Selected serum biochemical parameters and acute phase protein levels in a herd of Saanen goats showing signs of pregnancy toxaemia

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ABSTRACT: The purpose of this study was to examine selected serum biochemical parameters and acute phase protein levels in a herd of Saanen goats showing signs of pregnancy toxaemia. Seventy five female goats were used and divided into three groups. Group 1 (n = 57) (blood serum glucose levels were within the physiological range), Group 2 (n = 11) (serum glucose values were low) and Group 3 (n = 7) (serum glucose values were high). Goats in Groups 2 and 3 were diagnosed with pregnancy toxaemia. Apart from serum glucose, β-hydroxybutyrate (BHB), triglycerides, blood pH, calcium (Ca), sodium (Na), potassium (K), aspartate aminotransferase (AST), alanine aminotransferase (ALT), haptoglobin (Hp), serum amyloid A (SAA) and tumour necrosis factor-α (TNF-α) were measured in all animals. In Group 3 average Hp and SAA values were found to be significantly (P < 0.001) higher than in Groups 1 and 2, and also higher in Group 2 than in Group 1. Acute phase proteins in goats with pregnancy toxaemia may be used in the course and the prognosis of the disease. The evaluation of acute phase proteins is useful and also quicker in cases of suspected pregnancy intoxication.

Keywords: goat; serum glucose; β-hydroxybutyrate; cytokines; enzymes; minerals; aciduria; ketonuria; acute phase proteins

Pregnancy toxaemia is a metabolic disorder of pregnant small ruminants, caused by an abnormal metabolism of carbohydrates and fats, which occurs at the final stage of pregnancy (Brozos et al. 2011). The condition is usually seen in females carrying multiple foetuses and may result from their inability to consume enough energy (Mobini et al. 2002). Energy requirements for ewes and does carrying twins or triplets are greatly increased during the final two months of gestation because 70–80% of foetal grow occurs during this time. The disease occurs in association with anorexia caused by other diseases or sudden stresses (Navarrei and Pugh 2002). Animals that are predisposed to the disease exhibit an ineffective gluconeogenic response to the continuous, preferential demands for glucose by the growing foetuses resulting in hypoglycaemia, lipid mobilisation and the accumulation of ketone bodies. Acute phase proteins (APP) are a group of blood proteins that change in concentration in animals subjected to external or internal challenges, such as infection, inflammation, surgical trauma or stress (Eckersall 2000). The acute phase response has been studied in ruminant species, and Hp and SAA are considered the most important and useful indicators of inflammatory processes in these animals (Gruys et al. 1994; Gonzalez et al. 2008; Eckersall and Bell 2010).

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MATERIAL AND METHODS

Animals. Goat breeding is, economically and socially, important in the Burdur province which is situated in the south-west part of Turkey. This province harbours one of the highest concentrations of farm animals in the country. Although cattle are more numerous than small ruminants, a considerable number of goats are reared in this area.

Saanen goats are one of the best known dairy goat breeds and have been successfully used to increase the milk yields of indigenous breeds of goats. They were introduced into Turkey in 1979 in order to upgrade the local breeds.

This study was conducted in March 2009. Six dead goats, from a group of 81 Saanen goats were brought to the Veterinary Medical Teaching Hospital for necropsy and diagnosis from a farm in Burdur province. According to the history taken from the owner, the dead goats had showed anorexia, depression, some of them blindness, followed by recumbency and coma before death. At necropsy, in two of six goats foetuses showed autolysis, in the other four goats there was severe fatty degeneration of the liver and abdominal cavity, dehydration, and the presence of more than one foetus. Ruminal fluid and urine samples were collected from these four dead goats. Ruminal fluids were acidic and pH values were 5.2, 5.3, 5.5 and 5.6, respectively. Urine samples were analysed using a dipstick; all four samples were 5.2, 5.3, 5.5 and 5.6, respectively. Urine samples were obtained by voluntary micturition or induced by covering the nose and the mouth of the goat for a few seconds. Urine samples were analysed using the Rothera test and Idexx UA Strips (urine test strips) for the diagnosis of ketosis (IDEXX UA Strips, IDEXX Laboratories Inc., Westbrook, ME, USA).

