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ARTICLE

Blood haemogram in *Ovis aries* and *Capra hyrcus*: effect of storage time

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Abstract: The aim of the present study was to assess the effect of storage time at +4 °C on haematological profile in goat ($n = 25$) and sheep ($n = 25$). After collection, blood samples were immediately analyzed and then divided into four aliquots that were stored at 4 °C and tested at 24 h (T1), 48 h (T2), 72 h (T3), and 1 wk (T4), respectively. One-way repeated-measures analysis of variance (ANOVA) was used to determine statistically significant effect of storage conditions both in goats and in sheep. Our results showed that among the two species studied, goats showed highest blood stability after refrigeration at +4 °C. In goats, all hematological parameters, except PLT, showed no significant changes during all days of monitoring with respect to basal values (T0). In sheep, no significantly effect of storage time on RBC and WBC levels were found, whereas the other hematological parameters change significantly over the time. Our findings suggest that the blood storage time reported for goat may not be applied to sheep's blood, which underscore the differences between these two species that are erroneously considered similar.

Key words: blood stability, haematological parameters, goat, sheep, storage time.

Résumé : Le but de cette étude était d'évaluer l'effet du temps de stockage à +4 °C sur le profile hématologique des chèvres ($n = 25$) et des moutons ($n = 25$). Après les prélèvaments, des échantillons de sang ont été immédiatement analisé et après divisés en quatre aliquotes qui ont été stockés à 4 °C et testés à 24 h (T1), 48 h (T2), 72 h (T3) et à une semaine (T4), respectivement. Une analyse de la variance (ANOVA) à un facteur à mesures répétées a été utilise pour déterminer l'effect statistiquement significative des conditions de stockage chez les chèvres et les moutons. Nos resultants ont montré que entre les deux espèces étudiées, les chèvres ont montré une stabilité sanguine plus grande après la réfrigération à +4 °C. Chez les chèvres, tous les paramètres hématologiques, excepté PLT, ont pas montré changements significatives durant tous les jours de surveillance par rapport aux valeurs de base (T0). Chez les moutons, n'a pas été détecté aucun effet significantif du temps de stockage sur les niveaux de RBC et WBC, tandis que les autres paramètres hématologique changent significantivement dans le temps. Notre découverte suggère que le temps de stockage de sangue indiqué pour les chèvres ne peut pas être appliqué sur le sang du moutoun soulignant les différences entre les deux espèces étudiées qui sont à tort considérés similaires. [Traduit par la Rédaction]

Mots-clés : stabilité sanguine, paramètres hématologiques, chèvres, moutons, temps de stockage.

Introduction

In Mediterranean areas, sheep and goat represents one of the most important resources for the agriculture economy. Extensive grazing methods represent an ancient, traditional practice for using poor lands. This sector involves more than 10 000 farms of sheep and goats (6% and 15%, respectively, of the Italian national herds) (Caracappa 1999). Considering the social and economic values of goats and sheep, it is important to perform, in animal farms, clinic and paraclinic exams to guarantee sanitary control strategies, prevention or treatment of diseases, and ensure good management practices. In small ruminants (sheep and goat), the haematological analysis is an important and reliable tool

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Abbreviations: **RBC**, red blood cell; **WBC**, white blood cell; **Hgb**, haemoglobin; **Hct**, haematocrit; **PLT**, platelets; **MCV**, mean corpuscular volume; **MCH**, mean cell haemoglobin; **MCHC**, mean cell haemoglobin concentration.

