Evaluation of metabolic profiles of Saanen goats in the transition period

F. Akkaya¹, S. Senturk², Z. Mecitoğlu², S. Kasap², S. Ertunc², C. Kandemir¹

¹Department of Animal Science, İzmir Ege University, 35040, İzmir, Turkey
²Department of Internal Medicine, Faculty of Veterinary Medicine, Bursa Uludag University, 16059, Bursa, Turkey

ABSTRACT: Healthy Saanen goats (n=30) in periparturient period were used in the present study. Blood samples were collected 21, 14 and 7 days before parturition, at the time of birth and postpartum days 7, 14 and 21. Non-esterified fatty acids (NEFA), betahydroxy butyric acid (BHBA), Total protein (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CREA) aspartate amino transferase (AST), gamma glutamyl transferase (GGT), sorbitol dehydrogenase (SDH), glucose (GLU), cholesterol (CHOL), triglyceride (TG), calcium (Ca), phosphorus (P), and magnesium (Mg) levels were evaluated. During the study period, NEFA, SDH, CREA levels increased and CHOL and TG levels decreased at time of parturition. When the changes of parameters in prepartum and postpartum period were compared, the concentrations of NEFA, CHOL, GLU, TG, Ca were higher (p <0.001) in prepartum period; however BHBA, Mg, ALB, GGT, AST, TP, P, BUN, SDH and CREA concentrations were detected to be higher (p <0.001) in postpartum period. Metabolic Profile Test based on biochemical parameters evaluated in our study would be beneficial for diagnosis, prevention and control of diseases such as pregnancy toxemia, hypocalcemia, infertility in goats.

Keywords: BHBA, Metabolic profile test, NEFA, Saanen goat, Transition period
INTRODUCTION

Milk production levels in excess of the metabolic reserve capacity of the animal leads to many metabolic diseases in periparturient period (Gilbert et al., 1998). The transition period, 3 weeks before and 3 weeks after birth, is a period in which many metabolic changes occur. If this period is not well managed and the nutritional needs of animals are not met, metabolic diseases will inevitably occur (Caldeira et al., 2007, Soutor et al., 2013, Araujo et al., 2014). Metabolic profile test is widely used evaluate and take early preventive measures in periparturient period problems of cattle. Unfortunately, in small ruminants these studies are mostly conducted on sheep and studies conducted with goats are rare.

Metabolic profile test (MPT) is a quantitative test to typically performed to assist in the early diagnosis of many metabolic diseases and to monitor animal health status on a flock basis (Ghergariu et al., 1984; Boginet al., 1988; Batmaz et al., 1992; Kida, 2002a, 2002b). MPT scores when used in conjunction with environment, nutrition, and body condition evaluation could be employed for the assessment of yield characteristics, as well as in the improvement of milk quality and quantity, elimination of fertility problems, and prevention of many subclinical diseases. In sum, protection of herd health and reduction of possible economic losses could be achieved by regular MPT implementation (Ghergariu et al., 1984; Boginet al., 1988; Ivanov et al., 1993).

In order to check MPT in goats; parameters such as glucose (GLU), beta hydroxybutyrate (BHBA), non-esterified fatty acid (NEFA), cholesterol (CHOL), total protein (TP), albumin (ALB), globulin, blood urea nitrogen (BUN), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), sorbitol dehydrogenase (SDH), triglyceride (TG), calcium (Ca), magnesium (Mg) and inorganic phosphorus (P) could be evaluated (Borges et al., 1997; Kida, 2002a, 2002b). By measuring these, it is possible to comprehensively evaluate the nutritional status of animals, especially during the transition period (Batmaz et al., 1992; Ivanov et al., 1993).

Although there have been many studies on metabolic profile testing in cattle, especially in the Holstein cattle (Cozzi et al., 2010; Samanc et al., 2011), limited research has been conducted on high milk producing Saanen goats. This gap in the extant knowledge has been motivated the present study, the aim of which is to obtain a preliminary reference for the biochemical parameters and allow monitoring of changes in metabolic profiles of the Saanen goats during the transition period.

MATERIALS AND METHODS

Animal material: The study sample consisted of 30 dairy Saanen goats in second and third lactation from a flock of 300 animals. All goats were from the same herd and yield group, and the management and feeding conditions were same for all. They ranged in age from two to four years. Lactation milk yield average was about 850 lt per goat in the previous lactation. It was stated that the rate of twins in the previous pregnancy was about 64%. Further, the previous average lactation period was reported as 273 days.

Routine clinical examinations (body temperature, pulsation and respiration rates, lymph nodes, tracheal palpation, lung auscultation and percussion) were performed, and only clinically healthy animals were selected for inclusion in the study and these routine clinical examinations were repeated before each sampling (Diffay et al., 2005). Animals suffering from any diseases that cause such as ketosis, pneumonia, enteritis, mastitis, metritis, lameness that might affect the biochemical parameters were excluded from the study.

After birth, the kids were left with their mother. They were allowed to suckle their mothers freely. Goats were milked twice a day during and after the study period. All animals included in the study were fed with the same rations (Table 1) during the study, and their housing and environmental conditions were identical. All goats were provided with 1 kg of hay per animal daily. A concentrate containing 12% protein was also offered each morning (0.9 kg for each goat). All animals had unlimited access to water and salt blocks. Animals included in the study were given antiparasitic treatment one month before conception.

Sample collection and evaluation

Blood samples were collected from the animals 21, 14, and 7 days before expected parturition, at the time of parturition, as well as 7, 14 and 21 days postpartum. While all blood samples for the measurement of BHB were taken in the morning, approximately 6 hours after the morning feed, for other biochemical parameters including albumin, phosphorus, magnesium, AST, GGT, TP, BUN, and NEFA were taken before the morning feeding.

