Usefulness of serum protein electrophoretic pattern in the assessing the body homeostasis maintenance in sheep and goat housed in different management conditions

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ABSTRACT

The study aimed to evaluate the total plasma proteins of sheep and goats subjected to two different management conditions. A total of 40 clinically healthy, pluriparous animals were selected: 10 Maltese goats and 10 Comisana sheep from Farm A; 10 Maltese goats and 10 Comisana sheep from Farm B. Animals from Farm A were housed in a barn with access to an outdoor pen. Animals from Farm B grazed on improved natural pasture characterized by a botanical composition typical of Mediterranean semi-natural grasslands. Blood samples were collected from all animals via jugular venipuncture and the serum total proteins together with serum protein fractions (i.e. albumin, α-globulins, β1-globulins, β2-globulins, and γ-globulins) were assessed. Statistical analysis showed significant higher values of serum total proteins in pasture-raised sheep compared to those housed in a barn with access to an outdoor pen ($P < 0.05$), higher β2- and γ-globulin content in sheep housed in a barn with access to an outdoor pen compared to pasture-raised sheep ($P < 0.05$), in pasture-raised sheep compared to stalled sheep ($P < 0.05$). According to the results obtained in the present study, the management condition could be led to stress condition in farmed animals, and, it is well established that stress provokes a response of the animal which involves a cascade of reactions, including acute phase protein response. The onset of the stress response represents an adaptive reaction with the goal of reestablishing the homeostasis.

KEY WORDS

Farm, goat, serum proteins, sheep, stressors, protein electrophoretic fractions, livestock.

INTRODUCTION

Under modern production systems animals must familiarize to environmental conditions in order to maintain health status and production. Farm animals are daily exposed to a wide range of abiotic stressors such as social interactions or rough handling, common farm practices, exposure to adverse climatic conditions, work and transportation [1], [2]. In-depth knowledge of stress responses in farm animals lets to understand the welfare status of the animals and allows the design of housing and husbandry requirements which will advantage the production efficiency and product quality as well [3]. Small ruminant species as goat and sheep have undergone an evolutionary species-specific adaptation due to environmental needs and management requirements [4]. Several previous research studies have explored and investigated chronobiology, a scientific discipline that examines the temporal processes of living organism [5], [6]. During the evolutionary process, mammalian species developed specific biological mechanism to anticipate environmental changes and activate a range of physiological responses aimed at ensuring the homeostasis maintenance, by promoting food intake and ensuring reproductive success. Therefore, it is important to identify a possible correlation between stress and the management conditions of confined animals in a farming system, in which the biological clock or the so-called endogenous pacemaker of the animal organism is affected by periodic factors or external cycles present in nature [7]. These may vary within a farming system that involves the prolonged confinement of animals. The electrophoretic profile of proteins allows to obtain a graph displaying the albumin, α-, β1, β2-, γ-globulin fractions recognized as inflammation-related proteins as they contain indirect markers of inflammation activation (acute phase proteins or APP) and immunity (antibodies) [8-16]. The α- and β2-globulins fractions increase in cases of inflammation, while γ-globulins rise in response to the immune reaction of the animal. In small ruminants, the major positive APPs are haptoglobin and serum amyloid A, while among the negative APPs, albumin is the most representative [17], [18], [19]. These proteins together with other APPs are the actors of the well-orchestrated and synchronized process, known as acute phase response (APR), where several cell types and a network of proteins initiate, amplify, sustain, control and eventually resolve the in-

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flammatory reaction [20]. Acute phase proteins are commonly considered as blood proteins primarily synthesized by hepatocytes as part of the acute phase response. To date, there are numerous species-specific ELISA kits commercially available for the determination of APPs, however they are expensive and require an operator with some experience in ELISA investigations. Noteworthy, the electrophoresis of plasma proteins is an easy, fast and inexpensive technique that generates a photograph of the animal's globulin fractions giving a first idea of the animal's immune-inflammatory status and/or stress status, possibly suggesting further and in-depth investigations to evaluate the animal's health status [17-19].

The aim of the study was to assess whether the serum protein electrophoretic profile including the albumin, α-, β1-, β2- and γ-globulin fractions, of sheep and goats is influenced by different management conditions.

