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Investigation of Vitamin B_{12} and Folic Acid Levels in Goats with Pregnancy Toxemia

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This study aimed to determine beta hydroxybutyric acid (BHBA), vitamin B_{12}, folate and glucose levels in Aleppo goats with pregnancy toxemia and to reveal their relationship with the disease. The study included 54 Aleppo goats ranging in age from one to four years old and in the last six weeks of pregnancy. Clinical examinations and BHBA measurements of all goats were performed. Goats are divided into 3 different groups based on their blood β-hydroxybutyrate (BHBA) as follows: non-ketonemic (<0.8 mmol/L, n= 15), moderate hyperketonemic (0.8-1.6 mmol/L, n= 13) and severe hyperketonemic (>1.6 mmol/L, n= 26). While no significant difference was found between the non-ketonemic group and moderate hyperketonemic group in BHBA concentration (P>0.05), a significant difference was found between the non-ketonemic group and the severe hyperketonemic group (P<0.001). The difference in vitamin B_{12} concentration between the 3 groups was significant (P<0.005). There was no significant difference between the 3 groups in terms of folate and glucose values (P>0.05). In the correlation graph between the variables in goats with pregnancy toxemia, a positive correlation was observed between BHBA concentration and vitamin B_{12} (r=0.392, P<0.05) and glucose (r=0.394, P<0.05). In addition, a positive correlation was observed between vitamin B_{12} value and folate (r=0.206, P<0.05) and glucose (r=0.323, P<0.05). In conclusion, our results suggest that hyperketonemia associated with serum B_{12} and glucose concentrations. Future studies are needed to understand the underlying mechanism of B_{12} increase in goats with pregnancy toxemia.

Key Words: *Pregnancy toxemia, aleppo goat, glucose, folate, B_{12}*

Gebelik Toksemisi Olan Keçilerde Vitamin B_{12} ve Folik Asit Düzeylerinin Araştırılması

Bu çalışmada gebelik toksemili halep keçilerinde beta hidroksi butirik asit (BHBA), vitamin B_{12}, folat ve glikoz seviyeleri belirlenip hastalık ile ilişkisinin ortaya konulması amaçlandı. Bu amaçla tahmini gebeliğin son 6 haftasında bulunan, 1-4 yaş aralığında toplam 54 Halep keçisi çalışmaya dahil edildi. Tüm keçilerin klinik muayeneleri ve BHBA ölçümleri yapıldı. Keçiler kan β-hidroksibütirat (BHBA) değerlerine göre ketonemik olmayan (<0.8 mmol/L, n= 15), orta derecede hiperketonemik (0.8-1.6 mmol/L, n= 13) ve şiddetli hiperketonemik (>1.6 mmol/L, n= 26) olarak sınıflandırıldı. BHBA değerinde ketonemik olmayan grup ile orta derecede hiperketonemik grup arasında anlamlı fark bulunamazken (P>0.05), ketonemik olmayan grup ile şiddetli hiperketonemik grup arasında anlamlı fark tespit edildi (P<0.001). Vitamin B_{12} konsantrasyonu üç grup arasındaki fark anlamlı tespit edildi (P<0.005). Folate ve glukoz konsantrasyonu ise 3 grup arasındaki fark anlamlı bulunmadı (P>0.05). Gebelik toksemisi olan keçilerde değişkenler arasındaki korelasyon grafiğinde BHBA konsantrasyonu ile vitamin B_{12} (r=0.392, P<0.05) ve glikoz (r=0.394, P<0.05) arasında pozitif korelasyon gözlendi. Ayrıca vitamin B_{12} konsantrasyonu ile folat (r=0.206, P<0.05) ve glikoz (r=0.323, P<0.05) arasında pozitif korelasyon gözlendi. Sonuç olarak, elde edilen bulgular hiperketoneminin serum B_{12} ve glukoz konsantrasyonları ile ilişkili olduğunu düşündürmektedir. Gebelik toksemisi olan keçilerde B_{12} yüksekliğinin altında yatan mekanizmayı anlamak için ilave çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: *Gebelik toksemisi, halep keçisi, glukoz, folat, B_{12}*

