains, and were milked twice a day in a milking parlour with a milking machine.

A complete physical examination was carried out to check the health status of the ewes, and the results of the CBC and chemistry profiles for the ewes included in the study were within the established RIs for the Clinical Pathology Laboratory of the Veterinary Faculty of Perugia (Italy).

The animals were free from internal and external ~~parasite~~ and had been treated for endoparasites twice a year.

Blood samples were collected from the jugular vein using Vacutainer tubes (Terumo Corporation, Tokyo, Japan) with no additive. Blood samples were allowed to clot at room temperature (25°C) for at least 1 hour and centrifuged at 3000*g* for 10 minutes in order to separate the serum. The serum samples were neither lipemic nor haemolysed; they were refrigerated and electrophoresis was always run within 12 hours after blood collection, following gentle homogenization by vortexing.

Serum Protein electrophoresis

Total protein concentrations were determined by the Biuret method using an automated analyser (Hitachi 904 automatic analyser, Boehringer Mannheim GmbH, Mannheim, GERMANY). The protein calibrator was prepared from human serum (c.f.a.s. calibrator, Roche). The control serum used was Precinorm U (Roche). Electrophoresis was performed using a semi-automated AGE system (Hydragel-Hydrasis, Sebia PN 4100, Calenzano, Florence, Italy) according to the procedure described by the manufacturer (Hydragel 15 Protein Kit, Sebia PN 4100). Ten microlitres of each serum sample were applied to preformed, numbered, sample wells on the agarose films. Each film could accommodate up to 15 samples. The films were electrophoresed for 15 minutes constantly at 90 Volt. Subsequently, the films were simultaneously fixed and stained in amid-black staining solution, and then dried at 37°C. A control serum (control serum P human, Sebia, Calenzano, Firenze, Italy), was included in each run of samples. After de-staining in acetic acid and drying completely, the films were scanned in a densitometer (Epson Expression 1680 Professional Scanner, Epson America Inc., Long Beach, CA, USA). The

electrophoretic curve plus the related quantitative specific protein results for each sample were displayed using the computer software Phoresis (Sebia). The programme identified and verified the protein fraction and a visual inspection of the electrophoretogram corrected it. All samples were analysed by the same person and separation of the various protein fractions were detected using the quality control standard established by Osbaldiston [25] for sheep. The relative protein concentration within each fraction was determined as the optical absorbance percentage (%), and the absolute concentration (g/dL) of the same fractions was calculated from the total serum protein concentration.

Statistical analysis

The mean, minimum and maximum values, the standard error (SE) and standard deviation (SD) of total proteins (TP), albumin (Alb), each globulin fraction and Albumin/Globulin ratio (A/G) were calculated both for Sarda and Lacaune ewes to describe the central location and the spread of the data. The 99% confidence intervals (99% CI) of all parameters were calculated. The Shapiro-Wilk test was used to assess normality. The Wilcoxon-Mann-Whitney *U* test was applied to compare the concentrations of TP, protein fractions and A/G between S and L breeds. Values of $P \leq .05$ were considered statistically significant. All the statistical analyses were carried out using STATA 9.1 software.

Results

The mean values ± SD, SE, 99% CI, minimum and maximum values for the TP concentrations and for the relative and absolute concentrations of protein fractions, including the A/G in Sarda and Lacaune sheep are reported in Tables 1 and 2, respectively. In all sera analysed, albumin,

α_1-, α_2-, β_1-, β_2- and γ_1- and γ_2-globulin fractions were clearly resolved and accurately identified (Figure 1). Figure 1 shows a representative serum protein electrophoretogram observed in a healthy, adult, dairy sheep during the mid-lactation phase.

Albumin was easily identified as a thick band, which highlighted its high serum concentration, homogeneous electric charge, and high staining affinity.

All data were not normally distributed. Significant differences ($P \leq .05$) were present between S and L sheep breeds (Table 3). S showed significantly higher absolute values for total proteins, α_2-globulins, β_1-globulins, β_2-globulins and γ_1-globulins, whereas L had significantly higher absolute values for albumin, γ_2-globulins and A/G ratio. Differences in the concentration of α_1-globulin were undetected, despite it was slightly higher in the Sarda breed.

Discussion

Despite the non-specificity of protein patterns, SPE provides a simple means to evaluate the clinical status of sheep. This technique may serve to identify clinically unapparent disease in sheep prior to transplanting. Moreover, SPE at regular intervals could be useful to monitoring the clinical status of sheep in a herd and the response of the animal to treatment. The dynamic change in the globulin concentrations, especially the alpha and gamma globulin fractions, may be of prognostic significance.

