

Research Article

Activity of Phosphatase Enzymes, Concentration of Protein and Divalent Ions in Sheep Sera during different Physiological Status

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Abstract | The aim of present study was to investigate the probable effects of the reproductive state on the sera component and enzymes in lactating and pregnant sheep. All the blood tests were carried out in 46 healthy sheep. The animals were divided into three groups: first group (G1; n = 15) pregnant, the second group (G2; n = 16) lactating and third group (G3; n= 15) non-pregnant and non-lactating (control group). The results showed that higher concentrations ($P < 0.05$) of total protein were determined in the blood of pregnant sheep compared to lactating group sheep. Moreover, the alkaline phosphatase (Alp) activities were higher ($P < 0.01$) in lactating sheep than pregnant and control group, while higher ($P < 0.01$) concentrations of acid phosphatase (Acp) activity were determined in sera of pregnant compared to lactating and control sheep. The results further showed the higher ($P < 0.05$) specific activity of Acp in pregnant compared to lactating and control. The higher ($P < 0.05$) specific activity of Alp were also determined in sera of lactating compared to pregnant and control. The lowest ($P < 0.05$) sera Zn+2 and Mg+2 levels in pregnant sheep were observed compared with lactating and control group A. Results of this study showed that values of Acp, Alp activities and total protein could be used as an indicator of pregnancy in sheep. We suggest that pregnancy and lactation periods must be taken into consideration for the true interpretation of sera chemistry and ions status in sheep.

Keywords | Phosphatase enzymes, Sheep, Protein, Divalent ions

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INTRODUCTION

Sera chemistry is essential on diagnosis, treatment and estimation of diseases. In order to explain biochemical data correctly, the results obtained in the lab must be compared with values alike to healthy material, these called reference values. A big number of factors such as breed, species, age, sex, illness, reproductive state (pregnancy) and seasonal difference can effect on the sera chemistry values (Swanson et al., 2004; Nazifi et al., 2003; Yokus et al., 2006). There are many studies on the effects of different stages of the reproduction cycle on biochemical data in local animal species. In sheep studies were also carried out in relation to lactation and pregnancy (Iriadam, 2007; Giuseppe et al., 2009). These physiological states were considered to regulate metabolism in animals (Krajnicakova et al., 1993). Lactation is a very demanding period for the animal when they have increased nutritional needs (Antunovic et al., 2011).

Enzymes are a list of proteins of great biological importance. The presence, or partly the activity, of these catalysts (at times also called ferments) facilitates biochemical processes in the organism, the total of which is referred to as metabolism (Berg et al., 2006; Rochling, 2011; Šoch et al., 2008). Phosphatases enzyme have been classified as alkaline phosphatase (Alp) and acid phosphatase (Acp) depending on optimum pH required for their catalytic activity (Homayon et al., 2009). Alkaline phosphatase (Alp) belongs to hydrolytic category that results the hydrolysis of monoesters of phosphoric acid (Mohr et al., 2007). Alp is made up of many isoenzymes – the, carcinoplacental, placental, intestinal, renal and bone isoenzymes (Sato et al., 2005). Alp is present in all tissues of the body, especially in the cell membrane and kidney tubules; and it occurs at high levels in epithelium, liver, intestine, placenta and bone (Beckett et al., 2000; Valocky et al., 2007). Acp is a hydrolytic enzyme, catalyse the hydrolysis of different phospho-monoesters in acidic pH and cause liberation of an inorgan-

ic phosphate (Miteva et al., 2010). Phosphatases enzymes are involved in many biological processes such as signal transduction pathways and energy metabolism (Shan, 2002). Divalent ions are essential for vitamin synthesis, enzyme activity, hormone production, collagen formation, tissue synthesis, energy production, oxygen transport and some physiological processes linked to growth, health and reproduction (Gürdoğan et al., 2006). The zinc ion is an important constituent of several enzymes mainly carbonic anhydrase and alkaline phosphatase, in the organic matrix (Gürbüz et al., 2002). Magnesium ion play vital role in the body and is mostly deposited in the skeleton. Magnesium is one of the 4 bulk metals in the body. It is a co-factor for about 300 cellular-enzymes such as alkaline phosphatase (Funda et al., 2004). The present study was accomplished to contrast the levels of metabolites in sera of pregnant and lactating sheep. We measured the levels of alkaline phosphatase, acid phosphatase, total protein, Zn⁺² and Mg⁺², in addition to calculation of specific activity of Alp and Acp.

MATERIAL AND METHODS

STUDY SUBJECTS

This study was applied in 46 healthy female sheep aged 2–4 years with average body weight of 42.1±3.4 kg. Animals were divided into three groups: First group (G₁; n = 15) were pregnancy, the second group (G₂; n = 16) were lactating and third group (G₃; n = 15) were non pregnant and non-lactating (control group).

SAMPLE COLLECTION

A 10 ml of blood was collected via jugular venipuncture of each animal, disposable syringes were used and sterile needles 18 gauge x 1 1/2 inches. The blood samples were placed in glass tubes, sera were separated by centrifugation (10 min) at 3000 rpm. Sera concentrations of total protein and acid phosphatase (Acp) were measured by using a commercial kits (Biolabo SA, France). The serum concentrations of alkaline phosphatase (Alp) was determined using the commercial available kits (BioSystems S.A. Spain) according to a previous report (Sema et al., 2009).