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Introduction

Pregnancy toxemia occurs in over-conditioned sheep and goats carrying twins, triplets, or large fetuses during last 4-6 weeks of pregnancy (1). Goats are socioeconomically significant animals because they can adapt to a wide range of climatic conditions, can be grazed even in stony places that cannot be appraised in any other manner, and are especially preferred by family companies (2). Due to the herd-based nature of pregnancy toxemia, it results in significant economic losses due to the loss of both goats and offspring when preventive and treatment are not carried out appropriately. In the early stages of the disease, the majority of animals are asymptomatic or exhibit anorexia, dry and mucous feces, ataxia, and blindness. Some animals may exhibit convulsions and respiratory distress in the last stages, along with an inability to stand or lateral recumbency. If timely and proper treatment is not provided, the mortality rate is around 80% (3, 4). The underlying mechanism of pregnancy toxemia is the inability of the pregnant ewe to meet the increased energy demands of the growing fetus during the final stages of pregnancy. To meet energy imbalance, body fat stores are breakdown to produce sufficient energy by gluconeogenesis. However excessive lipolytic activity results in an increase of keton bodies in the circulation. Fetuses cannot synthesis glucose to meet their energy

requirements and are therefore dependent on glucose given by the maternal circulation (4, 5). The underlying mechanism of pregnancy toxemia is the inability of the pregnant ewe to meet the increased energy demands of the growing fetus during the final stages of pregnancy. Body fat stores are broken down to produce sufficient energy. However excessive lipolysis results in an increase of ketone bodies in the circulation (6-8).

Vitamin B_{12}, also known as cyanocobalamin, is a water-soluble vitamin and is an important part of enzyme systems involved in multiple metabolic pathways and energy production, mainly through rumen fermentation (9, 10). This vitamin functions as a coenzyme and is involved in energy metabolism and cell replication (11). In particular, methylmalonyl CoA mutase is a cobalamine-dependent mitochondrial enzyme and plays a regulatory role in gluconeogenesis and fatty acid oxidation in ruminants (12). If the cobalt concentration in the ruminal fluid is below 0.5 mg/mL, the ruminal synthesis of vitamin B_{12} by ruminal flora is inhibited and its passage into blood and other tissues is reduced (10, 13). When ration does not contain cobalt, B_{12} production in the rumen decreases within a few days, however, the vitamin B_{12} stored in the liver is enough for a few days (14). Synthesized cobalamin is absorbed from intestine into the the portal circulation and transported to the liver with folates. While some of the folates are utilized by liver cells, others are methylated and then released into the bloodstream for use by peripheral tissues (15). Similar to vitamin B_{12}, folate is also a water-soluble vitamin, and has vital functions such as protein synthesis and red blood cell production (16). Unlike other water-soluble vitamins, approximately 60% of B_{12} is stored in the liver (17). In the case of B_{12} deficiency, it has been reported that folate concentration is depleted in liver cells (18). Excretion of absorbed vitamin B_{12} occurs via the urinary, biliary and fecal routes, but most is excreted in the urine (10, 11, 19). Serum B_{12} level can vary with factors such as feed, heredity, body reserve and herd management. In normal cobalt intake, serum B_{12} level is between 1-3 ng/mL. The most important factors for preventing vitamin B_{12} and folic acid deficiency are adequate and balanced feed intake as well as healthy rumen, liver, and kidney functions (20).

The studies on serum vitamin B_{12} and folate status in goat are limited. In this study, it was aimed to determine the beta hydroxy butyric acid (BHBA), B_{12}, folate and glucose levels of Aleppo goats with pregnancy toxemia and to reveal their relationship with the disease and thus to contribute to prophylaxis and treatment.

Materials and Methods

Research and Publication Ethics: Before starting the study, ethical approval was obtained from Dokuz Eylul University Animal Experiments Local Ethics Committee, dated 30.11.2022, decision number 48/2022.

Animal Selection: This study was conducted on 54 Aleppo goats raised in countryside in the city of Izmir. Goats are from different farms and their care and feeding vary. Clinical examinations and BHBA measurements of all goats were performed. BHBA measurements in blood were made using a ketone meter (FreeStyle Optium B-ketone, Abbott, ABD). Goats are divided into 3 different groups based on their blood β-hydroxybutyrate (BHBA) as follows: non-ketonemic (<0.8 mmol/L, n= 15), moderate hyperketonemic (0.8-1.6 mmol/L, n= 13) and severe hyperketonemic (>1.6 mmol/L, n= 26) (21, 22).