MATERIALS AND METHODS

Animals and study design

All treatments, housing conditions, and animal care procedures described in this study were conducted in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes. The study was carried out in September during routine official veterinary inspections on two ovine-caprine farms located in Sicily, Italy, which differed in their management systems: Farm A (38°15 N; 15°27 E, 150 m above sea level) followed an intensive farming model, whereas Farm B (38°02 N; 15°42 E, 100 m above sea level) employed an extensive grazing system. A total of forty clinically healthy, pluriparous animals were selected: ten Maltese goats and ten Comisana sheep from Farm A, and ten Maltese goats and ten Comisana sheep from Farm B. All animals were between 3 and 5 years of age, with a mean body weight of 45.4 ± 5.6 kg for goats and 48.0 ± 3.0 kg for sheep. Prior to the start of the trial, all animals underwent clinical evaluations, including assessments of body condition score (BCS), rectal temperature, heart rate, respiratory rate, appetite, and fecal consistency. Animals were free from internal and external parasites and were maintained under natural photoperiod (sunrise at 06:30, sunset at 19:17 over the study period) and environmental conditions. Thermal and hygrometric records were carried out for the whole study by means of a data logger (Gemini, UK), and they followed the normal autumnal seasonal pattern for the location. The temperature-humidity index (THI) value, an indicator of thermal comfort, was calculated using the National Weather Service temperature humidity index formula for ruminant species (Potter and Jacobsen, 2000):

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

The temperature-humidity index (THI) values calculated for Farm A and Farm B with the respective climatic conditions are reported in Figure 1.

Animals from Farm A were housed in a barn with access to an outdoor pen and were provided with a balanced diet formulated to meet their physiological and productive requirements, maintaining a consistent dry matter (DM) content. The concentrate portion of the diet included 12% oats, 15% fava beans, 25% barley, 10% peas, 20% sugar beet pulp, 5% molasses, and 3% mineral and vitamin supplements. The forage component consisted of alfalfa (Medicago sativa L.). The estimated nutritional composition of the total ration was approximately 89% DM, 17-18% crude protein (CP), 32-35% neutral detergent fiber (NDF), and a gross energy content of about 2.7 Mcal/kg DM. Concentrate was administered at a rate of approximately 250 g for animal, twice daily. Fresh water was available ad libitum throughout the study period.

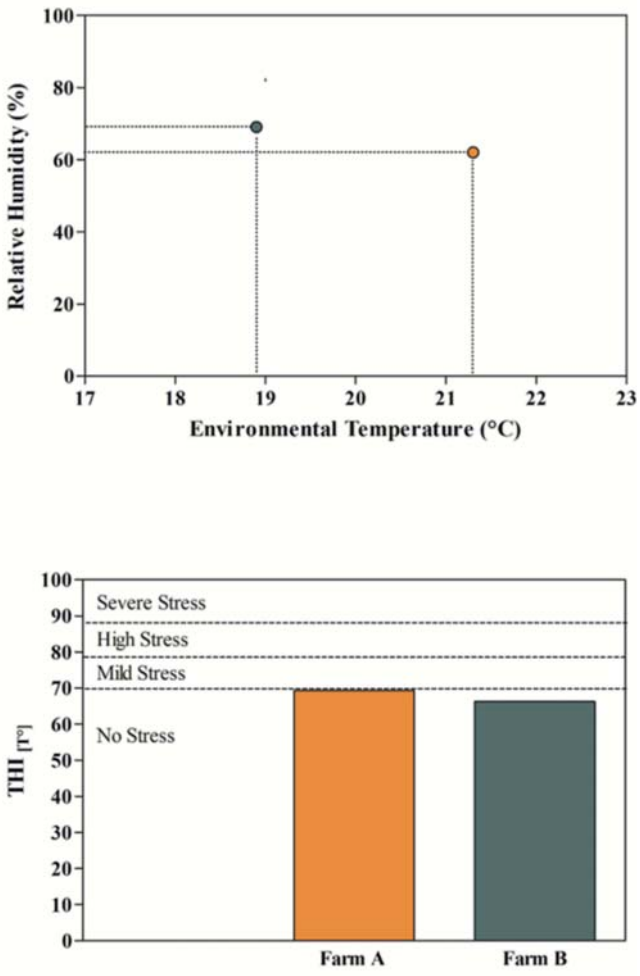

Figure 1. Temperature-humidity index (THI) values calculated for Farm A and Farm B with the respective environmental conditions.

