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# Review article Clinical biochemistry in sheep: A selected review<sup>\*</sup>

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# ABSTRACT

As in other species, the first point in sheep clinical biochemistry is the correct selection of the appropriate tests and, consequently, the optimal management of the pre-analytical phase from the collection of the samples to their management and possible transport or storage before analysis. There are so many different breeds and breeding systems in sheep, as well as laboratory techniques, that no universally acceptable reference values and ranges can be provided. Each laboratory should determine its own reference values and ranges, according to recommended methods. The main uses of clinical biochemistry in sheep health management are in the diagnosis of liver, muscle and nutritional disorders, for which selected examples are discussed in this paper.

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# 1. Introduction

Clinical pathology is mainly used in individual medicine and more rarely in flock health management, which is probably why available information concerning sheep is mostly based on experimental studies, series of cases and observations during disease outbreaks. To our knowledge, there is no general review on ovine clinical biochemistry, although general information can be found in chapters of the major medicine and clinical pathology textbooks (Kaneko et al., 2008) and also in some topic related reviews. However, the applications of clinical pathology in sheep are numerous, making a comprehensive review of all the information available impossible and probably of little use. The following survey has been compiled from a subjective selection of references concerning the main uses of clinical biochemistry in sheep.

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### 2. Pre-analytical factors of variation

As in other species, blood analyses can be greatly influenced by the conditions of specimen collection, even though the "meal effect", reported in monogastric animals, is not observed in sheep, except in young lambs at the preruminant stage.

#### 2.1. Anticoagulants and processing of specimens

A recent study of possible effects of anticoagulants on sheep serum/plasma biochemical analyses recommends the use of serum, but not of EDTA or citrate plasmas (Mohri et al., 2007). They also found little difference between serum and heparin plasma. However, this is not true for all analytes, as copper is reported to be partly lost during coagulation, so that the copper concentration is lower in serum than in plasma (Laven and Smith, 2008). Moreover, it takes longer to prepare a serum than a plasma sample and the risk of haemolysis is higher due to the fragility of sheep red blood cells, which is further increased in the case of selenium deficiency (Rezaei and Dalir-Naghadeh, 2009). This may explain why potassium cannot be analyzed from whole blood with test strips in sheep, in which the intracellular potassium concentration is high (Diquelou et al., 2004).

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Fig. 1. Circadian rhythms of urea concentrations in blood plasma or saliva of sheep (arrow: feeding time; grey area: night period) (from Piccione et al., 2006).

Correct processing of specimens, especially absence of haemolysis and appropriate storage conditions, is critical for energy metabolites, blood gases and pH (Szenci et al., 1991; Morris et al., 2002; Piccione et al., 2007).

#### 2.2. Time of sampling

The influence of season is often difficult to separate from confusion factors, such as food supply, and/or reproductive status in female animals (Yokus et al., 2004, 2006; Obidike et al., 2009). Accordingly, different reference intervals should be considered (Karapehlivan et al., 2007). For some analytes, temporal changes can be related to seasonal influences, such as formation/resorption of bone and corresponding changes of bone turnover markers (Arens et al., 2007). In other cases, large variations are observed and no seasonal and/or physiological cause may be found. For instance, mean plasma lactate concentration was reported to range from 1.7 to 2.9 mmol/L in a group of 20 ewes sampled monthly for one year (Allison et al., 2008). In some cases, circadian variations of analytes cannot be unambiguously linked to an endogenous rhythm, but may also result from the rhythm of food administration, as observed for plasma and salivary urea concentrations (Fig. 1) (Piccione et al., 2006).

#### 2.3. Nutrition/food supply

Effects of food supply and, possibly, of parasitic status are especially marked on analytes that reflect energy and mineral metabolism (Marley et al., 2005). For instance, the plasma concentration of non-esterified fatty acids (NEFA) was reported to be three times higher in the first month post-partum in ewes fed indoors than in grazing ewes, whereas concentration of triglycerides was identical and urea 30% lower than that; this is due to the difference in the alimentary supply, which is lower indoors than outdoors (Alvarez-Rodriguez et al., 2008). Similar effects on NEFA, a lowering of leptin and no change in blood glucose and insulin were also observed in ewes fed at half maintenance requirements for four weeks at the beginning of pregnancy (Sosa et al., 2009). Under-nutrition in ewes also had consequences on their foetuses, for which the plasma glucose and lactate concentrations were lower than in controls (Oliver et al., 2005).

Minerals and vitamins, supplied at supra-nutritional levels in order to enhance growth rate, can lead to changes in blood biochemistry, not only in relation to the minerals and vitamins supplied, but also for other, directly related markers, such as glutathione peroxidase for selenium (Yu et al., 2008). They can also alter many other analytes, for instance, by increasing the concentration of thyroid hormones or by reducing the cortisol concentration in selenium-supplemented lambs (Dominguez-Vara et al., 2009). A dramatic increase in the renal fractional excretion of sodium was reported in the case of increased sodium chloride intake (Meintjes and Engelbrecht, 1993). In pregnant ewes, restricted feeding led to a significant reduction in body weight compared to control feeding (means of 65 and 78 kg, respectively), but to minor or no changes in the concentrations of plasma glucose, total proteins, triglycerides, cholesterol and lactate or in lactate dehydrogenase activity (Tanaka et al., 2008).

## 2.4. Effect of transport and lairage

Transport and duration of lairage can affect some plasma variables, for instance by increasing glucose and creatine kinase and decreasing cortisol in young lambs ( $\sim$ 12 kg bodyweight), but not in older ones ( $\sim$ 25 kg bodyweight) (Ali et al., 2006; Bornez et al., 2009). Such alterations are used to monitor transport stress and provide bases for improving animal welfare (Cockram et al., 2000; Tadich et al., 2009).

#### 3. Reference intervals and decision limits

The issue of reference values is, as recently reviewed, the same in sheep as in other species (CLSI, 2008). Many reference intervals have been published in textbooks, review

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