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Evaluation of Serum 25-(OH) D Vitamin Concentration in Healthy and Pregnancy Toxemic Goats

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Abstract

The goal of the research was to assess serum 25-(OH) D concentration in healthy goats and goats with pregnancy toxemia.This research used 30 goats during the final three weeks of gestation. 10 of them were evaluated as a control group (CG), 10 as subclinical pregnancy toxemia (SPT), and the remaining 10 as clinical pregnancy toxemia (CPT). Pregnancy toxemia in goats was diagnosed by analysis of blood beta-hydroxybutyric acid (BHBA) concentration in the Freestyle Optium Neo-H device. While glucose, iron, and unsaturated iron-binding capacity (UIBC) concentrations were analyzed by an automatic biochemistry analyzer, serum 25-(OH) D concentration was investigation by liquid chromatography-mass spectrometry. Blood BHBA concentrations in SPT and CPT were statistically elevated versus the control group (P<0.001). The 25-(OH) D vitamin level in goats with pregnant toxemia had been found to be substantially lower than that in the control group (P<0.001). The serum iron concentration in goats with pregnant toxemia was substantially lower than that in the CG (P<0.010). The sensitivity of serum 25-(OH) D in the diagnosis of pregnancy toxemia was 90%, specificity was 95%, the threshold value was 26.12 µg/L, and the AUC value was 0.95. It was observed that pregnancy toxemia in goats showed a strong negative correlation with 25-(OH) D and BHBA and a significant positive correlation with glucose and iron. This study concluded that the 25-(OH) D levels in goats with pregnant toxemia were dramatically reduced and exhibited a notable correlation with inflammation.

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Sağlıklı ve Gebelik Toksemili Keçilerde Serum 25-(OH) D Konsantrasyonunun Değerlendirilmesi

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Öz

Çalışmanın amacı sağlıklı keçilerde ve gebelik toksemisine sahip keçilerde serum 25-(OH) konsantrasyonunu değerlendirmektir. Bu araştırmada gebeliklerinin son üç haftasında olan 30 keçi kullanıldı. Bunlardan 10 tanesi kontrol grubu (CG), 10 tanesi subklinik gebelik toksemi (SPT) ve kalan 10 tanesi ise klinik gebelik toksemi (CPT) olarak değerlendirildi. Keçilerde gebelik toksemi tanısı, Freestyle Optium Neo-H cihazında kan beta-hidroksibütirik asit (BHBA) konsantrasyonunun analizi ile konuldu. Glikoz, demir ve doymamış demir bağlama kapasitesi (UIBC) konsantrasyonları otomatik biyokimya analizörü ile analiz edilirken, serum 25-(OH) D konsantrasyonu sıvı kromatografisi-kütle spektrometrisi ile analiz edildi. SPT ve CPT kan BHBA konsantrasyonlarının kontrol grubunda yükselmiş olduğu bulundu (P<0.001). Gebe toksemi olan keçilerde 25-(OH) D vitamin düzeyi CG'dekinden önemli ölçüde düşük olarak belirlendi (P<0.001). Gebe toksemi olan keçilerde serum demir konsantrasyonu CG'dekinden

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önemli ölçüde düşük bulundu (P<0.010). Serum 25-(OH) D'nin gebelik toksemisinin tanısında duyarlılığı % 90, özgüllüğü % 95, eşik değeri 26,12 ve AUC değeri 0,95 olarak bulundu. Keçilerde gebelik toksemisinin 25-(OH) D ve BHBA ile güçlü negatif korelasyon, glikoz ve demir ile anlamlı pozitif korelasyon gösterdiği görüldü. Bu çalışmada gebe toksemi olan keçilerde 25-(OH) D düzeylerinin önemli ölçüde azaldığı ve inflamasyonla belirgin bir korelasyon gösterdiği sonucuna varılmıştır.