used to monitor and evaluate health and welfare status of animals (Babatunde et al.1992; Gupta et al. 2007; Polizopoulou 2010). For complete blood counts, after blood collection in ethylenediaminetetra-acetic acid (EDTA) tubes, samples should be rolled gently several times to ensure adequate anticoagulant mixing and processed as soon as possible (Brockus and Andreasen 2003; Morris 2008a, b). Although it is recommended to perform laboratory analysis immediately upon collection, if this is not possible especially when blood samples are collected from remotely located farms, then the samples must be refrigerated at 4 °C. Blood samples forwarded by mail to a laboratory may produce artefactual results; therefore, it is imperative to ensure packing with ice or cold packs in insulated containers to minimize these effects (Jones and Allison 2007; Topper and Welles 2003). It is well known that prolonged storage could compromise red blood cell (RBC) properties, in particular storage condition could lead to metabolic depletion, disturbed ion homeostasis, protein and lipid modifications (e.g., oxidation, degradation, cross-linking), and volume changes accompanied by alterations in intracellular hemoglobin concentrations (Ho et al. 2003; vanWijk and van Solinge 2005). RBC mechanical properties have also been shown to be altered during the time period between sampling and measurement (Bartoli et al. 1986; Zhang et al. 2004). Room temperature also caused greater RBC swelling to occur after 6–24 h, may lead to aberrations, such as increased PCV and decreased MCHC. Buttarello (2004) and Goosens (1994) in their experimental protocol suggested that refrigeration of human blood samples from 24 to 72 h is recommended to stabilize blood and minimize artifactual changes. Other studies (Ihedioha and Ibeachu 2005; Ihedioha and Onwubuche 2007) conducted on different animal species showed significant differences in the stability of blood samples stored at room or refrigerator temperature. Particularly, a study conducted on bovine blood (Bluel et al. 2002) showed that refrigeration had a stabilizing effect on RBC and a decrease on white blood cells (WBC) during 24 h of storage, whereas equine blood samples stored at room temperature were more stable when compared with refrigerated samples. In fish, haematological parameters can be assessed within 6 h from blood collection when samples are stored at +4 °C because long-term storage modifies the results of the analyses (Faggio et al. 2013). As shown by the literature, the period during which the assessed parameters change significantly from baseline values varies from one species to the other. Much of the available information on the haematology of small ruminants are known from data on sheep and goats (Piccione et al. 2007, 2011, 2014) because of their economic importance; however, there are relatively few reports regarding blood storage in these species. In view of this lack of information, the objective of this study was to evaluate the stability of hematological parameters in goats and sheep blood stored at 4 °C at different times: T0 (within 3 h after sampling), T1 (24 h after sampling), T2 (48 h after sampling), T3 (72 h after sampling), and T4 (1 wk after sampling).

