Analysis of correlations between acetylcholinesterase, haptoglobin, tumor necrosis factor, serum amyloid A, and paraoxonase in Saanen goats with pregnancy toxemia*

İSMAIL AYTEKIN1, BIROL GÜNGÖR2, SEZAI ARSLAN3

1Department of Laboratory Animals, Faculty of Veterinary Medicine, Afyon Kocatepe University, TR03200, Afyonkarahisar, Türkiye
2Clinician Veterinarian, TR10100 Balıkesir, Türkiye
3Department of Internal Medicine, Faculty of Veterinary Medicine, Tekirdag Namik Kemal University, TR59030, Suleymanpasa, Türkiye

Received 05.08.2023 Accepted 27.10.2023

Aytekin İ., Güngör B., Arslan S.

Analysis of correlations between acetylcholinesterase, haptoglobin, tumor necrosis factor, serum amyloid A, and paraoxonase in Saanen goats with pregnancy toxemia

Summary

The present study investigated the levels of acetylcholinesterase, tumor necrosis factor-α (TNF), paraoxonase (PON1), haptoglobin (HPT), serum amyloid A (SAA), beta-hydroxybutyric acid (BHBA), non-esterified fatty acid (NEFA), glucose, total protein, cholesterol, and triglyceride in Saanen goats with pregnancy toxemia. The animal material included Saanen goats aged 2-5 years. The study was conducted on a total of 20 Saanen goats: 10 pregnant and 10 healthy animals. After clinical examination, 10 goats with BHBA levels of more than 1.5 mmol/L of blood were included in the pregnancy toxemia group, and 10 healthy goats with BHBA levels of less than 1 mmol/L were included in the control group. There were no significant differences between the total protein, cholesterol, and triglyceride levels in the two groups. It was determined that, while the glucose level was low in the goats with pregnancy toxemia, BHBA, NEFA, acetylcholinesterase, tumor necrosis factor-α, paraoxonase, serum amyloid A, and haptoglobin levels were higher than they were in the healthy goats. In conclusion, it appears that ACHE, TNFa, PON, SAA, and HPT may be useful as additional indicators in the diagnosis of pregnancy toxemia and in the development of methods for its prevention.

Keywords: acetylcholinesterase, haptoglobin, paraoxonase, serum amyloid A, tumor necrosis factor-α, goat, pregnancy toxemia, inflammation

Pregnancy toxemia is a metabolic disease due to the disruption of carbohydrate, glucose, and fat metabolisms observed in sheep and goats during the final stages of pregnancy. The disease is characterized by partial anorexia and depression, often with neurological signs, progressing to recumbency and death. It occurs more often in animals carrying multiple fetuses. In goats, the disease develops during the last stage of pregnancy and the milking period (4, 24, 28).

There are two forms of cholinesterase in mammals: acetylcholinesterase and pseudocholinesterase. Acetylcholinesterase (ACHE) and pseudocholinesterase are produced mainly in the liver (17, 28). Cholinesterase has been measured in the plasma and liver of cattle, camels, goats, and sheep, and its highest levels were determined in goats (23).

Haptoglobin (Hp) and serum amyloid A (SAA) are regarded as major acute phase proteins (APPs) in various animals, including goats (9). It was observed that serum haptoglobin (HPT) and serum amyloid A (SAA) levels increased in liver diseases, infectious diseases, and parasitic diseases (20, 21). It was determined that SAA and HPT levels were higher in Saanen goats with pregnancy toxemia than they were in healthy goats (2).

*This study funded is by the Scientific Research Projects Unit of Balikesir University, Turkey (Grant no: 2019/124).
Serum paraoxonase (PON) enzyme, associated with high-density lipoprotein (HDL) cholesterol in mammals, protects the body against several harmful chemicals (19). PON levels are lower in sheep with fatty liver than they are in healthy sheep (7). Paraoxonase-1 (PON-1) levels decrease on the first day of the last month of pregnancy in Turkish Saanen goats and increase on prenatal days 15 and 30 (24).

Tumor necrosis factor-alpha (TNFα) is induced by various stimuli, such as tumor cells, bacteria, viruses, parasites, cytokines, and inflammation. TNFα is responsible for neoplastic tissue destruction and certain local and systemic effects induced by tumors (8). TNF levels reported in Saanen goats with pregnancy toxemia were higher than they were in healthy pregnant goats (2).