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Introduction

Feeding animals incorrectly inadequately and in an unbalanced manner causes serious health problems in animal husbandry. The prenatal period, also known as the prepartum period, is a very important phase in the health of goats and their offspring (Ünal and Uztimür, 2025; Uztimür and Ünal, 2024b). Goats typically exhibit metabolic diseases at this stage due to poor nutrition. Among these diseases, pregnancy toxemia is a prevalent metabolic condition in goats (Ünal and Uztimür, 2025). Factors such as goats being pregnant with more than one kid and the mother's inability to meet the energy needs of these kids, inactivity, intensive nutrition, heavy parasitic invasions, malnutrition, transportation, and stress lead pregnant goats to use their existing energy reserves (Bani Ismail et al., 2008). The occurrence of the above-mentioned conditions in pregnant goats causes significant disruption of the carbohydrate and fat metabolism, and, accordingly, serious vital problems in pregnant goats (Fırat and Özpınar, 2002).
Vitamin D is a vitamin with a sterol structure and a fat-soluble hormone-like structure (Lippolis et al., 2011). Sunlight contains UV light which triggers the production of cholecalciferol, commonly referred to as vitamin D3. The 25-hydroxylase enzyme change it into 25-(OH) vitamin D here. 25-(OH) D vitamin plays a role in important events such as absorption of calcium from the small intestines, maintenance of calcium phosphorus balance, calcium homeostasis with the skeletal muscle system, as well as regulation of the immune system, skin, apoptosis, and cell differentiation (Lippolis et al., 2011; Horst et al., 1994). Research has found a notable correlation between a lack of vitamin D and medical issues including hypocalcemia, osteomalacia, laminitis, and fractures of the pelvis (Horst et al., 1994; Goff, 2006; Martinez et al., 2018). Furthermore, studies have reported that 25(OH)D supplementation or infusion enhances the oxidative capacity of neutrophils, effectively combating transition period diseases like metritis and mastitis, and significantly lowering the number of bacteria in milk and retentio secundinarum (Goff et al., 1991; Martinez et al., 2018; Lippolis et al., 2011). There are a small number of studies evaluating the 25-(OH) level on metabolism in goats, but no study has been found to determine the 25-(OH) concentration, especially in goats with pregnancy toxemia. This study targeted to assess serum 25-(OH) D levels in goats impacted by SPT and CPT.

Materials and Methods

The study had commenced following approval from the B.Ü Regional Ethics Committee (Meeting No: 2024/01, Decision No: 01/03).

Selection Criteria

Animals exhibiting BHBA concentrations between 0.8 and 1.6 mmol/L were identified as having subclinical pregnancy toxemia (SPT) (Fırat and Özpınar, 2002; Uztimür and Ünal, 2024a), whereas those with blood BHBA levels of 1.6 mmol/L or higher were categorized as having clinical pregnancy toxemia (CPT) (Uztimür and Ünal, 2024a). Animals exhibiting serum BHBA levels below 0.8 mmol/L and devoid of any other disorders or issues identified after comprehensive clinical examination, hematological, and biochemical analyses were designated as the control group (CG).

Working Groups

Thirty goats in the last three weeks of pregnancy were used in the study. Of these 10 were placed into the control group (CG), 10 into the SPT group and the remaining 10 into the CPT group. Animals diagnosed with any additional ailment during the systematic clinical assessment alongside CPT and SPT were eliminated from the study. Pregnancy toxemia in goats was diagnosed by analysis of blood BHBA concentration using Freestyle Optium Neo-H (Abbott, UK).

25-(OH) D and Chemical Analysis

Eight milliliters of blood were extracted from the jugular vein of healthy and pregnancy-toxemic goats utilizing vacutainer tubes devoid of additives, following the specified technique. Blood samples were permitted to coagulate at ambient temperature (20°C). The samples underwent centrifugation at 2000 rpm for 10 minutes to separate the serum. Samples were stored at -20 °C until the beginning of the study. Biochemical parameters including glucose, iron and unsaturated iron binding capacity (UIBC) concentrations were evaluated using chemistry instrument (Mindray BS-2000m, China). 25(OH)D measurements in serum samples were analyzed within 5 months after blood collection. 25(OH)D was analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) device. It has been reported that the LC-MS/MS device which was used to take the measurements has an intraassay coefficient of variation (CV) <5%, interassay CV <7%, recovery >94-105% and detection limit of 5.1 nmol/L.

Statistical Analysis

Data were evaluated utilizing commercial software (GraphPad Prism 9 and IBM SPSS Statistics for Windows, Version 22.0). The Shapiro-Wilk test was employed to assess the normality of the data distribution. One-way analysis of variance and the post hoc Tukey multiple comparisons test were employed to ascertain the differences and comparisons between the groups and the control group. Receiver Operating Characteristic (ROC) analysis was employed to determine sensitivity, specificity, and threshold values. The association between variables was assessed using Spearman's rank correlation test. The threshold for statistical significance between groups was established at a P value of less than 0.05.

