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The effect of pregnancy toxemia on serum carnitine and amino acid levels in goats

[*O efeito da toxemia na gravidez nos níveis séricos de carnitina e aminoácidos em cabras*]

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ABSTRACT

The aim of this study was to determine changes in the carnitine and amino acid profile of goats with clinical pregnancy toxemia. The study included a total of 40 Aleppo breed goats, 20 with clinical pregnancy toxemia and 20 healthy goats. The goats with low glucose level and BHBA of 1.6-5mmol/L formed the group with clinical pregnancy toxemia, and goats with high glucose level and BHBA <0.8mmol/L formed the control group. Carnitine and amino acid profiles were determined in the groups. The results showed that the serum BHBA level was significantly higher, and the glucose level was lower in the clinical pregnancy toxemia group ($p<0.001$). From the serum amino acid profiles, the levels of Methyl Glutaryl, Valine, Methionine, Phenylalanine, Tyrosine, Asparagine, Arginine, Glutamic Acid, Alanine and Ornithine were determined to be statistically significantly low in the pregnancy toxemia group ($p<0.01$), and lysine was determined to be significantly high ($p<0.01$). It was determined that all serum carnitine profiles, except Decenoylcarnitine and Propionylcarnitine, were higher in goats with clinical pregnancy toxemia ($p<0.05$). In conclusion, it was concluded that these two profiles can be used as biomarkers in the diagnosis of pregnancy toxemia.

Keywords: pregnancy toxemia, amino acid, carnitine, goat

RESUMO

O objetivo deste estudo foi determinar alterações no perfil de carnitina e aminoácidos de cabras com toxemia clínica de gestação. O estudo incluiu um total de 40 cabras da raça Aleppo, 20 com toxemia clínica de gestação e 20 saudáveis. As cabras com baixo nível de glicose e BHBA de 1,6-5 mmol/L formaram o grupo com toxemia clínica da gestação, e as cabras com alto nível de glicose e BHBA <0,8 mmol/L formaram o grupo controle. Os perfis de carnitina e aminoácidos foram determinados nos grupos. Os resultados mostraram que o nível sérico de BHBA foi significativamente maior e o nível de glicose foi menor no grupo de toxemia clínica da gravidez (P<0,001). Com base nos perfis de aminoácidos séricos, os níveis de metil-glutaril, valina, metionina, fenilalanina, tirosina, asparagina, arginina, ácido glutâmico, alanina e ornitina foram determinados como sendo estatística e significativamente baixos no grupo de toxemia da gravidez (P<0,01), e a lisina foi determinada como significativamente alta (P<0,01). Foi determinado que todos os perfis séricos de carnitina, exceto decenoilcarnitina e propionilcarnitina, foram maiores em cabras com toxemia clínica de gestação (P<0,05). Concluiu-se que esses dois perfis podem ser utilizados como biomarcadores no diagnóstico de toxemia gestacional.

Palavras-chave: toxemia da gravidez, aminoácido, carnitina, cabra

INTRODUCTION

Pregnancy toxemia is a metabolic disease in sheep and goats, which forms in the last stage of pregnancy, especially within 15 days before birth or in the first weeks of lactation when the energy requirement increases by 70-80%. Pregnancy toxemia not only creates treatment costs but by leading to losses of both mother and offspring also causes economic losses. In cases that are not treated, especially in the early stages of the disease, the mortality rate can reach 100% (Rook, 2000).

The disease can develop for multifactorial reasons such as genetic factors and decreased rumen capacity because of poor and insufficient nutrition, multiple pregnancies, and rapid growth of the fetus. The basic mechanism in the occurrence of the disease is a negative energy

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balance characterized by hypoglycemia and adipose tissue mobilization, which leads to loss of maternal glucose balance control (Duehlmeier *et al*., 2011). With gluconeogenesis of body fat deposits to provide glucose balance control, lipolysis occurs to produce the energy required. As a result of this lipolytic activity there is an increase in ketone bodies in circulation (Fthenakis *et al*., 2012).