Compared to the electrophoretograms obtained using AC by other Authors [1,4,5,11,14,16,25] our results suggest that AG as a supporting matrix for SPE gives a higher resolution, good reproducibility, improved clarity of electrophoretic bands and a sharper separation of the serum fractions in agreement with previous reports. [17,18,26-28] Furthermore, AG has numerous

advantages: it is non-toxic, easy to perform on a routine basis, it can be made clear for scanning and it can easily be combined with immunofixation techniques. Thus, the use of a Sebia instrument simplified this complex technique and the present study obtained results of great clarity.

We studied total protein concentrations and protein electrophoretic patterns for sheep by using AGE, currently a well-established and diffuse technique for SPE in veterinary medicine.

It is important to underline that the distribution of the protein fractions within a species conforms to the same pattern and this pattern differs in each species, as previously shown. [5] The SPE densitometric trace in sheep, in particular, appears to be unusual compared to those of other species for its noticeably greater length, due to a faster anodal migration of the corresponding zones and a pronounced cathodal migration of the gamma globulin zone which creates a distinctive protein-rich zone. [3,4,15,26,29,30]

The method of dividing protein fractions is pivotal for the interpretation of the results. There are significant differences in fractionation, depending on the method used. Furthermore, technologists could be inconsistent in their subdivision of the electrophoretogram, making arbitrary decisions based on general experience. As a result, not all electrophoretic analysis of serum provides clear, overlapping separations of protein fractions. Moreover, there is no way to guarantee accuracy and quality control using electrophoresis, due to the absence of quantitated standard sera available for the various animal species. Different techniques to fractionate the electrophoretic curve have been described [5,28] and a mandatory standardization of this step is absolutely necessary in order to obtain precise, reproducible results. Our data was ~~been~~ obtained according to the method of Osbaldiston, [25] since it has recently been demonstrated to be an accurate way to identify individual peaks, even using AGE: [29] the distance of migration (mm) of

the albumin from the point of application (d^{alb}) was taken as the reference standard and the relative distances of migrations of the other protein-rich zones (d^{zone}/d^{alb}) were calculated to determine the various protein fractions.

In agreement with Osbaldiston, [25] a visual examination of the SPE results in our study enabled 7 isolated bands (albumin, α1-globulin, α2-globulin, β1-globulin, β2-globulin, γ1-globulin and γ2-globulin) to be the clearly and easily separated in all samples. Thus, we can confirm the ~~e~~as being the ruminant species with the highest number of protein fractions. This characteristic is typical of the adult ~~shee~~since the appearance of the γ2-region and its magnitude in the AG densitometric trace is age-related and becomes evident starting approximately from 9 weeks to 1 year after birth, [16] as it is larger in the older animal. [17]

The actual number of protein peaks in the ovine SPE curve is also a matter of discussion. Literature has shown that the number of separate fractions in sheep ranges from 4 to 8. [1,6,14,16] Using AC as the supporting matrix, different patterns are shown in adult sheep: some authors recognize 1 α-, 1 β- and 1 γ-globulin fractions, [5,11] whereas others recognize 2 α-, 1 β- and 1 γ-globulin fractions.[14] Some authors, in particular, ~~recogniz~~ 2 β- and 1 γ-globulin fractions in lactating ewes, [16] others recognize 1 α-, 2 β- and 2 γ-globulin fractions, [1] others detect 2 α-, 1 β- and 2 γ-globulin fractions [4] and yet others 2 α-, 2 β- and 2 γ-globulin fractions. [25] All previous studies carried out on adult ewes using AGE as the supporting matrix recognized 2 α- and 2 γ-globulin fractions, but only a single β zone. [6,17,18] Thus, our results differ in that ours recognize 2 β zones. A possible reason for this discrepancy is the incorporation of the β2-globulin fraction in the γ1-globulin zo~~n~~as suggested by the higher mean value of the latter compared to our results.