Results and Discussion

The statistical significance level and mean-standard deviation values between the groups with pregnancy toxemia and the CG are presented in Table 1 and Figure 1. Serum 25-(OH) vitamin D levels were determined to be statistically decreased in goats with CPT compared to those with CG ($P<0.001$). No substantial difference was observed among SPT, CPT, and CG for UIBC ($P>0.477$). BHBA levels in goats with CPT were considerably greater compared to both those with CG and SPT ($P<0.001$). A statistically significant difference was determined between the SPT group and CG ($P<0.025$) and the SPT and CPT groups ($P<0.001$).

Serum iron concentration was found to be statistically different between the CPT and SPT groups ($P<0.010$) and the CG group. However, no statistically significant difference was observed in iron concentration between the SPT and CPT groups ($P>0.477$). Serum glucose concentration in the control group was significantly higher than that in the SPT and CPT groups ($P<0.001$). Furthermore, there was a statistically significant difference in serum glucose concentration between the SPT and CPT groups.

Table 1. Statistical significance level and mean-standard deviation values between groups of pregnancy toxemia and CG

Variable	SPT	CPT	CG	P value
BHBA (mmol/L)	1.24±0.23[a]	3.17±1[b]	0.5±0.20[c]	0.001
25-(OH) D (µg/L)	12.19±8.52[a]	10.33±5.18[a]	27.45±1.41[b]	0.001
Iron (µmol/L)	102.20±31.48[a]	103.90±30.60[a]	153.30±51.47[b]	0.010
UIBC (µmol/L)	109.31±63.96	123.70±71.17	89.90±47.07	0.477
Glucose (mg/dL)	44.16±6.33[a]	30.64±6.72[b]	51.91±8.66[c]	0.001

BHBA: beta-hydroxybutyric acid, UIBC: Unsaturated iron binding capacity, SPT: subclinical pregnancy toxemia, CPT: clinical pregnancy toxemia; Control group (CG). Differences between groups with different letters in the same row are significant (P<0.05). Data are given as mean ±standard deviation

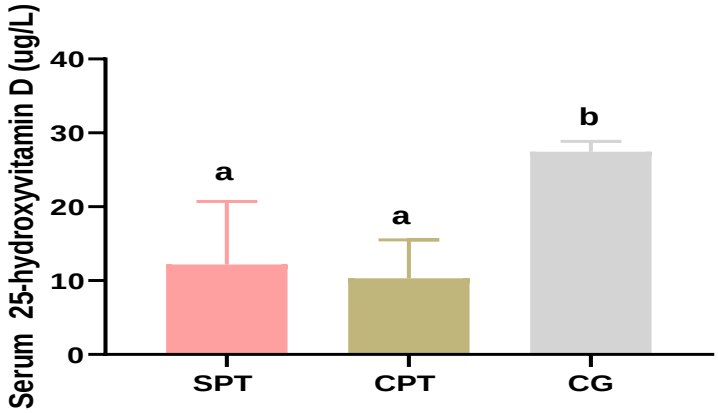

Figure 1. Serum 25-(OH) D levels of pregnancy toxemia groups. SPT: subclinical pregnancy toxemia, CPT: clinical pregnancy toxemia; Control group (CG). Statistical significance differences between groups P<0.05. In the graph, data are given as mean±standard deviation.

In pregnancy toxemic goats, a high degree of negative correlation with serum 25-(OH) D and BHBA (r= 0.-650, P= 0.001) was observed, while a moderate level of positive correlation was seen with glucose (r= 0.516, P= 0.003) and iron (r= 0.607, P= 0.001).

Sensitivity, specificity, AUC and cut-off values of serum 25-(OH) D, iron and glucose parameters in determining pregnancy toxemia using the Roc curve are shown (Table 2 and Figure 2). In the diagnosis of pregnancy toxemia, serum 25-(OH)D AUC was found to be 0.95, sensitivity was 90%, specificity was 95% and cut-off value was 26.12 µg/L; iron concentration AUC was found to be 0.79, sensitivity was 70%, specificity was 70% and cut-off value was 113 µmol/L; glucose AUC was found to be 0.88, sensitivity was 80%, specificity was 75% and cut-off value was 44.02 mg/dL.

Table 2. Sensitivity, specificity, AUC and cut-off values of 25-(OH) D, iron and glucose parameters in determining pregnancy toxemia.