Carnitine is a structural material that plays an important role in energy production and fatty acid metabolism (Kendler, 1986). Carnitine cannot be obtained from food and is synthesized endogenously by the two basic amino acids of lysine and methionine. Ascorbic acid, ferrous iron, pyridoxine and niacin are necessary co-factors, and a deficiency of any one of these can lead to carnitine deficiency (Cave *et al*., 2008). Almost all (99%) carnitine is within the cell and affects carbohydrate metabolism. Carnitine plays a critical role in the energy balance across cell membranes and in the energy metabolism of tissues such as the heart and skeletal muscles, which take most of their energy from fatty acid oxidation (Cave *et al*., 2008).

It facilitates the entry of long-chain fatty acids into mitochondria and provides the formation of energy because of beta-oxidation (Topaloğlu and Gündüz, 2011). In addition to playing a role in the metabolism of free fatty acids, carnitine affects carbohydrate use by regulating the acyl coenzyme A (CoA)/CoA ratio (Özdemir and Aydın, 2013). Problems occurring in carnitine regulation have been associated with diabetes mellitus, hemodialysis, trauma, malnutrition, cardiomyopathy, obesity, hunger, drug interactions, endocrine imbalances, and complications of other disorders (Flanagan *et al*., 2010).

Amino acids (AA) are defined as organic substances containing both amino and acid groups. Of the more than 300 AAs found in nature, only 20 (α-AA) function as keystones of protein structure (Wu, 2009). AA metabolism, as one of the most important sources for energy production in the cell, is used for protein synthesis, and when necessary is oxidized as an energy source (Martínez-Reyes and Chandel, 2020). As a result of the oxidation of fatty acids, glucose, and amino acids, acetyl-CoA is produced, and this acetyl-CoA citric acid cycle (TCA) is used to produce nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide 2 ($FADH_2$). NADH and $FADH_2$ are then used for ATP production. Amino acid deficiency leads to interruptions in energy metabolism by lowering mitochondrial membrane potential (Owen *et al*., 2002).

The aim of this study was to contribute new information to the literature by revealing the changes in carnitine and amino acid profiles caused by clinical pregnancy toxemia in goats, and to determine whether these can be used as biomarkers for disease diagnosis.

MATERIALS AND METHODS

Approval for the study was granted by the Animal Experiments Local Ethics Committee of Harran University (decision no: 2023/005).

The study material consisted of a total of 40 Aleppo breed goats with a history of at least one birth, in the 120-150th day of pregnancy, which were brought to the Veterinary Faculty Animal Hospital of Harran University. The goats were separated into two groups according to the presence or absence of clinical signs of pregnancy toxemia, because of beta hydroxybutyric acid (BHBA) (Andrews, 1997) and glucose concentrations obtained from blood samples taken from the vena jugularis of the goats.

The groups formed were Group 1 (n=20) as the clinical pregnancy toxemia group of goats with low glucose level and BHBA of 1.6-5mmol/L, and Group 2 (n=20) as the healthy control group of goats with high glucose level and BHBA <0.8 mmol/L. Pregnancy toxemia was diagnosed according to clinical examination results such as tremors, the smell of acetone in the respiratory tract and teeth grinding, together with reduced appetite, inertia, remaining behind the herd, and co-ordination dysfunction reported in the anamnesis of the owner, and the measurements of BHBA and glucose determined with rapid testing kits (Free Style Optium Neo H - Abbott®) from the blood samples taken from the vena jugularis of the goats. Any goats that were in a coma or had BHBA value >5 were excluded from the study (Figure 1).