Animals from Farm B grazed on improved natural pasture characterized by a botanical composition typical of Mediterranean semi-natural grasslands. The sward primarily included perennial grasses such as *Lolium perenne* and *Dactylis glomerata* (approximately 40%), spontaneous and sown legumes including *Trifoliu*m *subterraneum* and *Medicago polymorpha* (around 30%), and various herbaceous species native to Mediterranean shrublands, such as *Plantago* spp. and *Eryngium campestre* (approximately 30%). The pasture provided an average DM yield of 1,800 to 2,200 kg/ha. Nutritional analysis of the forage showed 25-30% DM, 12-15% crude protein (CP), 45-50% neutral detergent fiber (NDF), and an estimated gross energy content of 2.3 to 2.6 Mcal/kg DM. During the hottest hours of the day, animals had access to shaded areas, either natural or artificial. Fresh water was available ad libitum throughout the study period.

Blood sampling and analysis

Blood samples were collected from all animals via jugular venipuncture into 8-mL vacutainer tubes containing a clot ac-

tivator (Terumo Co., Tokyo, Japan). Immediately after collection, the samples were placed in refrigerated bags and transported to the laboratory for analysis. Serum was separated from the blood by centrifugation within 1 hour of venipuncture. The tubes were allowed to stand at room temperature for 20 minutes before centrifugation at 3,000 rpm for 10 minutes. The obtained serum was then stored at -25°C until further analysis. The serum samples were neither lipemic nor hemolyzed. The total protein concentration of the serum was determined using the biuret method, employing a commercial kit and an automated ultraviolet-visible spectrophotometer (Model Slim, SEAC, Florence, Italy). Protein electrophoresis was performed using an automated system (Sel Vet 24, SELEO Engineering, Naples, Italy), following the manufacturer's instructions. For each sample, 25 L of serum were applied to numbered sample wells on an acetate cellulose film. The holder accommodated up to 24 samples. The films were electrophoresed for 28 minutes at 180 V. After electrophoresis, the films were fixed simultaneously using an automated system, stained with a red acid solution (Ponceau S 0.2%, trichloroacetic acid 3%) for 10 minutes, and then dried at 37°C. After de-staining in acetic acid, the films were dried completely for 15 minutes. The films were then scanned using a densitometer, and the electrophoretic curves along with the corresponding quantitative protein concentrations for each sample were displayed using the software (Sel Vet 24, SELEO).

To ensure assay precision, all serum samples were tested in duplicate by the same operator, and reproducibility between runs was assessed by testing the same serum sample within 24 hours. The intra-assay coefficient of variation was less than 6% for all measured parameters. All samples were analyzed by the same operator, who determined the fraction boundaries based on the densitometer tracings. Relative protein concentrations within each fraction were determined as the optical absorbance percentage, and absolute concentrations (g/L) were calculated using the total protein concentration. The major protein fractions were separated from cathode to anode as albumin, α-globulins, β1-globulins, β2-globulins, and γ-globulins.

Statistical analysis

Data were tested for normality using the Shapiro-Wilk test. All data were normally distributed ($P > 0.05$), allowing for statistical analysis. One-way analysis of variance (ANOVA) was conducted to study the influence of the management condition on the studied parameters. When significant differences were found ($P < 0.05$), Bonferroni post-hoc comparison test was applied to determine whether there were significant differences between management conditions. All data were analyzed using PRISM 9 statistical software (GraphPad Software Inc., California).

RESULTS

The obtained data were expressed as mean values ± standard deviation and were shown in Table 1. Representative serum protein electrophoretograms obtained from sheep and goats housed in a barn with access to an outdoor pen (Farm A) and from sheep and goats grazed on improved natural pasture (Farm B) are shown in Figure 2. The statistical analysis applied on data recorded from sheep and goats showed higher values of serum total proteins in pasture-raised sheep compared to those housed in a barn with access to an outdoor pen ($P < 0.05$, Table 1), higher β2-globulins content in sheep housed in a barn with access to an outdoor pen compared to pasture-raised sheep ($P < 0.05$, Table 1), higher γ-globulin levels in sheep housed in a barn with access to an outdoor pen compared to pasture-raised sheep ($P < 0.05$, Table 1).