The identification of 2 α- and 2 γ-globulin fractions in all bibliographic data using AGE, including our results, demonstrates the greater ability and good reproducibility of AGE to subdivide the globulin fractions. This could be particularly advantageous and useful for clinicians. For example, the detection of an increased amount of the α2-region can be indicative of an increased production of acute phase proteins (Serum Amyloid A, haptoglobin) in cases of stress and inflammatory status; [1] the increase in the γ1-region is probably observed in cases of IgA, IgM or IgE production, whereas the increase in the γ2-region can be indicative of IgG production in response to the antigenic stimulus during chronic infectious and parasitic diseases. [1, 14] Our results further demonstrate that AGE in sheep is equally able to discriminate two β fractions with good reliability and reproducibility. The increase in the β1 zone could be correlated with the increase not only in transferrin during anaemia, pregnancy, iron deficiency and acute hepatitis, but also in casein-protein during lactation. Conversely, the increase in the β2 zone could be explained by the increase in Igs (IgM, IgA) in the course of infectious diseases, complement and C-reactive protein involved in acute inflammatory status and stress response, and β2-lipoprotein in the course of hepatobiliary disease and colestasis. [1,35]

Interestingly, for the first time we can observe significant differences in the concentration of serum protein fractions and in the A/G ratio between the two sheep breeds (Table 3). Thus, separate RIs for the relative and absolute values of TP and of each serum protein fraction were established for Sarda (Table 1) and Lacaune (Table 2) ewes.

In fact, recent, similar studies have emphasised the importance of breed-specific RIs in dogs and other species. [15,30,31] This could be explained because proteins are synthesised under genetic control and variations in protein between breeds and species are to be expected.

RIs are central to the veterinarian's decision process, as they are used for diagnostic and follow-up purposes. The use of inadequate RIs may lead to erroneous clinical decisions. Normal values for serum protein fractions of sheep reported in literature show a wide variation. Much of this variation depends on the technique used to carry out SPE and on the method used to divide protein fractions. Thus, each laboratory must use a standardised procedure to establish its own normal values. In the present trial, the analysis of serum proteins for lactating sheep was studied to establish RIs by using AGE. As recommended, [19-21] we attempted to obtain a reference sample group as representative as possible of the mid-lactating Sarda and Lacaune populations, by selecting breeders in central Italy to attenuate a potential geographic effect on SPE.

The mean values of TP detected in this study were slightly lower than those reported by Keay and Doxey [17] and higher than those shown in Kaneko.[1]

Only Keay and Doxey [6,17] performed SPE using AGE in healthy sheep. However, they only reported the mean values of protein fractions. [17] Compared to these results, mean albumin concentrations in our trial were consistently higher, whereas all the globulins concentrations were lower (except for β-globulins that were higher in the Sarda sheep breed). These differences could be due to the small number of animals and the different and varied breeds, age and physiological states of the ewes included in the study by these authors.[17]

Our results also disagree with the concentrations of protein fractions reported by Kaneko [1] using AC. The different technique used and the unknown age, sex, breed, physiological phase and nutritional status of the animals could account for the discrepancies. Furthermore, we suppose that this technique induces the overlap of the α2-globulin zone inside the β1-globulin fraction, suggested by the higher mean value of the latter compared to our results.

The A/G ratio is of special interest for clinical pathologists, because it enables a systematic

classification of the electrophoretic profile and identification of dysproteinaemia.[28] A reversal of the A/G due to a decrease in serum albumin and an increase in- and γ-regions may be associated with chronic and subclinical diseases in sheep, such as chronic pulmonary (chronic pneumonia syndrome or lungworm infestations) and liver diseases.[11, 14] The A/G ratios found in the current study are higher than the values reported by Kaneko [1] and lower than those identified by Woolf et al.[7] No data is available regarding the A/G ratio obtained using AGE.

The physiological and reproductive status, which significantly influences metabolism in ruminants and requires varying level of energy and amino-acids according to the different stages of reproduction, should not be underestimated.[12, 32] In particular, the lactation phase strongly affects the protein reserves in the sheep, due to the maximal requirements of the mother. TP shows a significant increase in serum with a higher A/G ratio compared to non-lactating sheep, as a result of the protein catabolism for milk synthesis.[1,4,12,16,33-37]

Moreover, continual physiological changes occur in sheep during the lactation periods (early, mid and end). Albumin is higher in the end phase, [12] α-globulins reach their peaks in the early and mid phases, and β- and γ-globulins decrease during all phases.[16]

Thus, specific RIs for SPE in lactating ewes are required, since TP, protein fractions and A/G are fundamental parameters to investigate the metabolic state in this physiologic period. [12,16,34,37-39]

Our results are in contrast with Piccione et al., [16] who used AC to calculate the mean values of protein fractions in 10 mid-lactating Comisana ewes. We found higher concentrations for TP, Albumin, α-, γ-globulins and A/G and lower values for β1- and β2-globulins. Also in this study, AC did not allow the authors to separate neither α- nor γ-globulins into 2 zones and the α2 and γ1 zones were probably included within the β1 fraction.