In recent years, the identification of markers for assessing the inflammatory process has emerged as a valuable avenue of research. These markers hold the potential to provide insights into the underlying dynamics of inflammatory conditions and aid in their estimation. However, while numerous studies have explored the utility of these markers in various contexts, a significant research gap persists in the domain of pregnancy toxemia in goats. Despite the evident importance of understanding and managing pregnancy toxemia, there is a conspicuous absence of investigations focused on unraveling the intricate relationship between inflammatory processes and this condition in goats. This dearth of research highlights the pressing need to explore the potential of inflammatory markers within the specific context of pregnancy toxemia in goats, which could contribute to enhanced diagnostic and therapeutic strategies for this condition.

The present study investigated the levels of ACHE, TNFα, PON, SAA, beta-hydroxybutyric acid (BHBA), non-esterified fatty acids (NEFA), glucose, total protein, cholesterol, and triglyceride in Saanen goats with pregnancy toxemia.

### Material and methods

**Animal material.** This study was conducted on a total of 20 Saanen goats bred on a farm in the Balikesir province in Turkey: 10 goats with pregnancy toxemia (group I) and 10 healthy goats (group II). They were 2-5 years old, had completed two or more lactations, and weighed 40-60 kg. The goats were clinically examined. The pregnant goats were in the last 4 weeks of pregnancy, confined to the ground and exhibited loss of appetite, depression, lethargy, muscle dysfunction, lack of coordination, and ataxia. After clinical examination, 10 goats with blood levels of BHBA above 1.5 mmol/L were assigned to the pregnancy toxemia group, and 10 healthy non-pregnant goats with BHBA levels of less than 1 mmol/L were assigned to the control group. All goats in both groups were kept under the same feeding, care, and environmental conditions. Standard daily rations given to goats included barley (500 g), wheat bran (400 g), and alfalfa hay (1 kg). The ration was fed twice a day (at 6 a.m. and 6 p.m.). Fresh water was available all the time.

**Blood analysis.** Blood samples of 10 ml were collected from v. jugularis of the goats diagnosed with pregnancy toxemia into tubes (Vacusera, Türkiye) without anticoagulant after the clinical examination. The blood samples were immediately centrifuged (with Biosan LMC4200R, Türkiye) at 5000 rpm for 5 min to obtain the serum. Two samples were transferred into Eppendorf tubes and stored at −80°C until analysis. After the blood serum samples were collected, they were sent to a private laboratory for analysis.

**Biochemical analysis.** The levels of acetylcholinesterase, tumor necrosis factor-α, paraoxonase, haptoglobin, and serum amyloid A were measured by the ELISA (Enzyme-Linked Immunosorbent Assay) method (Goat SunRed ELISA Kit cat. No: E90440, Eastbiopharm, China).

Biochemical analyses were conducted with a Randox brand Daytona model device (United Kingdom). Randox brand kits were used to study the biochemical parameters. BHBA (Cat. No: RB1007) was determined by the enzymatic kinetic method, total cholesterol (Cat. No: CH3810) was determined by the enzymatic endpoint method, glucose (Cat. No: GL 3815) and NEFA (Cat. No: FA1115) were determined by the colorimetric method, total protein (Cat. No: TP38669) was determined by the Biuret Reagent endpoint method, and triglycerides (Cat. No: TR3823) were determined by the lipase/GPO-PAP method.

**Ethical statement.** This study conformed to instructions from the Balikesir University Animal Experiments Local Ethics Committee Presidency and was approved (Approval number: BAUHADYEK 2019/12-7).

**Statistical analysis.** The results are expressed as means ± SD. The data were analyzed with the SPSS 20 statistical software (IBM, USA). An independent samples T test was used to determine the statistical significance between the two groups. The difference between the groups in terms of the parameters examined was considered statistically significant at the P < 0.05 level.

### Results and discussion

**Clinical findings.** The goats with pregnancy toxemia included in the study were characterized by anorexia, lagging behind the herd, lethargy, bad breath, shaggy gait, teeth grinding, ataxia, difficulty in standing, blindness, confinement to the ground, head resting, muscle tremors, loss of consciousness and mortality.