Blood and urine samples were collected into vacuum tubes with heparin. Venous blood pH was analysed using an automated blood gas analyser (Roche OPTI CCA blood gas analyser, Roche, Mannheim, Germany).

Serum concentrations of haptoglobin, serum amyloid A and tumour necrosis factor were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer’s instructions (Goat Hpt/HP, SAA and TNF-α ELISA KIT, Cusabio Biotech Co., Suffolk, UK).

Statistical analyses. The one-way analysis of variance test was used to statistically evaluate any differences between groups. For the determination of differences, Duncan’s test was used. Calculations were made using the SPSS 10.0 program pack. A P < 0.05 value was regarded as statistically significant.

RESULTS

A total of 75 pregnant Saanen goats were included in this study. On the basis of serum glucose concentrations goats were classified as having physiological, low or high glucose values (Tables 1 and 2).

Fifty seven goats had glucose concentrations between 3.04–4.68 mmol/l, and were included in the healthy control group (Group 1). Eleven goats had kept in a thermal box with ice during their transportation to the laboratory.

Clinical biochemistry. Blood samples were collected in tubes without anticoagulant and were centrifuged at 3000 rpm, at 4 °C for 10 min. Serum samples were carefully harvested and stored at −20 °C until used. These sera were then used to establish the concentrations of glucose, Ca, ALT, AST and triglyceride levels using an auto analyser and commercial kits (VET TEST 8008, IDEXX Laboratories Inc., Westbrook, ME, USA).

Blood concentrations of D-3-hydroxybutyrate level (BHBA) were determined using a commercially available test kit spectrophotometrically according to the manufacturer’s instructions (Randox Laboratories Limited, Crumlin, UK).

Samples for determination of blood pH values were collected into vacuum tubes with heparin. Venous blood pH was analysed using an automated blood gas analyser (Roche OPTI CCA blood gas analyser, Roche, Mannheim, Germany).

Urine samples were obtained by voluntary micturition or induced by covering the nose and the mouth of the goat for a few seconds. Urine samples were analysed using the Rothera test and Idexx UA Strips (urine test strips) for the diagnosis of ketosis (IDEXX UA Strips, IDEXX Laboratories, Westbrook, ME, USA).

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glucose concentrations between 1.07–2.91 mmol/l; these animals were categorised as having subclinical pregnancy toxaemia (Group 2). Seven goats had serum glucose concentrations between 7.37–32.06 mmol/l; the animals in this group were categorised as having clinical pregnancy toxaemia (Group 3).

**Goats in Group 1 (n = 57) were between days 100–110 of gestation. In this group there was no ketonuria or any clinical signs.**

**Goats in Group 2 (n = 11) were between days 110–130 of gestation. In these animals anorexia, grinding on the teeth, depression, in some of them blindness, and ketonuria (+++) were seen.**

Three of the goats in Group 3 had serum glucose concentrations above 30 mmol/l; these animals were classified as having severe clinical pregnancy toxaemia. One of the goats in this group had a serum glucose concentration of 86.2 mmol/l.

**Table 1. Serum biochemical values in 75 pregnant goats with minimum and maximum values**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n = 57) normal glucose</th>
<th>Group 2 (n = 11) low glucose</th>
<th>Group 3 (n = 7) high glucose</th>
<th>Reference P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>3.04–4.68</td>
<td>1.07–2.91</td>
<td>7.37–32.06</td>
<td>3–5.17</td>
</tr>
<tr>
<td>BHB (mmol/l)</td>
<td>0.13–0.84</td>
<td>0.90–8.08</td>
<td>3.35–14.07</td>
<td>&gt; 0.86* ≤ 0.86**</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>0.17–0.83</td>
<td>0.26–1.38</td>
<td>0.50–2.82</td>
<td>0.11–0.33</td>
</tr>
<tr>
<td>Ca (mmol/l)</td>
<td>1.31–3.19</td>
<td>0.80–1.90</td>
<td>0.92–1.86</td>
<td>2.05–2.45</td>
</tr>
<tr>
<td>Na (mmol/l)</td>
<td>138–159</td>
<td>139–152</td>
<td>125–130</td>
<td>142–155</td>
</tr>
<tr>
<td>K (mmol/l)</td>
<td>3.50–5.70</td>
<td>3.50–5.70</td>
<td>1.10–2.90</td>
<td>3.90–5.4</td>
</tr>
<tr>
<td>AST (µkat/l)</td>
<td>0.595–8.279</td>
<td>0.986–5.185</td>
<td>4.233–10.642</td>
<td>1.02–4.76</td>
</tr>
<tr>
<td>ALT (µkat/l)</td>
<td>0.153–0.663</td>
<td>0.221–2.278</td>
<td>1.955–2.924</td>
<td>0.374–0.646</td>
</tr>
<tr>
<td>HP (g/l)</td>
<td>0.00–0.07</td>
<td>0.74–2.70</td>
<td>1.30–2.95</td>
<td>0.00–0.05***</td>
</tr>
<tr>
<td>SAA (mg/l)</td>
<td>2.22–9.45</td>
<td>1.75–25.17</td>
<td>213.21–520.11</td>
<td>1.69–11.94***</td>
</tr>
<tr>
<td>TNF–α (pg/ml)</td>
<td>32.63–57.72</td>
<td>68.90–99.45</td>
<td>317.72–420.21</td>
<td></td>
</tr>
</tbody>
</table>