In comparison with the data found in 12 lactating Barki ewes under semi-arid conditions, [35] we detected consistently lower concentrations of TP and albumin. Barki ewes maintain a higher A/G ratio for fluid equilibrium as an adaptive response to the higher need for water mobilisation by the blood to the mammary glands for milk production. Thus, even the climatic conditions and the geographic locations could affect the trend of change in plasma proteins and are likely to impact the relevance of general ovine RIs. [1,7,8,21]

Based on the above, the significant differences found in our study between Sarda and Lacaune ewes were probably correlated with breed, since age, the physiological state, nutritional intake and geographical area were the same.

In conclusion, SPE has so far not been sufficiently standardised in sheep. To our knowledge, we present for the first time the RIs for SPE in ewes using AG, with an analysis of a representative number of mid-lactating sheep and with the calculation of absolute and relative values. These results are likely to be of value to clinical pathologists to investigate the health condition of these animals and provide a basis for further investigation into the value of this technique to investigate disease in ewes. AGE of serum proteins in sheep, if properly standardised, could be a useful diagnostic aid for clinicians, since it can be recommended as a qualitative screening procedure to detect abnormalities of the major proteins. Although SPE using AGE is not as sensitive and specific as more modern techniques, it is an easy and cheap technique that can also be performed in field stations in areas of extensive and intensive breeding. Moreover, the results obtained contribute to improving the knowledge of the biochemical processes and electrophoretic profile during the lactation phase in ewes. Our results provide a picture of the protein profile during mid-lactation, which could be considered as guidelines for the management strategies to guarantee nutritional needs and to avoid a decline in

the productive performance of ewes during this physiological phase.

Since these results underline possible differences in concentrations of TP and protein fractions in breeds, further studies are required to explore SPE RIs in other ovine breeds.

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Figure legends:

Figure 1: Agarose gel electrophoresis of serum proteins from a 5-year-old, clinically healthy, mid-lactating Lacaune sheep. Bands on agarose gel are visualised by amido black staining and a densitometer scan of the electrophoretogram is presented.

Table 1: Measurements of variability (mean, standard deviation-SD, standard error-SE, minimum-Min, maximum-Max) and 99% confidence interval for relative (%) and absolute (g/dL) concentrations of serum total protein and protein fractions and for the A/G ratio, obtained by means of agarose gel electrophoresis in mid-lactating Sarda sheep (n=56). AGE: agarose gel electrophoresis, AC: acetate cellulose.

Table 2: Measurements of variability (mean, standard deviation-SD, standard error-SE, minimum-Min, maximum-Max) and 99% confidence interval for relative (%) and absolute (g/dL) concentrations of serum total protein and protein fractions and for the A/G ratio, obtained by means of agarose gel electrophoresis in mid-lactating Lacaune sheep (n=119). AGE: agarose gel electrophoresis, AC: acetate cellulose

Table 3: Wilcoxon-Mann-Whitney test (*P value* <.05). Differences in concentration (g/dL) of serum total protein and protein fractions between Sarda and Lacaune sheep.

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464 **Table 1:**

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Sarda Sheep breed		Mean+SD	Standard error (SE)	Minimum	Maximum	99%CI
Total protein	**g/dL**	7,96±0,80	0,11	6,3	9,8	7,67-8,24
Albumin	**%**	45,82±9,6	1,28	31,2	65,9	42,39-49,24
	g/dL	3,60±0,56	0,07	2,44	4,75	3,40-3,79
α_1-globulins	**%**	4,43±0,75	0,10	2,5	6,1	4,17-4,70
	g/dL	0,35±0,06	0,009	0,23	0,52	0,33-0,38
α_2-globulins	**%**	10,45±1,27	0,17	8,4	17,6	10-10,9
	g/dL	0,83±0,11	0,01	0,63	1,36	0,79-0,87
β_1-globulins	**%**	3,28±0,88	0,12	1,7	5,5	2,96-3,60
	g/dL	0,26±0,08	0,01	0,12	0,45	0,24-0,29
β_2-globulins	**%**	7,71±3,12	0,42	2,8	16,3	6,60-8,83
	g/dL	0,63±0,28	0,04	0,19	1,21	0,53-0,73
γ_1-globulins	**%**	23,44±7,66	1,02	8,8	38,9	20,71-26,17
	g/dL	1,91±0,76	0,10	0,57	3,81	1,64-2,18
γ_2-globulins	**%**	4,86±1,90	0,25	1,5	10,4	4,18-5,54
	g/dL	0,38±0,14	0,02	0,09	0,77	0,33-0,43
A/G		0,91±0,39	0,05	0,45	1,93	0,77-1,05