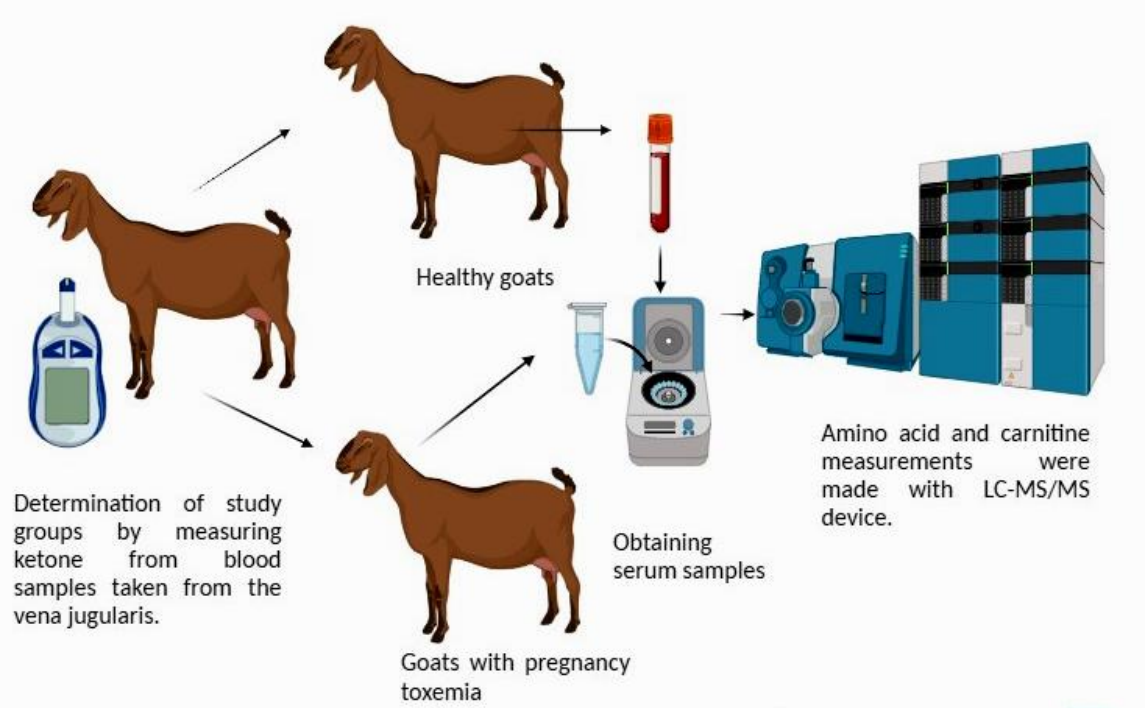

Figure 1. Study design (The study material consisted of a total of 40 Aleppo breed goats with a history of at least one birth, in the 120-150th day of pregnancy. The goats were separated into two groups according to the presence or absence of clinical signs of pregnancy toxemia, because of beta hydroxybutyric acid (BHBA) and glucose concentrations obtained from blood samples taken from the vena jugularis of the goats).

The blood samples withdrawn from the vena jugularis of the goats were centrifuged at 3000 rpm for 10 minutes. The serum samples obtained were transferred to Eppendorf tubes and then stored at -20°C until the day of analysis. The derivation method was used to analyze the presence of free amino acids in the biological fluids. In this method, a sample of 100µL was mixed with a standard mixture containing C13 and N15-marked atoms formed from 20 amino acids prepared in 0.1M HCl. At the second stage, basic organic buffer components prepared in propanol were added to balance the pH and increase the effectiveness of the derivation reaction. Protein breakdown occurred at this stage. Then a chloroform/iso-octane mixture containing 5% alkaline chloroformate as the active substance was added and this was left at room temperature for 3 minutes. During this procedure, gas formation was observed because carbon dioxide is produced as a by-product of the esterisation reaction of alkaline chloroformate and amino acids (Tammo *et al.*, 2021). Through centrifugation the amino acids derived were transferred to the upper phase containing organic solvents and then 1µL of this phase was injected into the LC-MS/MS device (Shimadzu 8045 MS/MS, Shimadzu North America, Columbia). Following the extraction and derivation processes, the molecular weight of the amino acids increased, and as they became more volatile, the signal given to the MS device increased. Chromatographic separation was performed using a Trimaris Amino Acid LC-MS/MS column (250mm x 2mm, 3µM) containing a C18 reverse phase filling substance. Water, methyl alcohol, and 1M ammonium format in the ratio of 85:14:1 were used as mobile phase A, and methyl alcohol was used as mobile phase B. In the analysis of the dried blood samples performed with acylcarnitine, a method based on derivation using butanolic-HCl was applied. In this method, a spot sample 3.2mm in diameter taken from the dried blood sample was left in an organic extraction solution containing standard marked acylcarnitine doterium and amino acid at a certain concentration. After 30 minutes of extraction, organic solvents were removed via solvent evaporation under nitrogen until dried. In the