*subclinical pregnancy toxaemia, **healthy animals, ***limit of detection of the assay (Gonzalez et al. 2008)

**Table 2. Mean blood biochemical values in healthy, subclinical and clinical pregnancy toxaemia goats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n = 57) normal glucose</th>
<th>Group 2 (n = 11) low glucose</th>
<th>Group 3 (n = 7) high glucose</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>3.71 ± 0.44^b</td>
<td>2.13 ± 0.69^b</td>
<td>13.01 ± 9.04^a</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>BHB (mmol/l)</td>
<td>0.47 ± 0.58^c</td>
<td>2.34 ± 2.29^b</td>
<td>6.63 ± 3.44^a</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>0.53 ± 0.49^c</td>
<td>0.70 ± 0.47^b</td>
<td>1.60 ± 0.91^a</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>Blood pH</td>
<td>7.36 ± 0.01^c</td>
<td>7.16 ± 0.10^b</td>
<td>6.80 ± 0.16^a</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>Ca (mmol/l)</td>
<td>2.15 ± 0.43^b</td>
<td>1.48 ± 0.38^a</td>
<td>1.14 ± 0.46^a</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>Na (mmol/l)</td>
<td>146.80 ± 5.52^b</td>
<td>145.18 ± 4.44^b</td>
<td>127.85 ± 1.77^a</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>K (mmol/l)</td>
<td>4.56 ± 0.58^b</td>
<td>4.32 ± 0.73^b</td>
<td>2.21 ± 0.57^a</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>AST (µkat/l)</td>
<td>1.47 ± 1.07^b</td>
<td>2.46 ± 1.30^b</td>
<td>7.10 ± 2.39^a</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>ALT (µkat/l)</td>
<td>0.40 ± 0.12^c</td>
<td>0.88 ± 0.78^b</td>
<td>2.27 ± 0.37^a</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>HP (g/l)</td>
<td>0.01 ± 0.01^c</td>
<td>1.20 ± 0.67^b</td>
<td>1.86 ± 0.69^a</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>SAA (mg/l)</td>
<td>5.02 ± 2.19^b</td>
<td>10.23 ± 6.35^b</td>
<td>357.75 ± 105.50^a</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>TNF–α (pg/ml)</td>
<td>46.68 ± 6.45^c</td>
<td>84.75 ± 12.33^b</td>
<td>352.79 ± 38.66^a</td>
<td>&lt; 0.001***</td>
</tr>
</tbody>
</table>

^b^c^ mean values marked with different superscripts in the same line are significantly different from each other (P < 0.001) ***statistically highly significant.
them exhibited more severe clinical signs. Serum BHBA values in three goats were 3.60, 4.54 and 8.08 mmol/l. Caesarean sections were not performed on these animals because they were in the recumbent stage and their chance of survival was low. After injecting a combination of dexamethasone (1 mg/10 kg b.w., i.m.), and dexcloprostenol (125 μl, i.m.) (Lima et al. 2012) two dead foetuses were delivered. Recovery was not seen in three goats. They died 48–72 h after treatment. Other goats in this group were treated with 5–7 g of glucose (i.v.) 6–8 times a day in conjunction with 30–40 units of zinc protamine insulin (i.m.) every other day for three days (Radostits et al. 2008).