Collection of Serum Samples and Biochemical Analysis: 10 mL blood was collected from all goats from *V. jugularis* into tubes without anticoagulant. The samples were centrifuged at 3000 rpm for 10 minutes and the serum obtained was stored at –20 °C until analysis. Serum vitamin B_{12} (pg/mL), folate (ng/mL) and glucose (mg/dL) concentrations were measured using an automatic immunoassay analyzer (ADVIA Centaur XPT Immunoassay System, Siemens, Germany).

Statistical Analysis: The conformity of the data to the normal distribution was evaluated with Histogram, Q-Q plots and Shapiro-Wilk test. The Kruskal Wallis test was used for comparisons between groups of more than two. Dunn-Bonferroni test was used for multiple comparisons. The relationship between quantitative variables was evaluated with Spearman correlation analysis. Analysis of the data was performed in an open source software (R 4.2.2, RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.). Significance level was accepted as $P<0.05$.

Results

In the clinical examination, symptoms of anorexia, depression, muscle weakness, and ataxia were observed in the moderately hyperketonemic group. In the severe hyperketonemic group, muscle tremors, opisthotonos, grinding of the teeth, blindness, ataxia, and sternal recumbency were prominent symptoms. Table 1 shows the median, minimum and maximum values of serum BHBA, vitamin B_{12}, folate and glucose concentrations for study groups as well as the statistical difference between groups. While no significant difference was found between the non-ketonemic (median= 0.4 mmol/L; range= 0.3-0.6 mmol/L) and moderate hyperketonemic group (median= 1.2 mmol/L; range= 0.9-1.6 mmol/L) in BHBA value, there was a significant difference between the non-ketonemic and severe hyperketonemic group (median= 6.8 mmol/L; range= 1.9-26.2 mmol/L) and between moderate hyperketonemic and severe hyperketonemic group ($P<0.001$). Serum vitamin B_{12} concentrations were higher in moderate hyperketonemic group (median= 416 pg/mL; range= 186-677 pg/mL) compared to non-ketonemic group (median= 266 pg/mL; range= 138-394 pg/mL). Serum folate and glucose levels did not differ between groups.

Tablo 1. Mean, minimum, maximum values of biochemical parameters in pregnancy toxemia and healthy goats

Parameters	Non-ketonemic (n=15)	Moderate Hyperketonemic (n=13)	Severe Hyperketonemic (n=26)	P
BHBA (mmol/L)	0.4 (0.3-0.6)[b]	1.2 (0.9-1.6)[b]	6.8 (1.9-26.2)[a]	**<0.001**
B_{12} (pg/mL)	266 (138-394)[b]	416 (186-677)[a]	325.5 (165-1122)[ab]	**0.048**
Folate (ng/mL)	0.8 (0.6-2.7)	0.8 (0.5-25)	0.8 (0.4-22)	0.600
Glucose (mg/dL)	52 (40-96)	41 (27-343)	45.5 (7-278)	0.397

[a,b,ab]: Different letters on the same line indicate statistical difference. ***: P<0.001.

Figure 1 illustrates correlation graphs, correlation coefficients and statistical significance between various variables in goats with pregnancy toxemia. Serum BHBA levels correlated positively with serum vitamin B_{12} (r=0.392, P<0.05) an glucose (r=0.394, P<0.05) levels. In addition, serum vitamin B_{12} levels showed a positive correlation with serum folate (r=0.206, P<0.05) and glucose (r=0.323, P<0.05) levels.

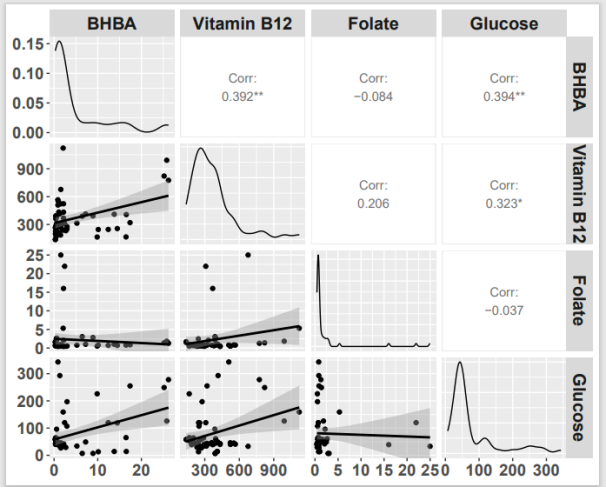

Figure 1. Correlation plots, correlation coefficients and statistical significance between several variables in goats with pregnancy toxemia