Variable	AUC	Sensitivity	Specificity	Cutt-off
25-(OH) D (µg/L)	0.95	90	95	26.12
Iron (µmol/L)	0.79	70	70	113
Glucose (mg/dL)	0.88	80	75	44.02

AUC: area under the curve. Statistical significance differences between groups P<0.05

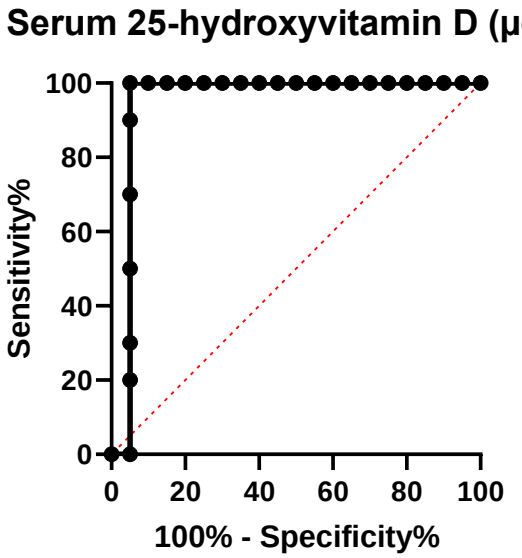

Figure 2. ROC curve of serum 25-(OH) D in the diagnosis of pregnancy toxemia

This is the first study to evaluate serum 25-(OH) D concentration in pregnancy toxemia goats. The study found a significant change in serum 25-(OH) D and iron levels with an increase in BHBA concentration. Additionally, a significant relationship was determined between serum 25-(OH) D levels and BHBA, iron and glucose.

Fats are metabolized as a result of the occurrence of negative energy balance (NEB) in animals. Consequently, the body produces BHBA, acetone, and acetoacetic acid, which are referred to as ketone bodies (Bani Ismail et al., 2008). Due to its greater stability in the bloodstream compared to other ketone bodies, BHBA is commonly used for diagnosing ketosis (Scott et al., 1998). The blood BHBA cut-off value for diagnosing SPT in goats is determined to be ≥0.8 mmo/L (Bani Ismail et al., 2008). However, a value of ≥1.6 mmol/L can be used for diagnosing CPT (Fırat and Özpınar, 2002). This study focused on the use of BHBA concentration, which is widely accepted as the most reliable method for diagnosing pregnancy toxemia. This study found that the levels of BHBA in the groups with SPT and CPT were considerably increased compared to the CG. In addition, the BHBA level of the CPT group was

significantly higher than the SPT group. The findings obtained in this study are also supported by other reported results (Bani Ismail et al., 2008; Uztimür et al., 2024; Uztimür and Ünal, 2024b).

Pregnancy toxemia can be caused by various factors, including multiple kids during pregnancy, physical inactivity, excessive feeding, severe parasite infestations, starvation, transportation, and stress. NEB occurs when the mother is unable to provide sufficient energy for the kids. Hypoglycemia, a significant factor in NEB, is observed in animals experiencing pregnancy toxemia. However, in studies on pregnancy toxemia by various researchers, hypoglycemia, normoglycemia, and hyperglycemia have all been reported (Iqbal et al., 2022; Souto et al., 2019). For example, Iqbal et al. (2022) stated that 55% of sheep with pregnancy toxemia were hypoglycemic. In another study conducted on goats with pregnancy toxemia, it was determined that 22.72% of the goats were hypoglycemic. In parallel, Hefnaw et al. (2011) reported that the glucose concentration of goats with experimental pregnancy toxemia was significantly low. Lima et al. (2012), in a study conducted on goats with pregnancy toxemia, stated that the hypoglycemic state led to an increase in the survival rate after cesarean section in goats. In this study, the serum glucose concentration in the CPT and SPT groups was found to be significantly lower than in the CG. In addition, it was determined that all the animals in the goat groups with pregnancy toxemia were hypoglycemic (100%). The fact that the hypoglycemic rate/concentration in goats with pregnancy toxemia in this study was much higher than the literature reports is thought to be related to the development of severe NEB due to the animals in the study experiencing severe hyperketonemia and poor nutritional status.