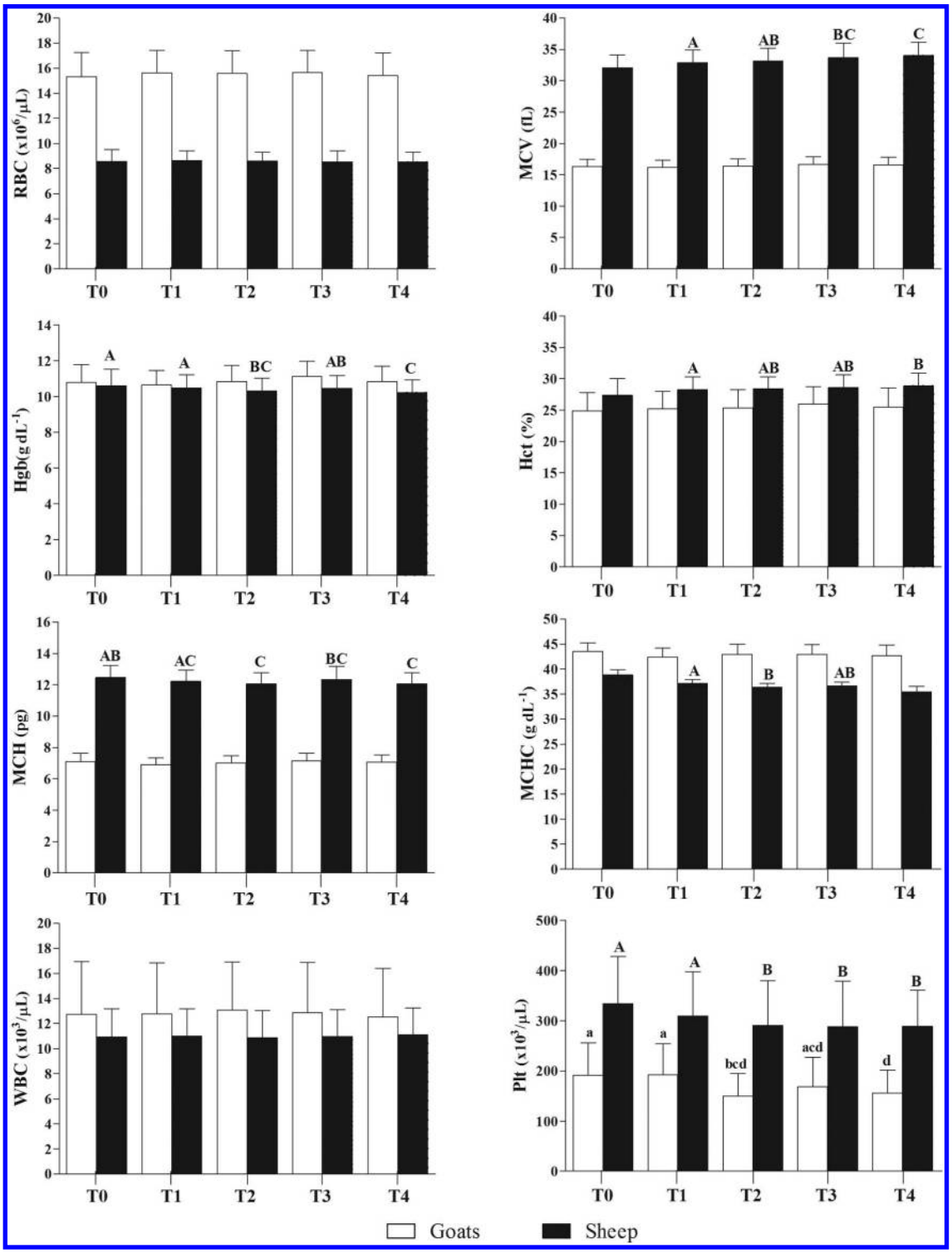
Materials and Methods

Twenty-five Maltese goats (2–3 yr, 42 ± 5 kg body weight) and twenty-five Valle del Belice sheep (2–3 yr, 68 ± 4 kg body weight) were enrolled in this study. Goats were reared in a farm located in Messina (37°87′N, 14°30′E; Sicily) at an altitude of 723 m above sea level. Sheep were reared in a farm situated in Palermo (37°7′N, 13°30′E; Sicily) at an altitude of 734 m above sea level. All subjects were milked in the morning at 0700 and in the evening at 1700 and they were subjected to the same management condition. All animals were clinically healthy and free from internal and external parasites. They were treated for endoparasites twice a year. Their health status was evaluated based on rectal temperature, heart rate, respiratory profile (data not shown), appetite, and fecal consistency. In both goats and sheep, blood samples (3.5 mL) were withdrawn at 0800, at 15 ± 3 d after delivery, from the external jugular vein using Vacutainer tubes (Terumo Corporation, Tokyo, Japan) containing K_3-EDTA as the anticoagulant agent. Blood samples were stored on "wet ice" (4~6 °C) in a small insulated container. The ice was contained in plastic bags and the sample tubes were in a tray on the ice bags, and thus the tubes did not directly contact the ice. The temperature inside the container was continuously monitored. Blood samples were tested within 3 h after sampling (T0) and then were divided into four aliquots that were stored at 4 °C and tested at 24 h (T1), 48 h (T2), 72 h (T3), and 1 wk (T4), respectively. Prior to testing, the cooled samples were removed from the insulated container and left at room temperature for 20 min, then each blood sample was gently mixed and measured. An automated analyser for haematology (HECO Vet C, SEAC, Florence, Italy) was used to assessed blood heamogram that included the following parameters: RBC, WBC, haemoglobin (Hgb), haematocrit (Hct), platelets (PLT), mean corpuscular volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC). All treatments, housing, and animal care were carried out in accordance with the guidelines established by the Canadian Council on Animal Care (2009) for animal experiments.

Statistical analysis

All results are expressed as means ± SD. Data were normally distributed ($P > 0.05$; Kolmogorov–Smirnov test). One-way repeated-measures analysis of variance (ANOVA) was used to determine statistically significant effect of storage conditions in both goats and sheep. A P value <0.05 was considered statistically significant. Bonferroni's multiple comparison test was applied for post hoc comparison. The data were analyzed using the STATISTICA version 8 software (StatSoft, Inc., Tulsa, OK, USA).

Fig. 1. Patterns of haematological parameters recorded in goats and sheep during the experimental period. Mean values without the same letters at different time points represent statistical differences.