Goats in Group 3 (n = 7) were between days 120–140 of gestation and they had shown recumbency and coma. Ketonuria in these goats was severe (+++).

All goats in Group 3 (n = 7) died within 4 h after the farm was visited. In this group all animals carried two dead foetuses.

**DISCUSSION**

Pregnancy toxaemia has a significant economic impact on goat enterprises due to loss of foetuses, veterinary costs, and loss of the dams (Rook 2000). In severe cases, morbidity and mortality rates can reach up to 20% and 80%, respectively (Andrews 1997; Rook 2000).

The last six weeks of gestation in goats (and ewes) are a critical period for the pregnant animal because approximately 80% of the foetal growth occurs during this period (Bergman 1993).

Pregnancy toxaemia in small ruminants occurs because of the competition for glucose between the pregnant animals and their foetuses, as the latter undergo intensive growth (Bulgin 2005).

Hypoglycaemia and hyperketonaemia are the primary metabolic disturbances in pregnancy toxoaemia (Radostits et al. 2008). However, hyperglycaemia may also develop. Blood levels of glucose in goats with pregnancy toxoaemia vary dramatically and this gave rise to the idea that hypoglycaemia might indicate that the foetuses are alive and hyperglycaemia that the foetuses are dead (Bulgin 2005; Lima et al. 2012). In this study all goats with high serum glucose concentrations and which were in the last four weeks of the gestation period (Group 3), had two dead foetuses and these results are similar to previous reports. Hyperglycaemia occurs because foetal death removes the suppressing effect of the foetus on hepatic gluconeogenesis (Wastney et al. 1983). Compared with the control (Group 1), glucose values were decreased significantly (P < 0.001) in Group 2 and only three of them delivered dead foetuses.

The rate of hepatic ketone body production usually increases 4–5 times in sheep during late gestation and in early lactation. Under conditions of an insufficient energy supply (starvation, spontaneous ketosis etc.), alimentary ketogenesis decreases and the rate of hepatic ketogenesis from NEFA disproportionately increases. The liver now becomes the main and eventually almost the sole ketogenic organ. Such metabolic changes are associated with marked increases in the rate of mobilisation of long chain fatty acids from adipose tissues and a marked rise in circulating concentrations of NEFA and ketone bodies. During hyperketonaemia ketone bodies are readily oxidised serving as fuels for energy production. Many peripheral tissues, like the heart, skeletal muscle, kidney, non-foetal uterine tissues and the lactating mammary gland can harvest large amounts of energy from the oxidation of ketone bodies (Harmeyer and Schlumbohm 2006).

In late gestation, the abdominal space is filled with accumulated fat and an ever-expanding uterus. Because of the lack of rumen space, these females have difficulty consuming enough feedstuffs to satisfy their energy requirements (Pugh 2002). In our study, huge accumulations in fat were observed in the abdominal cavities at the necropsies.

In sheep and goats, pregnancy toxoaemia is much more common in highly prolific breeds (Smith and Sherman 1994) such as the Saanen breed goats in this study.

Ewes and goats with BHB concentrations of 0.86–1.6 mmol/l are classified as having mild or subclinical pregnancy toxoaemia (Ramin et al. 2005; Bani et al. 2008). According to the serum glucose and BHB concentrations in this study we classified goats as normal (Group 1), exhibiting subclinical pregnancy toxoaemia (Group 2), or exhibiting clinical toxoaemia (Group 3) (Table 1).

In this study all goats with high serum glucose and BHBA had two dead foetuses (Group 3); mean serum glucose and BHBA values were found to be 13.01 ± 9.04 and 6.63 ± 3.44, respectively. Glucose and BHB values were significantly (P < 0.001) higher in the subclinical (Group 2), and clinical (Group 3) pregnancy toxoaemia groups than in the normal group.
In goats, it has been reported that ketonaemia leads to a metabolic acidosis and changes in acid base parameters could be used as indicators of early pregnancy toxoaemia (Gonzalez et al. 2011). In this study in Group 1 serum BHB levels and blood pH values were within normal ranges; in Groups 2 and 3 serum BHB levels increased and blood pH values then gradually decreased.