DISCUSSION

The results obtained in this study help to renew the current knowledge regarding the electrophoretic profile of serum proteins in sheep and goats. The serum proteins herein evaluated included the fractions named albumin, α-, β1-, β2- and γ globulins which reflect the inflammatory and immune status of the animals [21]. The aim of the study was to determine whether farm management practices can influence the health status of the animals, particularly in terms of inflammatory and immune system responses [22], [23]. The significance of this study also lies in the aspect of animal adaptation to farm management practices, especially in terms of guiding certain species-typically raised in semi-wild conditions toward more structured and housed management systems [24]. The results,

Table 1 - Mean values standard deviation of serum protein electrophoretic pattern (i.e. albumin, α1-globulins, β1-globulins, β2-globulins, γ-globulins, total proteins, A/G) obtained from sheep and goats housed in a barn with access to an outdoor pen (Farm A) and from sheep and goats grazed on improved natural pasture (Farm B).

	Serum protein electrophoretic pattern						
	Albumin (g/dL)	*α1-globulins (g/dL)*	*β1-globulins (g/dL)*	*β2-globulins (g/dL)*	*γ-globulins (g/dL)*	*Total proteins (g/dL)*	*A/G*
Sheep							
Farm A	2.67 ± 0.87	0.41 ± 0.29	0.88 ± 0.54	1.31 ± 0.31*	0.63 ± 0.39*	6.96 ± 0.48*	1.07 ± 0.40
Farm B	2.65 ± 1.43	0.71 ± 0.48	0.93 ± 0.44	0.49 ± 0.16	1.99 ± 0.91	5.89 ± 0.38	0.69 ± 0.42
Goats							
Farm A	2.79 ± 0.71	0.40 ± 0.09	0.81 ± 0.47	0.72 ± 0.19	2.00 ± 0.72	6.36 ± 0.73	0.83 ± 0.36
Farm B	2.70 ± 1.09	0.60 ± 0.28	1.03 ± 0.63	0.45 ± 0.15	2.34 ± 0.93	6.87 ± 0.78	0.60 ± 0.32
Reference range (Kaneko et al., 1997)							
Sheep	2.40-3.00	0.30-0.60	0.70-1.20	0.40-1.40	0.90-3.30	6.00-7.90	0.42-0.76
Goat	2.70-3.90	0.50-0.70	0.70-1.20	0.30-0.60	0.90-3.20	6.40-7.00	0.63-1.26

Statistically significant differences: * vs sheep of Farm B ($P < 0.05$)