**Biochemical findings.** The results of biochemical analyses for the goats with pregnancy toxemia and the healthy goats are presented in Tables 1 and 2.

The analysis of blood serum samples revealed that SAA, HPT, TNF, PON1, and ACHE levels were higher in the goats with pregnancy toxemia than they were in the healthy non-pregnant goats (P < 0.001), (Tab. 1).

The analyses conducted on the blood serum samples demonstrated that BHBA and NEFA levels were higher in the goats with pregnancy toxemia than they were in the healthy non-pregnant goats (P < 0.001). The glucose levels were lower in the goats with pregnancy toxemia.
Tab. 1. Acetylcholinesterase, haptoglobin, tumor necrosis factor-α, serum amyloid A, and paraoxonase levels in Saanen goats with pregnancy toxemia and healthy Saanen goats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Saanen goats (n = 10)</th>
<th>Saanen goats with pregnancy toxemia (n = 10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA (µg/mL)</td>
<td>5.91 ± 1.08a</td>
<td>18.56 ± 0.81a</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>HPT (µg/mL)</td>
<td>149.58 ± 23.56a</td>
<td>341.32 ± 18.76a</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>TNFα (µg/mL)</td>
<td>50.44 ± 7.32a</td>
<td>166.66 ± 6.81a</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>PON 1 (µg/mL)</td>
<td>81.35 ± 15.56a</td>
<td>247.96 ± 13.74a</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>ACHE (ng/mL)</td>
<td>12.27 ± 2.41a</td>
<td>25.12 ± 1.31a</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Explanations: a-b – values within a row with different superscripts differ significantly at P < 0.05; NS – not significant; BHBA – beta-hydroxybutyric acid; NEFA – non-esterified fatty acid

Tab. 2. Biochemical parameters in Saanen goats with pregnancy toxemia and healthy Saanen goats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Saanen goats (n = 10)</th>
<th>Saanen goats with pregnancy toxemia (n = 10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>56.50 ± 9.59a</td>
<td>31.20 ± 5.41a</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>BHBA (mM/L)</td>
<td>0.40 ± 0.11a</td>
<td>7.95 ± 2.09a</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>54.20 ± 13.61a</td>
<td>57.70 ± 11.14a</td>
<td>NS</td>
</tr>
<tr>
<td>NEFA (mM/L)</td>
<td>0.42 ± 0.25a</td>
<td>1.35 ± 0.34a</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>22.50 ± 9.66a</td>
<td>17.80 ± 4.18a</td>
<td>NS</td>
</tr>
<tr>
<td>Total Protein (g/dL)</td>
<td>6.56 ± 0.52a</td>
<td>6.33 ± 0.53a</td>
<td>NS</td>
</tr>
</tbody>
</table>

Explanations: a-b – values within a row with different superscripts differ significantly at P < 0.05; NS – not significant; BHBA – beta-hydroxybutyric acid; NEFA – non-esterified fatty acid

toxemia than they were in the healthy goats (P < 0.001). There were no significant differences between the cholesterol, triglyceride, and total protein levels of the goats with pregnancy toxemia and the healthy goats (Tab. 2).

We hypothesized that important changes in levels of acetylcholinesterase, tumor necrosis factor-α, paraoxonase, haptoglobin, and serum amyloid A can occur in Saanen goats with pregnancy toxemia. In the study, it was determined that, while the glucose level was low in the goats with pregnancy toxemia, their BHBA, NEFA, ACHE, TNFα, PON, SAA, and HPT levels were higher than those in the healthy goats. It was determined that SAA, TNF, and HPT levels were higher in Saanen goats with pregnancy toxemia than they were in healthy goats (2). Acetylcholinesterase levels were lower in cows with clinical ketosis than they were in the control group (29). Paraoxonase levels were lower in sheep with fatty liver than they were in healthy sheep (7).

It was reported that lagging behind the herd, loss of appetite, lethargy, shaky gait, bad breath, difficulty in standing, teeth grinding, blindness, head resting, confinement to the ground, loss of consciousness, muscle tremors, and mortality are observed in goats with pregnancy toxemia (3, 14, 20, 24, 28). In this study, the above-mentioned signs were also observed in the goats with pregnancy toxemia.