DETERMINATION OF THE DIVALENT IONS

One ml of sera was digested with 3ml of a mixture

(6HNO₃: 1HClO₄) in a glass test tube. The mixture was analysed by atomic absorption spectrophotometer (GBC 933 plus), with air-acetylene flame and hollow cathode lamp (Medhat et al., 2013). Serum Mg and Zn concentrations were determined by using commercial kits with spectrophotometer (Oladipo and Temiye, 2005).

STATISTICAL ANALYSIS

Data were statistically analysed by SPSS (Statistical Package for the Social Sciences). All parameters were presented as means ± S.D (Sema et al., 2009).

RESULTS

Biochemical values obtained from pregnant sheep (G₁), lactating sheep (G₂) and control group (G₃) for different states are summarized in Table 1. Higher (P< 0.05) concentrations of total protein were determined in the serum of sheep in pregnant compared to lactating and control group. The activity and specific activity of Alp were higher (P< 0.01 or P< 0.05) in lactating than pregnant and control group. On the contrary a higher (P< 0.01 or P< 0.05) activity and specific activity of Acp were determined in the blood of pregnant compared to lactating and control group. Results further revealed that the lowest (P< 0.05) serum Zn⁺² and Mg⁺² levels in pregnant (G₁) compared with lactating (G₂) and control group (G₃).

DISCUSSION

There were some differences in biochemical values parallel to physiological change ruminants and sheep after and before parturition (Ali et al., 2014). The essential objective of present study was to investigate probable effects of reproductive states on the sera chemistry and divalent ions concentrations in sheep. Higher concentrations of total protein were determined in pregnant compared to lactating and control group. Same concentrations of serum total proteins in pregnant sheep and those in lactating sheep have been found by (Antunović et al., 2002). Decrease of serum total protein over the lactation could be explained by a fast extraction of immunoglobulin from the blood during the last months of pregnancy (Kaneko et al., 2008).

Our results showing that Alp activity was higher in lactating

Table 1: Phosphatase enzymes, concentration of protein and divalent ions in sheep sera

Group	Acp (U/l)	Alp (U/l)	Total Protein (mg/l)	Specific Activity of Acp (U/ mg)	Specific Activity of Alp (U/mg)	Zn ion (µg/dl)	Mg ion (mg/dl)
G ₁	18.12 ± 2.63 ^a	512.32 ± 36.50 ^b	70.01 ± 3.02 ^a	0.25 ± 0.02 ^a	7.30 ± 0.20 ^b	48.50 ± 0.41 ^c	2.47 ± 0.41 ^b
G ₂	14.32 ± 5.75 ^b	717.53 ± 53.11 ^a	63.12 ± 2.06 ^b	0.22 ± 0.08 ^b	11.37 ± 0.48 ^a	51.02 ± 0.31 ^b	2.94 ± 0.18 ^a
G ₃	7.24 ± 4.62 ^c	390.22 ± 57.50 ^c	74.03 ± 3.01 ^a	0.11 ± 0.07 ^c	5.62 ± 0.93 ^c	59.30 ± 0.22 ^a	2.50 ± 0.21 ^b

G₁= pregnant sheep; G₂= lactating sheep; G₃= control group; Means with different superscript letters differ significantly (P< 0.05); Acp= acid phosphatase; Alp= alkaline phosphatase

AUTHOR'S CONTRIBUTION

than pregnant and control group. Increase of Alp activity during lactation is due to an increase in the production of isoenzyme from bone (Sema et al., 2009). The authors (Yokus and Cakmr, 2006; Yokus et al., 2006) suggested that Alp activity in pregnancy were higher than those in lactation period. The result of this study were consistent with some literature (Birgel et al., 1997; Khan et al., 2002; Sema et al., 2009). Higher concentrations of Acp activity were determined in serum of pregnant compared to lactating and control group. Decreased concentrations of Acp activity in lactating sheep have to be considered as a result of constant energy loss with the milk (Antunovi, 2011). Calculate specific activity of enzyme to know the real activity of enzyme in different stages. Specific activity (S. activity) is the number of enzyme units / ml divided by protein concentration in mg per ml.

Results revealed that specific activity of Acp was higher in pregnant sheep compared to lactating sheep and control group, whereas a higher specific activity of Alp was determined in serum of lactating compared to pregnant and control group. These results of specific activity exactly matching with the result of enzyme activity. Moreover, present study showed the lowest serum Zn and Mg levels in pregnant (G_1) compared with lactated (G_2) and control group (G_3). The reason for the decrease in the concentration of Zn and Mg ions levels obtained in pregnant sheep compared with lactating could be belong to the foetus requirement. Due to the high Zn^{+2} and Mg^{+2} levels being released through involution of the uterus (Gürdoğan et al., 2006). It has been reported (Funda et al., 2004; Seyrek et al., 2006) that the Zn levels of serum in sheep should be between 80-120 $\mu\text{g}/\text{dl}$. Similar results were obtained from control group ($59.3 \pm 0.22\mu\text{g}/\text{dl}$). The levels of serum Zn ion in pregnant and lactating sheep were lower than in control group. Higher concentration of Zn and Mg ions in lactating sheep could be connected with higher activity of Alp that could be further contributed to the enzyme requires zinc (Zn^{+2}) and magnesium (Mg^{+2}) or calcium (Ca^{+2}) divalent ions for activity (Steitz, 1999).

In conclusion, results of this study showed that values of Acp, Alp activities and total protein could be used as an indicator of pregnancy in sheep.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Wissam Mahmud Mohamed designed the study and carried out laboratory work, Ahmed Salman Hamad facilitated the study by collecting and arranging samples Noor Khalid Zaidan carried out laboratory work and finalised the manuscript.

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