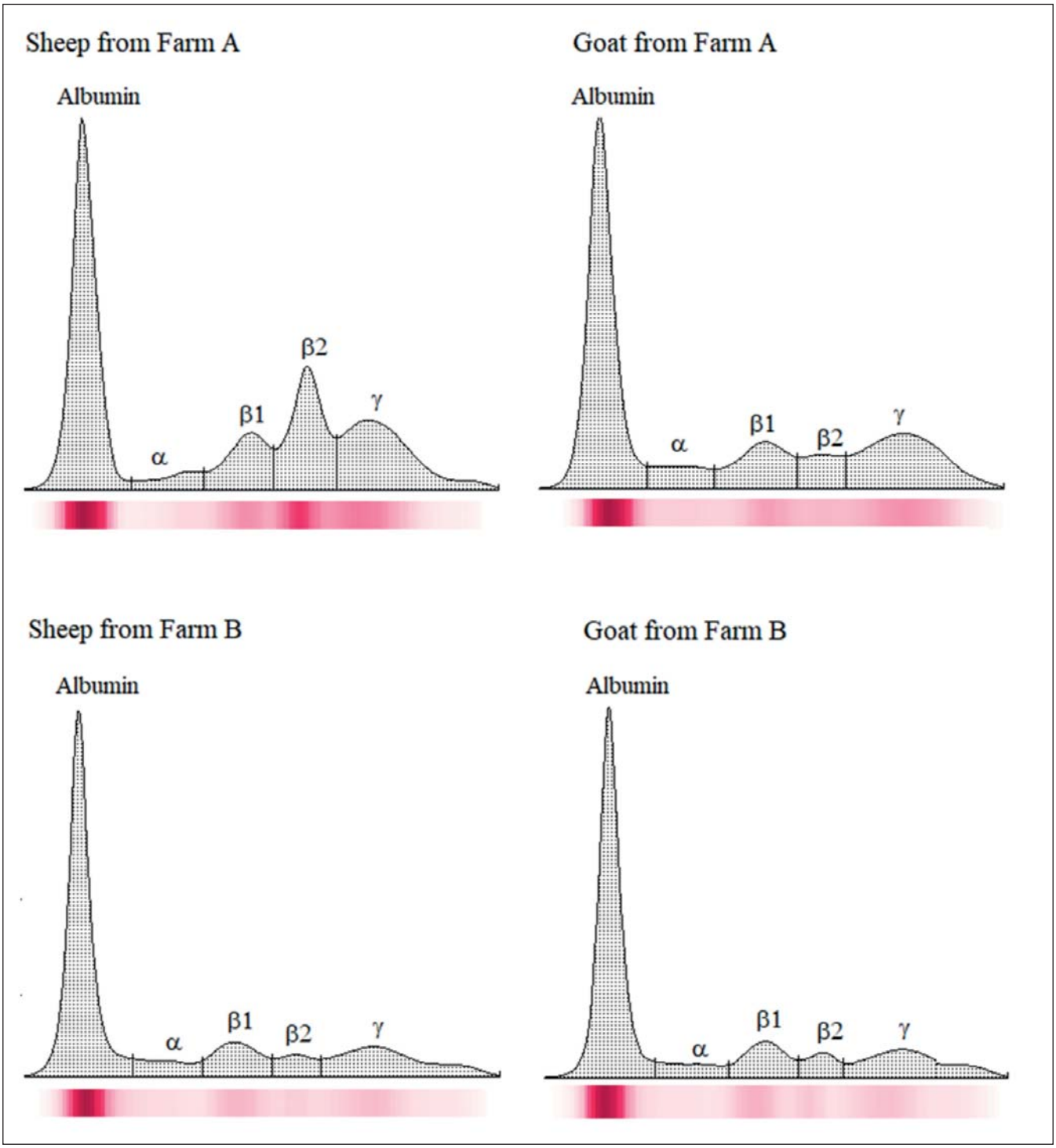

Figure 2. Representative serum protein electrophoretograms obtained from sheep and goats housed in a barn with access to an outdoor pen (Farm A) and from sheep and goats grazed on improved natural pasture (Farm B).

regarding the serum protein electrophoresis pattern showed higher values of serum total proteins in pasture-raised sheep compared to those housed in a barn with access to an outdoor pen, whereas the β2- and γ-globulin levels were higher in sheep housed in a barn with access to an outdoor pen compared to pasture-raised sheep. The differences found in the values of serum total proteins between groups referred to management conditions could be attributed to different diet of pasture-raised sheep/goat compared to stalled sheep/goat. As a matter of facts, animals from the pasture group inevitably had easier access to various herbaceous species, affecting the protein sequence. In contrast, the stalled sheep were fed a fixed and controlled protein diet provided by the farmer, which resulted in lower protein content. The higher β2-globulin content in sheep housed in a barn with access to an outdoor pen compared to pasture-raised sheep may be due to the alertness of the inflammatory system of the animals which can be activated not because there is an actual inflammation occurring, but because the organism is on alert. This response cannot be considered pathological but physiological. This value is also linked to the higher γ-globulin content in sheep housed in a barn with access to an outdoor pen than pasture-raised sheep, as the immune system

of the pasture-raised sheep was countering the alert state of the inflammatory system. It is well known that an animal experiencing a stressor condition react with a response including a phase of adaptation and alertness that often does not indicate a true inflammatory state, but rather a response to stimuli that the organism encounters. This leads to a modification of the inflammatory profile, which should be considered a physiological, not pathological, response. The management condition could be led to stress condition in farmed animals, and, it is well established that stress provokes a response of the animal which involves a cascade of reactions, including inflammation and acute phase protein response [25], [26], [27]. The onset of the stress response represents an adaptive reaction, which appears to have been more pronounced in goats than in sheep herein investigated, with the goal of reestablishing the homeostasis [28]. Noteworthy, the electrophoretic protein fractions measured in goats did not change between the two management conditions. Sufficient scientific evidence has established that goats are a more resilient animal model for survival in harsh ecosystems than other livestock species [29], [30].

CONCLUSION

The management conditions experienced by the two groups of sheep and goats seemed to activate the immune-inflammatory system of the animals in both groups, due both to the different types of management to which the animals had been exposed and to a consequent immune-inflammatory response. This response may reflect either an adaptive process or an activated inflammatory state that can be defined as physiological rather than pathological. According to the changes observed in levels of the serum protein fractions (i.e. β2- and γ-globulins) measured in sheep and goats enrolled in the current study seemed that the immune-inflammatory profile of these small ruminant species housed in stalls was in a state of alertness likely triggered by the stress of the management procedure.

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