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470 **Table 2**

Lacaune Sheep breed		Mean+SD	Standard error (SE)	Minimum	Maximum	99%CI
Total protein	**g/dL**	7,31±0,53	0,05	6,2	9,7	7,19-7,44
Albumin	**%**	53,19±5,79	0,53	29,5	63,4	51,80-54,58
	g/dL	3,88±0,42	0,04	2,24	5,63	3,78-3,98
α_1-globulins	**%**	4,52±0,99	0,91	2,6	7,3	4,28-4,75
	g/dL	0,33±0,08	0,007	0,18	0,54	0,31-0,35
α_2-globulins	**%**	10,48±1,16	0,11	8,1	13,7	10,20-10,75
	g/dL	0,77±0,09	0,009	0,58	1,04	0,74-0,79
β_1-globulins	**%**	2,48±0,98	0,09	1,3	13,7	2,24-2,71
	g/dL	0,18±0,07	0,007	0,08	0,68	0,16-0,20
β_2-globulins	**%**	5,01±1,28	0,12	2,5	10,4	4,70-5,32
	g/dL	0,37±0,10	0,009	0,19	0,79	0,34-0,39
γ_1-globulins	**%**	17,39±17,39	0,33	5	28	16,52-18,26
	g/dL	1,29±0,32	0,03	0,32	2,13	1,20-1,36
γ_2-globulins	**%**	6,94±2,52	0,23	2,8	15,3	6,34-7,55
	g/dL	0,51±0,20	0,02	0,19	1,08	0,46-0,56
A/G		1,17±0,25	0,02	0,42	1,73	1,11-1,23

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475 **Table 3**

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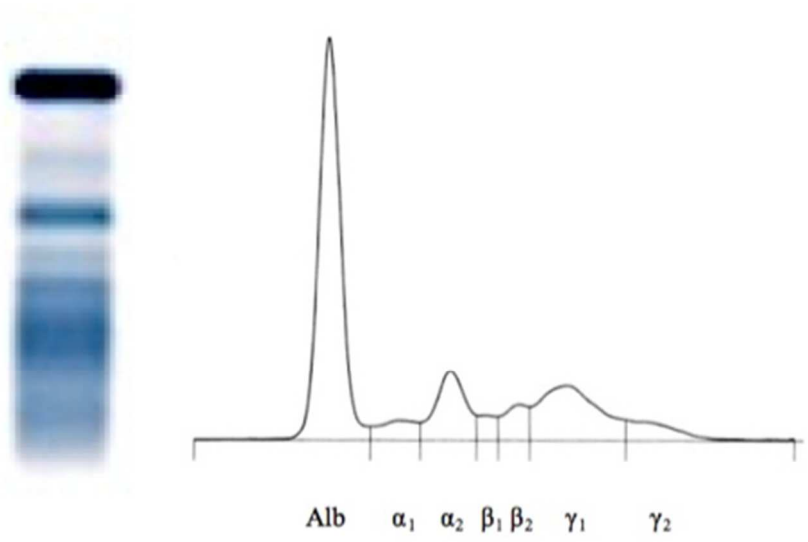
Analyte	Sarda	Lacaune	*P*-value
Total protein (g/dL)	7,96±0,80	7,31±0,53	**0,0000**
Albumin (g/dL)	3,60±0,56	3,88±0,42	**0,0007**
α_1-globulins (g/dL)	0,35±0,06	0,33±0,08	0,0602
α_2- globulins (g/dL)	0,83±0,11	0,77±0,09	**0,0001**
β_1-globulins (g/dL)	0,26±0,08	0,18±0,07	**0,0000**
β_2-globulins (g/dL)	0,63±0,28	0,37±0,10	**0,0000**
γ_1-globulins (g/dL)	1,91±0,76	1,29±0,32	**0,0000**
γ_2- globulins (g/dL)	0,38±0,14	0,51±0,20	**0,0000**
A/G	0,91±0,39	1,17±0,25	**0,0000**



Agarose gel electrophoresis of serum proteins from a 5-year-old, clinically healthy, mid-lactating Lacaune sheep. Bands on agarose gel are visualised by amido black staining and a densitometer scan of the electrophoretogram is presented.
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