Iron is an essential element for various enzyme functions, including immunological and metabolic function, DNA and RNA synthesis, cellular respiration, hemoglobin formation, and oxygen transport in both the host and pathogen organisms (Değirmençay et al., 2022; Borges et al., 2007). Moreover, recent research in the field of veterinary medicine has indicated that the measurement of iron levels can serve as a reliable indicator for assessing inflammation (Değirmençay et al., 2022). The reduction in iron concentration during inflammation is associated with the utilization of iron by the host's defensive mechanism to enhance bacterial virulence and replication (Borges et al., 2007). In a study on serum iron concentration as an inflammatory biomarker, it was established that iron level decreased markedly in calves with bovine respiratory disease complex and that this parameter is important in the evaluation and diagnosis of inflammation (Değirmençay et al., 2022). Borges et al. (2007) reported that serum iron concentration showed a very good performance in determining acute inflammation in their study on 97 patients with systemic inflammation. In parallel, in the study conducted by Neumann (2003) on cats and dogs with different diseases, it was determined that the serum iron concentration was markedly low. Abuajamieh et al. (2016) found that haptoglobulin and serum amyloid-A concentrations, which are important inflammatory markers, were significantly increased in dairy cows with ketosis during the periparturient period. Similarly, in the study conducted by El-Deeb and El-Bahr (2017), it was determined that haptoglobulin and serum amyloid-A levels were significantly increased in dairy cows with ketosis and there was also a significant positive correlation between BHBA concentration and inflammatory markers. In the present study, which is in line with the previous literature, serum iron concentration was found to be significantly low in goats with pregnancy toxemia. These results show that inflammation occurs in goats with SPT and CPT due to increased BHBA concentration (El-Deeb and El-Bahr, 2017; Abuajamieh et al., 2016).

It has been established that vitamin D plays a role in the process of calcium metabolism (Horst et al., 1994). However, studies conducted in recent years have shown that vitamin D also plays a role in metabolic, infectious and uterine diseases (Horst et al., 1994; Martinez et al. 2018). Vitamin D undergoes a two-step process to convert into its active form, 1,25-dihydroxyvitamin D. First, it is transformed to 25(OH) D3 by 25-hydroxylase in the liver. Then, 25(OH)D3 is further converted to 1,25-

dihydroxyvitamin D3 (1, 25(OH)2D3) (Horst et al., 1994). 25-(OH) D is a reliable indicator in evaluating the amount of vitamin D in the peripheral circulation (Martinez et al. 2018). In the present study, serum 25-(OH) D concentration was taken as reference in the evaluation of vitamin D concentration in goats with pregnancy toxemia. Additionally, in this study, 25-(OH) D vitamin was analyzed by LC-MS device, which is considered the gold standard. The body generally obtains the vitamin D it needs from sunlight and orally or parenterally. However, many studies have determined that there is a vitamin D deficiency in both humans and animals (Zhou et al., 2019; Dos Santos et al., 2017). In many different species, including goats, 25-(OH) concentration is defined as normal between 75 and 150 nmol/L, inadequate between 25 and 75 nmol/L, and as a true deficiency when <25 nmol/L (Nemeth et al., 2017). Nisar et al. (2024) reported that there was vitamin D deficiency in sheep with subclinical pregnancy toxemia and that it had a significant effect on energy metabolism and redox balance. In a study conducted on Romney sheep in New Zealand, it was determined that the 25-(OH)D3 concentration in sheep giving birth to twins and triplets was lower than in those giving birth to singletons (Dittmer et al., 2020). Başbuğ et al. (2014), examining sheep with caseous lymphadenitis, determined that the 25-(OH) D vitamin concentration was significantly lower than in the healthy group. In a study conducted by He et al. (2022) on patients with type 2 diabetes who were prone to ketosis, it was reported that serum 25-(OH) D concentration was significantly lower and there was a correlation between 25-(OH) D concentration and ketone bodies. In addition, researchers have associated the decrease in serum 25-(OH) D concentration in type 2 diabetes who were prone to ketosis with the development of protection against the disease. This study has several limitations. First, it needs to be conducted with a larger population of pregnant women with toxemia. Second, it requires the evaluation of different inflammatory cytokines and acute-phase proteins with BHBA. In future studies, there is a need for research with a larger study population and controlled experimental studies to evaluate the effect of serum 25-(OH)D vitamin concentration on disease.

Conclusions

In conclusion, this study found that the serum 25-(OH)D vitamin concentration in goats in the last trimester of pregnancy was significantly low and there was a significant relationship with inflammatory markers. In addition, the decrease in serum iron concentration in animals with pregnancy toxemia also indicates the development of inflammation.

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