next stage, the sample was incubated at 60°C for 20 minutes to separate butanolic HCl, amino acids, and acylcarnitine. Following further evaporation, the samples were dissolved in the mobile phase and 5µL was injected into the LC-MS/MS system. Using the electrospray ionization method, the amino acid and acylcarnitine molecules were analyzed in the multiple reaction tracking mode, and each acylcarnitine molecule was scanned according to the standards integrated in the software specific to the device. A column was not used in this analysis and 90%MeOH was used as the mobile phase (Xu *et al*., 2020).

Data obtained in the study were analyzed statistically using SPSS vn. 24.0 software (Statistical Package for the Social Sciences). Conformity of the variables to normal distribution was examined with visual (histogram and Q-Q Plot) and analytical methods (Shapiro-Wilk test). Descriptive analyses were reported as mean ± standard error mean (SEM) values for variables showing normal distribution. As the data showed normal distribution, independent groups were analyzed using the Student's t-test. Variance homogeneity was determined using the Levene test. A value of $p<0.05$ was accepted as statistically significant in all the analyses.

RESULTS

The mean BHBA, glucose and serum amino acid values of the study groups are shown in Table 1 and the serum carnitine profile values in Table 2. The serum BHBA value was determined to be significantly higher, and the glucose value was significantly lower in the group with clinical pregnancy toxemia than in the control group ($p<0.001$).

From the serum amino acid profiles, the values of Methyl Glutaryl, Valine, Methionine, Phenylalanine, Tyrosine, Asparagine, Arginine, and Glutamic Acid were determined to be significantly lower in the group with clinical pregnancy toxemia ($p<0.001$). From the other amino acid profiles, Alanine and Ornithine were seen to be significantly lower in the pregnancy toxemia group ($p<0.01$). Lysine was observed to be significantly higher in the group with pregnancy toxemia ($p<0.01$). No statistically significant difference was determined between the groups in respect of the Aspartic Acid, Glycine, and Citrulline profiles ($p>0.05$).

Table 1. Average BHBA, glucose, serum amino acid values of pregnancy toxemia and control groups

Amino Acid Profile (µmol/L)	Pregnancy Toxemia Group	Control Group	*P Value
	$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	
BHBA	3.69± 0.63	0.53± 0.11	**0.000**
Glikoz	28.26±1.75	58.11±1.83	**0.000**
Methy Glutaryl	0.015±0.001	0.042±0.002	**0.000**
Valine	220.73±1.885	239.51±1.881	**0.000**
Lizin	173.34±2.206	162.92±2.824	**0.006**
Methionine	23.28±0.536	32.53±0.965	**0.000**
Phenylalanine	35.40±0.686	50.43±1.028	**0.000**
Aspartic Acid	0.01±0.001	0.01±0.001	0.140
Tyrosine	33.26±0.535	46.27±0.682	**0.000**
Asparagine	26.87±0.656	35.38±0.492	**0.000**
Alanine	177.47±9.390	213.57±1.371	**0.001**
Arginine	283.56±2.638	317.96±2.727	**0.000**
Citrulline	60.36±4.935	69.56±3.251	0.129
Glycine	257.07±5.700	263.16±6.584	0.489
Ornithine	38.59±2.640	49.67±1.443	**0.001**
Glutamic Acid	257.19±5.268	207.29±1.627	**0.000**

*Significance levels according to Student's t-test results. Beta hydroxybutyric acid (BHBA).

Table 2. Average carnitine profile values of pregnancy toxemia and control groups.