Unlike previous findings in goats with subclinical pregnancy toxoaemia (Bani et al. 2008), serum concentrations of triglycerides and serum activities of AST in goats with subclinical and clinical pregnancy toxoaemia were significantly (P < 0.001) increased compared with those of normal animals in this animals. However, the serum activity of AST significantly increased (P < 0.001) in clinical pregnancy toxoaemia goats compared with normal and subclinical pregnancy toxoaemia animals.

In human patients with ketoacidosis and ketonuria, there is a marked loss of K\(^+\) in the urine leading to hypokalaemia (Rose and Post 2001). Hypokalaemia could be explained in part because the goats were not eating and, therefore, their dietary K\(^+\) intake would have been reduced. These two mechanisms, acting together, resulted in hypokalaemia (Lima et al. 2012).

Serum potassium concentrations are decreased in ewes with pregnancy toxoaemia (Halford and Sanson 1983); in this study in comparison to normal and subclinical pregnancy toxoaemia goats, serum potassium concentrations progressively decreased in the clinical pregnancy toxoaemia group. In addition, serum sodium concentrations in normal and subclinical pregnancy toxoaemic goats did not show significant differences, while a significant decrease in goats with clinical pregnancy toxoaemia was observed. Therefore, these parameters are good indicators of clinical pregnancy toxoaemia. On the other hand, serum calcium concentrations in both subclinical and clinic pregnancy toxoaemic goats were found to be significantly altered.

The acute phase response (APR) is defined by the secretion of fibrinogen, serum amyloid A (SAA), haptoglobin (Hp) and α-1 acid glycoprotein principally by the liver (Vels et al. 2009).

It is clear that determination of animal APP values are not only useful for monitoring inflammatory processes for diagnostic and prognostic purposes but also for analysing various non-inflammatory conditions such as pregnancy, parturition, metabolic diseases and stress, which have previously been considered as not affecting APP values (Kent 1992). Haptoglobin and serum amyloid A proteins could be used as valuable indicators of inflammation in goats (Gonzalez et al. 2008).

In ruminants the circulating levels of Hp are negligible in normal animals, but increase over 100-fold on immune stimulation (Conner et al. 1998). Hp is induced in cows with fatty liver syndrome (Katoh et al. 2002). Increases in serum haptoglobin values can be a potential indicator of acidosis in goats (Gonzalez et al. 2010). In this study four of six dead goats exhibited acidic ruminal fluids at necropsy. Further, haptoglobin concentrations were increased and blood pH values in goats with subclinical and clinical pregnancy toxoaemia were decreased. Previous studies have reported that there is a significant correlation between Hp and BHB in subclinical pregnancy toxoaemia in goats (Trevisi et al. 2005; Gonzalez et al. 2011).

SAA concentrations are not affected by the induction of pregnancy toxoaemia (Gonzalez et al. 2011). In our study, serum concentrations of SAA in normal goats and in animals with subclinical pregnancy toxoaemia were not significantly altered but in those with clinical pregnancy toxoaemia values were significantly increased (Table 2).

Of the proinflammatory cytokines, tumour necrosis factor-alpha (TNF-α) is one of the major mediators of APP synthesis in the liver (Alsemgeest et al. 1996; Yoshioka et al. 2002).

A previous study has shown that in early pregnant ewes, tumour necrosis factor-α and acute-phase proteins increased after challenge with peptidoglycan-polysaccharide (Dow et al. 2010). Similar findings were made in this study. A sharp increase was observed in serum TNF-α activity for the subclinical and especially clinical pregnancy toxoaemia groups.

In conclusion, the blood levels of glucose in goats with pregnancy toxoaemia can be a good indicator of the viability of the foetuses. Blood pH values and serum β-hydroxyl butyrate, triglyceride, calcium, ALT, haptoglobin and tumour necrosis factor-alpha values can be a good indicator of subclinical and clinical pregnancy toxoaemia. On the other hand, sodium, potassium, AST, and serum amyloid A values can be used for the diagnosis of clinical pregnancy toxoaemia in goats.

REFERENCES


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