Previous studies on sheep report that total protein in sheep with pregnancy toxemia was lower than it was in healthy non-pregnant sheep and healthy pregnant sheep (3, 14). In Turkish Saanen goats, serum total protein levels were low on days −30 and 0, and high on days −15, +15, and +30 during the last month of pregnancy and the one-month transition period after birth (24). In the present study, although total protein levels were lower in the Saanen goats with pregnancy toxemia than they were in the healthy sheep, the difference was insignificant.

Glucose levels were lower in sheep with pregnancy toxemia than they were in healthy sheep (14, 20, 22). It was reported that glucose levels were lower in Awassi sheep with pregnancy toxemia than they were in both healthy non-pregnant sheep and healthy pregnant sheep (18). The glucose level in Barki sheep with pregnancy toxemia was also lower than it was in both healthy non-pregnant sheep and healthy pregnant sheep (3). It was found that the glucose levels in goats with severe pregnancy toxemia were lower than those in goats with mild pregnancy toxemia (1). In the present study, it was determined that glucose levels in the goats with pregnancy toxemia were lower than they were in the healthy goats.

It was found that BHBA levels were higher in sheep with pregnancy toxemia than they were in healthy sheep (15, 18, 20). BHBA levels reported in sheep with pregnancy toxemia were higher than those in healthy non-pregnant sheep and healthy pregnant sheep in the last month of pregnancy (20). BHBA levels were higher in Awassi sheep with pregnancy toxemia than they were in both healthy and pregnant sheep with subclinical toxemia (15). It was reported that BHBA levels were higher in Awassi sheep with pregnancy toxemia than they were in healthy non-pregnant and healthy pregnant sheep (18). In goats with pregnancy toxemia induced by 72 h fasting, BHBA levels increased at hours 24, 48, and 72 (12). It was determined that BHBA levels in Saanen goats with pregnancy toxemia were higher than those in healthy pregnant goats (1). In the present study, it was established that BHBA levels in the goats with pregnancy toxemia were higher than they were in the healthy goats.

NEFA levels increased more in sheep with clinical pregnancy toxemia than they did in both healthy non-pregnant sheep and healthy pregnant sheep in the last period of pregnancy (3). It was reported that pregnancy toxemia induced in goats by 72 h fasting resulted in increased NEFA levels at hours 24, 48, and 72 (27). NEFA levels were higher in sheep with pregnancy toxemia than they were in healthy sheep (27). It was
found that NEFA levels were higher in sheep with pregnancy toxemia than they were in non-pregnant sheep and healthy sheep in the last month of pregnancy (20). NEFA levels were reported to be higher in goats with pregnancy toxemia than they were in healthy pregnant goats (22). In the present study, NEFA levels were higher in the goats with pregnancy toxemia than they were in the healthy goats.

A study conducted on Barki sheep indicated that cholinesterase levels were lower in healthy sheep in the last period of pregnancy than they were in healthy non-pregnant sheep (3). It was reported that cholinesterase levels in sheep with pregnancy toxemia were lower than those in healthy non-pregnant sheep and healthy pregnant sheep in the last month of pregnancy (14). It was found that cholesterol levels were lower in sheep with pregnancy toxemia compared to healthy sheep (16). In the present study, no statistically significant difference was found between the cholesterol levels of the goats with pregnancy toxemia and those of the healthy goats.

Triglyceride levels were higher in sheep with pregnancy toxemia than they were in healthy non-pregnant sheep and healthy pregnant sheep in the last month of pregnancy (20). Higher triglyceride levels were determined in sheep with pregnancy toxemia than in healthy sheep (16). It was found that triglyceride levels were higher in Saanen goats with pregnancy toxemia than they were in healthy pregnant goats (2). In the present study, it was found that triglyceride levels in the goats with pregnancy toxemia were not statistically significantly different from those in the healthy goats.

Pseudocholinesterase levels were determined in 40 Nubian goats, and those levels were reduced by topical administration of fenthion (13). Acetylcholinesterase levels were lower in cows with clinical ketosis than they were in the control group (26). In a study on the liver parameters of cows with ketosis, it was found that cholinesterase values for those cows were lower than they were for the control group (29). Three distinct types of cholinesterase levels were measured in the plasma and liver tissue of cattle, camels, goats, and sheep, and the highest cholinesterase levels were determined in goats (23). In the present study, acetylcholinesterase levels were found to be higher in the goats with pregnancy toxemia than they were in the healthy goats.