Carnitine Profile	Pregnancy Toxemia Group	Control Group	*P Value
	$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	
C0 (Free carnitine)	80.80±2.95	67.51± 2.09	**0.001**
C2 (Acetylcarnitine)	19.13±0.66	11.26±1.31	**0.000**
C3 (Propionylcarnitine)	2.82±0.20	2.35±0.15	0.080
C4 (Butyrylcarnitine)	0.76±0.20	0.30±0.02	**0.000**
C4DC (Methylmalonylcarnitine)	0.05±0.01	0.01±0.000	**0.036**
C5 (Isovalerylcarnitine)	0.92±0.04	0.53±0.01	**0.000**
C5:1 (Tiglylcarnitine)	0.08±0.002	0.05±0.002	**0.000**
C5OH (Hydroxyisovalerylcarnitine)	0.23±0.02	0.16±0.006	**0.017**
C5 DC (Glutarylcarnitine)	0.06±0.05	0.02±0.001	**0.000**
C6 (Hexanoylcarnitine)	0.09±0.02	0.02± 0.001	**0.015**
C6DC (Adipoylcarnitine)	0.34±0.07	0.07±0.02	**0.001**
C8 (Octanoylcarnitine)	0.18±0.05	0.02±0.002	**0.012**
C8:1 (Octenoylcarnitine)	0.02±0.005	0.01±0.002	**0.027**
C8 DC (Suberoylcarnitine)	0.11±0.03	0.008±0.000	**0.014**
C10 (Decanoylcarnitine)	0.12±0.02	0.04±0.004	**0.005**
C10:1 (Decenoylcarnitine)	0.03± 0.005	0.02±0.005	0.383
C10 DC (Sebacoylcarnitine)	0.03±0.005	0.01±0.000	**0.006**
C12 (Dodecanoylcarnitine)	0.05±0.009	0.01±0.001	**0.000**
C14 (Myristoylcarnitine)	0.22±0.05	0.03±0.003	**0.002**
C14:1 (Myristoleylcarnitine)	0.03±0.007	0.006±0.001	**0.001**
C14:2 (Tetradecadienoylcarnitine)	0.008±0.001	0.005±0.000	**0.030**
C16 (Palmitoylcarnitine)	0.28±0.04	0.06±0.005	**0.000**
C16:1 (Palmitoleylcarnitine)	0.04±0.005	0.009±0.000	**0.000**
C18 (Stearoylcarnitine)	0.33±0.04	0.09±0.009	**0.000**
C18:1 (Oleylcarnitine)	0.15±0.01	0.02±0.002	**0.000**
C18:2 (Linoleylcarnitine)	0.01±0.001	0.003±0.000	**0.000**
C18:1 OH (Hydroxyoleylcarnitine)	0.02±0.003	0.005±0.000	**0.000**

*Significance levels according to Student's t-test results.

From the serum carnitine profiles, the values of C2 (Acetylcarnitine), C4 (Butyrylcarnitine), C5 (Isovalerylcarnitine), C5:1 (Tiglycarnitine), C5 DC (Glutarylcarnitine), C12 (Dodecanoylcarnitine), C16 (Palmitoylcarnitine), C16:1 (Palmitoleylcarnitine), C18 (Stearoylcarnitine), C18:1 (Oleylcarnitine), C18:2 (Linoleylcarnitine), and C18:1 OH (Hydroxyoleylcarnitine) were determined to be significantly higher in the goats with clinical pregnancy toxemia ($p<0.001$). From the other carnitine profiles, C0 (Free carnitine), C6DC (Adipoylcarnitine), C10 (Decanoylcarnitine), C10 DC (Sebacoylcarnitine), C14 (Myristoylcarnitine), and C14:1 (Myristoleylcarnitine) were seen to be significantly higher in the pregnancy toxemia group ($p<0.01$). The values of C4 DC (Methylmalonylcarnitine), C5 OH (Hydroxyisovalerylcarnitine), C6 (Hexanoylcarnitine), C8 (Octanoylcarnitine), C8:1 (Octenoylcarnitine), C8DC (Suberoylcarnitine), and C14:2 (Tetradecadienoylcarnitine) were determined to be significantly higher in the pregnancy toxemia group ($p<0.05$). No significant difference was determined between the groups in respect of C3 (Propionylcarnitine) and C10:1 (Decenoylcarnitine) values ($p>0.05$).