Discussion

Pregnancy toxemia is a common metabolic disease in sheep and goats. It has high mortality rate and causes substantial economic losses. In addition to clinical symptoms, ketonemia, ketonuria and hypoglycemia are important biochemical findings associated with the disease (23).

Since BHBA level is more stable than acetone and acetoacetate, it is more preferred in the diagnosis of ketosis (24). In this study, study groups were formed based on blood BHBA levels. Serum BHBA levels were higher in the severe hyperketonemic group than in non-ketonemic group. The serum BHBA concentrations in moderate hyperketonemia group differ from those in non-ketonemic group and severe hyperketonemia group. The results obtained from the study were similar to those of the researchers (25-31).

B_{12} is crucial for the energy metabolism of sheep because it acts as a cofactor in processes that support the production of propionic acid and is the main precursor of glucose synthesis in the liver. If there is a shortage of B_{12} in ruminants, energy efficiency has also decreased (32, 33). Souto et al. (29) reported that serum vitamin B_{12} levels were within the reference intervals in sheep with pregnancy toxemia. In another study, researchers found that vitamin B_{12} levels associated with rumen microbiata status, the composition of the feed and rumen fermentation process (10). Similar to our results, Soeres et al. (34) also reported that sheep with pregnancy toxemia and fed with energy enriched ration had increased serum cobalamin and folate levels. They also argued that dietary energy content may hav an effect on serum cobalamin and folate levels.

Folate plays an important role in DNA and methionine metabolisms and is synthesied by rumen microorganism in ruminants. Its circulating levels is significantly altered by metabolic and nutritional disorders. In addition circulating folate concentrations closely related with circulating cobalamin levels (10, 35). Folic acid supplementation in pregnant sheep on certain days of gestation resulted in a dose dependent increases in serum folate and cobalamin (36). Studies of folic acid associated with pregnancy in the world (29, 36-38) are limited, and the value of folic acid for pregnancy toxemia in Aleppo goats has been reported for the first time. In a study in sheep, Soeres et al. investigated the amount of folic acid in energy-poor, energy-rich, and pregnancy toxemia sheep. They reported that the difference between the three groups was significant and that the pregnancy toxemia group had the highest folic acid level (34). Soeres et al. (34), on the contrary, no significant relationship was found between serum folate levels between the control and pregnancy toxemia groups. Girard et al. reported (38), folic acid is important on its own during pregnancy, and it can be added to pregnant animals since it also causes an increase in B_{12} levels.

Although hypoglycemia is frequently identified in pregnant toxemia, it is not a consistent finding (23, 25, 26, 39). Considering the reference range for serum glucose concentrations in goats (50-63 mg/dL), the median of serum glucose concentrations in moderate and severe hyperketonemic goats in the current study were below the lower reference range in the present study. However, the percentage of hyperglycemic cases

in moderate and severe hyperketonemia groups were 15.4% and 46.2%, respectively. Similar to our results, Souto et al. found that 51% of sheep pregnancy toxemia had hyperglycemia (29). Researchers hypothesized that glucose concentrations in pregnancy toxemia could indicate whether a fetus is viable (8). In 2012, Lima et al. (8) evaluated the chance of fetal survival in goats with pregnancy toxemia according to glycemic status and determined the fetal survival rate to be higher when cesarean section was performed in hypoglycemic goats. On the other hand, Jeffrey and Higgins reported in their study that hypoglycemia-induced nervous system damage may occur in pregnancy toxemia and these would be irreversible (40). Based on all these studies, it is thought that the glycemic status of pregnancy toxemia may change individually, and it is important to control the glycemic status of the patient before performing parenteral or oral glucose replacement.

In conclusion, our results suggest that hyperketonemia associated with serum cobalamin and glucose concentrations. Future studies are needed to understand the underlying mechanism of B_{12} increase in goats with pregnancy toxemia.

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