In a study conducted on Saanen goats, SAA levels in goats in the last period of pregnancy were not statistically significantly different from those in non-pregnant and non-milking sheep (25). Higher SAA levels were found in sheep with pregnancy toxemia than in healthy non-pregnant sheep (20). In a study conducted on goats, it was reported that there was no significant difference in SAA levels measured at hours 24, 48, and 72 in goats with pregnancy toxemia induced by 72 h fasting (12). In a study conducted on Barki sheep, SAA levels were found to be higher in healthy pregnant sheep in the last period of the pregnancy than in healthy non-pregnant sheep (3). Another study conducted on Awassi sheep also found that SAA levels increased in sheep with pregnancy toxemia when compared to healthy sheep (15). SAA levels were higher in Saanen goats with pregnancy toxemia than they were in healthy pregnant goats (2). In the present study, SAA levels in the goats with pregnancy toxemia were found to be higher than those in the healthy goats.

A study conducted on Barki sheep revealed that haptoglobin levels were higher in healthy sheep in the last period of pregnancy than they were in healthy non-pregnant sheep (3). It was also determined that haptoglobin levels increased more in sheep with pregnancy toxemia than they did in healthy non-pregnant sheep (3). Haptoglobin levels were found to be higher in sheep with pregnancy toxemia than they were in healthy non-pregnant sheep (20). In Awassi sheep with pregnancy toxemia, haptoglobin levels were higher than they were in healthy and pregnant sheep with subclinical pregnancy toxemia (15). It was found that haptoglobin levels were higher in Saanen goats compared to non-pregnancy in the late pregnancy (25). Haptoglobin levels were found to be higher in Saanen goats with pregnancy toxemia than they were in healthy pregnant goats (19). In the present study, it was found that the haptoglobin levels in the goats with pregnancy toxemia were higher than they were in the healthy goats.

The activity of serum PON1 in a fatty liver group was significantly decreased when compared with healthy control group (7). It was found that serum paraoxonase levels were lower in postpartum Holstein cows with fatty liver than they were in healthy non-pregnant cows (11). However, the authors also pointed out that there was no standardized method for the measurement of paraoxonase. A commonly used method was paraoxon hydrolysis, and different results could be obtained by methods that measured paraoxonase, so they concluded that the physiological interpretation of these levels was challenging (6, 11). In a study conducted on Saanen goats in Turkey, it was reported that serum paraoxonase-1 levels in the last month of pregnancy were lowest on day 0, high on day −15, and highest on day −30 (24). In the present study, paraoxonase levels in the goats with pregnancy toxemia were higher than those in the healthy goats. Since there were no studies on paraoxanase in goats with gestational toxemia, no comparison could be made. However, in view of the findings of a study on pregnant goats (24), we believe that the reason for the increase in PON in the goats with pregnancy toxemia was pregnancy toxemia.

It was found that TNF values were higher in animals with ketosis than they were in healthy animals (10, 31). TNF levels in cows with subclinical ketosis were higher than those in healthy cows (5). A study
Conducted on Barki sheep revealed that TNF levels in sheep with pregnancy toxemia were higher than those in healthy non-pregnant sheep and healthy pregnant sheep in the last period of pregnancy (3). TNF levels in sheep with mild and severe pregnancy toxemia were higher than those in healthy sheep (30). It was found that TNF levels were higher in Saanen goats with pregnancy toxemia than they were in healthy pregnant goats (2). In the present study, TNF levels in the goats with pregnancy toxemia were higher than those in the healthy goats.

In conclusion, it appears that ACHE, TNFa, PON, SA, and HPT may be useful as additional indicators in the diagnosis of pregnancy toxemia and in the development of methods for its prevention.

References


Corresponding author: Prof. Dr. Ismail Aytekin, Öğretim Üyesi, Veteriner Fakültesi, Afyon Kocatepe Üniversitesi, TR03200, Afyonkarahisar, Türkiye; e-mail: aytekin303@gmail.com