DISCUSSION

The body has developed an adaptive metabolism to protect the blood glucose balance, which is important for the normal functioning of organs. When necessary, the body deposits (fatty acids and amino acids) are activated to provide energy and participate in gluconeogenesis. In addition, acetyl-CoA, produced with β-oxidation of fatty acids, produces large amounts of ketone bodies, which can be cytotoxic at high concentrations (Bobe *et al*., 2004). Although subclinical pregnancy toxemia in goats has been relatively well researched there is a lack of information related to metabolic changes, especially in the

clinical form of the disease. The results of this study present important data that will contribute to understanding the effect on the amino acid and carnitine profiles of clinical pregnancy toxemia in Aleppo breed goats.

The results of this study showed a significant increase in the serum BHBA value in goats with clinical pregnancy toxemia compared to healthy pregnant goats. This increase in the serum BHBA level in goats with pregnancy toxemia has been previously reported (Hefnawy *et al*., 2010). These increases in the serum BHBA level in goats may be due to the expression of long-chain fatty acids converted to ketone bodies by the liver and tissue lipolysis. Moreover, hypoglycemia caused by the mobilization of fat deposits leading to hepatic ketogenesis and impaired carbohydrate and fat metabolism could be another reason for this increase (Rook, 2000). In the current study, there was also seen to be a significant decrease in the mean blood glucose value in the goats with clinical pregnancy toxemia. These findings were consistent with those of the previous study (Hefnawy *et al*., 2010). Just as hypoglycemia may be associated with long-term fasting, it can also be due to reduced hepatic gluconeogenesis with an increased BHBA level or increased glucose demand in pregnancies with developing twins or triplets (Andrews,1997).

The catabolism of amino acids is a necessary process in energy production in the context of negative energy balance (NEB). In addition to body fat mobilization, body protein can also be mobilized to ameliorate continuous NEB (Wu, 2009). Similarly, it is thought that some amino acids can be converted to intermediate substances of TCA, gluconeogenesis, and ketogenesis. In a study of pregnancy toxemia, Xue *et al*. (2019) demonstrated that valine, glutamic acid, and phenylalanine levels were decreased. Lisuzzo *et al*. (2022) reported that both alanine and valine were lower in sheep with hyperketonemia than in a control group. Leucine, isoleucine, and valine are amino acids that are used for protein synthesis in muscles. A low concentration of these amino acids has been shown to be positively correlated with the concentration of alanine, which is a glucogenic amino acid with a high rate of concentration in muscles (Liu *et al*., 2021).

Alanine represents one of the main sources for gluconeogenesis and is therefore effective in carbohydrate metabolism. In studies of dairy cattle, low concentration has been associated with ketosis and fatty liver (Luke *et al*., 2020). Consistent with the literature, alanine and valine were determined to be lower in goats with ketosis in the current study. Low concentrations of valine and alanine show amino acid mobilization (Lisuzzo *et al*., 2022). Asparagine is one of the precursors of oxaloacetate (Nelson and Cox, 2006). The current study results showed a decrease in the asparagine level of goats with clinical pregnancy toxemia, consistent with the findings of other studies (Lisuzzo *et al*., 2022). This metabolite plays a role in the cell functions of nerve and brain tissue and is a non-toxic transporter of excess ammonia in the body. Guo *et al*. (2019) showed a reduction in arginine, citrulline, and ornithine, which are amino acids in the urea cycle, in sheep with pregnancy toxemia. Arginine is converted to ornithine and urea in the last stage of the urea cycle (Albaugh *et al*., 2017). Consistent with the literature, the current study results showed a decrease in arginine, citrulline, and ornithine levels in the goats with clinical pregnancy toxemia. The dramatic decrease in amino acid levels of sheep with pregnancy toxemia causes the metabolizing of some amino acids in hepatic tissue to provide energy in the body and to try to meet the amino acid requirements of the mother for placental energy of the fetus (Bell, 1995).