Results

In goats, all hematological parameters, except PLT, showed no significant changes during different storage times with respect to basal values (T0). As reported in Fig. 1, PLT values in blood stored at 4 °C decreased already after 48 h sampling with respect to the baseline value ($P < 0.0001$). In sheep, no significant effect of storage time on RBC ($P > 0.05$) and WBC ($P > 0.05$) levels were found, whereas the other hematological parameters changed significantly in different way.

In particular, Hgb showed a small but significant decrease from the baseline value (10.62 ± 0.90 g dL^{-1}) already after 48 h (10.31 ± 0.71 g dL^{-1}) and it continued to decrease slightly attaining a value of 10.23 ± 0.69 g dL^{-1} after 1 wk ($P < 0.0001$). Hct significantly increased already after 24 h of sample collection and storage at 4 °C (28.06 ± 2.03%) with respect to the baseline values (27.38 ± 2.62%) and reached the highest value after 1 wk (28.88 ± 2.02%) ($P < 0.0001$). With respect to erythrocytes indices, our results showed a consistent increase over time in MCV values, and a decrease in MCHC and MCH values after 48 h ($P < 0.0001$). As well as in goats, PLT decreased already after 48 h sampling with respect to the baseline value ($P < 0.05$).

Discussion

Between the two species studied, goats showed the highest blood stability after refrigeration at 4 °C for 1 wk. In our study, both goats and sheep blood samples showed a decrease in PLT values starting at 48 h after collection and storage at 4 °C. It has been reported that PLT counts are the most unstable variable during storage of canine whole blood (Caillard 2002) with the decrease starting as early as 6 h after sampling. PLT stored at 4 °C are associated with an irreversible disk-to-sphere transformation. The loss of shape in platelets stored at 4 °C may be the result of microtubule disassembly, which may also contribute to the decreased survival of PLT stored at 4 °C (Kattlove et al. 1972). RBC and WBC showed no changes for all time of monitoring in both goats and sheep. With respect to other mammals (except for camels), these species have more resistant erythrocytes. This may be due partly to the shape of erythrocytes, which is oval rather than the circular discs seen in other mammalian erythrocytes, and partly to the composition of the erythrocyte membrane. RBC deformability and then cell resistance is determined by numerous factors such as internal viscosity, membrane rheologic behavior, cellular membrane surface area to cell volume ratio, and cell shape (Stoltz et al. 1999). In particular, it was suggested that the phospholipid classes and not the phospholipid concentrations are important in the determination of the physiological functions of the erythrocytes (Mirgani 1992; Schwartz et al. 1985; Warda and Zeisig 2000). Spingomyelin, phosphatidylcholine/phosphatidylethanolamine govern the fragility of erythrocytes. It was found that the sphingomyelin fraction is the most abundant in animals that we studied, followed by phosphatidylcholine (Al-Qarawi and Mousa 2004). The sum of these two classes constituted 74% and 72% in sheep and goats, respectively, and it is responsible for the stability of RBC. Even if RBC of sheep were resistant to storage condition, we found an increase in MCV values probably due to the higher deformability of RBC. According to other researches, the increase in MCV reflects the swelling of RBC (Hadzimusic et al. 2010). RBC swell and increases in size/volume are due to degenerative changes that permit ingress of water into the cells compromising the membrane stability (Hadzimusic et al. 2010). Wood et al. (1999) indicated that refrigerate storage prevented the swelling and in particular it was demonstrate that RBC membrane fragility decreases with storage time in goats (Okwusidi 2004); this could justify the absence of changes in goat RBC. The increase in MCV produced the same trend in HCT. Decrease in Hgb concentration was thought to be due to conversion of some of the Hgb to degradation intermediates as reported in pigs (Ihedioha and Onwubuche 2007). MCH decrease because the Hgb concentration decrease. Similarly, the decrease in MCHC values is related to the change of Hgb and the increase in Hct.

Our results provide new perspectives for veterinary practitioners, laboratory technologists, and research hematologists. Moreover, our findings underscore the importance of processing blood samples that take into account the species being analyzed and they confirm the existing hematological differences between goats and sheep that are erroneously considered similar.

Conflict of Interest Statement

We declare having no conflict of interest.

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