Huang *et al*. (2023) demonstrated that the lysine level was higher in sheep with subclinical pregnancy toxemia. The current study results also showed a higher lysine level in the sheep with clinical pregnancy toxemia. Lysine is an important ketogenic amino acid and high levels have been shown to indicate that the ketogenic pathway is active in subclinical pregnancy toxemia (Huang *et al*., 2023). In contrast, Marczuk *et al*. (2018) showed that the lysine value was lower in cows with primary ketosis compared to healthy cows. In another study, Lisuzzo *et al*. (2022) determined no difference in the lysine value between sheep with postpartum hyperketonemia and healthy sheep. The reason for this was thought to be that the use of lysine in milk protein synthesis in lactating animals prevented an increase in the level. The data in literature confirm that lysine deficiency leads to

a decrease in milk production and in the protein content of milk (Rulquin and Pisulewski, 2006).

Carnitine and acylcarnitine assist in the transport of amino acids to the mitochondria for β-oxidisation. Several recent metabolic studies have shown that carnitine and acylcarnitine could be used as potential diagnostic biomarkers in intensively reared cattle (Ghaffari *et al*., 2020). Elevated acylcarnitine in the plasma reflects irregularity of mitochondrial oxidization and emerges because of cell death or organ dysfunction (Mihalik *et al*., 2010). Previous studies have shown a significant increase in plasma acylcarnitine in sick cows (Hailemariam *et al*., 2014). It has been suggested that high plasma acylcarnitine could reflect the ability of hepatocytes to release excess carnitine and acylcarnitine from the mitochondria to prevent mitochondrial damage (Hüber *et al*., 2016). Huang *et al*. (2023) examined the carnitine profile in goats with subclinical pregnancy toxemia, and reported that only the C2, C5, C5:1, C6DC, C18:1 parameters were significantly increased. In contrast, the current study results showed that except for several parameters, there was determined to be an increase in most of the carnitine profile of the goats with clinical pregnancy toxemia. This difference could be since acylcarnitine in goats with subclinical pregnancy toxemia causes very little change in the serum markers of liver damage, and the increase in fatty acid oxidization may form an adaptive response (Huang *et al*., 2023). There is thought to be an accumulation of excess carnitine as a result of the inadequacy of this response in clinical pregnancy toxemia. It has been reported that to meet the energy requirements in early lactation, there is a significant increase in fatty acid oxidation genes and carnitine intake in the liver of dairy cattle and this causes an increase in acylcarnitine (Schlegel *et al*., 2012). Rico *et al*. (2018) suggested that the lipid-origin mitochondrial stress in overweight dairy cattle caused an increase in deficient β-oxidization and an increase in fatty acylcarnitine in circulation. High concentrations of medium and long-chain ACs (eg. C10, C16, C18) in the serum are due to irregular fatty acid oxidization in the mitochondria as a response to activation of the proinflammatory signaling pathways (Rutkowsky *et al*., 2014). As the liver cannot metabolize medium and long-chain ACs, these are expressed into the systemic circulation as a part of the detoxification process (Schooneman *et al*., 2013). There is increasing evidence showing that AC accumulation could play a role in insulin resistance in humans (Holland *et al*., 2007). There are also studies that have shown that serum acylcarnitine levels are elevated in animal models with obesity and insulin resistance and that this could be used to detect fatty acid oxidization irregularity (Mihalik *et al*., 2010). Insulin resistance has been reported in dairy cattle with ketosis, primarily type II (Kawashima *et al*., 2016). It has also been reported that insulin resistance up to a degree is seen in dairy goats with subclinical pregnancy toxemia (Liu *et al*., 2021). The higher level of insulin resistance in the current study was thought to be because the study was conducted on goats with clinical pregnancy toxemia. Insulin resistance can promote lipolysis can accelerate the progression to ketosis and increase acylcarnitine accumulation in the circulation, thereby creating a vicious circle (Kawashima *et al*., 2016).

CONCLUSIONS

In conclusion, the results of this study showed great changes in the amino acid and carnitine profiles of goats with clinical pregnancy toxemia. Significant decreases in the amino acid levels and significant increases in the carnitine profile constitute evidence that these two values could be used as biomarkers in the diagnosis of clinical pregnancy toxemia. Nevertheless, there is a need for further studies using more animals to determine which specific parameters should be measured to provide a clear diagnosis of the disease with the measurement of fewer parameters.

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