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The blood samples were collected by jugular venipuncture, with 25×8mm needles, using vacuum tubes with clot activator and gel for serum separation (Becton Dickinson and Company, Franklin Lakes, NJ). Serum samples were separated off by centrifugation for 15 minutes at 3000 rpm (for BHBA testing within 1/2 h of collection). Haemolysed samples were excluded from the study. Serum samples were stored at –20 ºC until analyzed. Samples were then centrifuged again after thawing before the analysis.

Serum biochemical parameters: Serum levels of albumin, phosphorus, magnesium, AST, GGT, TP and BUN were determined by Vet Scan-VS2® device (Abaxis, Inc.Union City, CA 94587) in Uludag University Veterinary Faculty Animal Hospital Central Laboratory. Glucose, triglyceride, calcium, cholesterol and creatinine levels were determined spectrophotometrically using an Abbott c16000 (Abbott ARCHITECT c16000, Abbott Park, Abbott Laboratories, Illinois-USA) in Uludag University, Faculty of Medicine Central Laboratory which is an accredited laboratory. BHBA levels were measured using on farm ketone test kit and corresponding reading device (Ketosite® BHBA test card, Ketosite® instruments, Stanbio Laboratory Texas-USA). NEFA levels were determined spectrophotometrically in Balikesir System Laboratory, using a commercial NEFA kit (NEFA-HR (2) Wako Chemicals GmbH, Germany). SDH levels were measured in Istanbul Bilim Laboratory, using a Goat SDH ELISA kit (Goat SDH ELISA kit, SunRed, Cat. No: 201-07-3106, Epoch microplate spectrophotometry SN:242136, Biotek Winooski, VT, USA).

Statistical analysis
The normality of the data was determined by Shapiro–Wilk test. The one-way repeated measures (RM) ANOVA test was used to determine whether there was a difference between the values of the same parameters on different days, using Sigma Plot 12 software. For all pairwise multiple comparison procedures, Holm-Sidak test was used for the data that was normally distributed and Tukey test was selected for the data with normality lower than <0.05. For all analyses, P < 0.05 was accepted as significant.

The study was approved by Uludag University Animal Experiments Local Ethics Committee (HADYEK), Bursa, Turkey (Decision no: 2014-16/04).

RESULTS
During the study, routine clinic examinations including body temperature, heart rate, respiratory rate, mucosal membranes, auscultation, and percussion of lungs of animals were normal in all animals. Also, the appetite of goats was very good during the working period. No animals were excluded from the study due to any disease during the study. Goats in farm in the scope of work were milked two times a day. The average daily milk production were determined as 3.8 ±0.324 liters during the study period. In this study, while 24 out of 30 pregnant goats bore twin kids, one pregnant goat bore triplets kids. Each of the remaining 5 pregnant goats bore a kid. The average live weight of the goats in the 21 days before birth was 51.45±4.1 kg. On the 21st postnatal day, their mean live weight was 47.1±5.4 kg.

NEFA concentrations were within normal range in the prepartum period, reaching the peak level (0.53 mmol/L) at the day of parturition, whereas the lowest level (0.15 mmol/L) was detected 21 days postpartum (Table 2). The difference between prepartum and postpartum NEFA levels was not statistically significant.
However, NEFA levels were significantly higher on the day of delivery when compared to prepartum levels (0.58 mmol/L). The mean BHBA (as well as measured levels) concentrations were found to be within the reference limits during the entire study period (Table 2).

However, while the BHBA levels steadily decreased in the periparturient period, increased significantly at the time of parturition (0.38 mmol/L) to reach the peak levels on day 21 (0.55 mmol/L) (Table 2).

<table>
<thead>
<tr>
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<th>-14</th>
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<td>NEFA</td>
<td>0.30±0.03b</td>
<td>0.17±0.01b</td>
<td>0.29±0.07b</td>
<td>0.53±0.05b</td>
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<td>0.31±0.04a</td>
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<td>0.38±0.05b</td>
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<tr>
<td>TP</td>
<td>6.6±0.13b</td>
<td>6.52±0.07b</td>
<td>6.69±0.10b</td>
<td>6.71±0.11b</td>
<td>6.92±0.12a</td>
<td>6.62±0.10b</td>
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<td>Alb</td>
<td>4.05±0.27a</td>
<td>4.19±0.25a</td>
<td>3.47±0.21b</td>
<td>3.71±0.20b</td>
<td>3.74±0.22a</td>
<td>4.57±0.20a</td>
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<tr>
<td>BUN</td>
<td>12.7±0.85b</td>
<td>13.6±0.94b</td>
<td>9.0±0.63b</td>
<td>11.1±0.68b</td>
<td>13.9±0.75a</td>
<td>16.4±0.85a</td>
<td>16.0±0.96a</td>
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<tr>
<td>CREA</td>
<td>0.56±0.01a</td>
<td>0.63±0.01a</td>
<td>0.64±0.01b</td>
<td>0.68±0.01a</td>
<td>0.63±0.01a</td>
<td>0.59±0.01b</td>
<td>0.60±0.01b</td>
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<td>AST</td>
<td>45.1±3.76a</td>
<td>52.7±3.08b</td>
<td>42.0±2.78a</td>
<td>52.7±3.30a</td>
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<td>27.0±2.64a</td>
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<td>29.3±1.85a</td>
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<td>29.9±4.43b</td>
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<td>57.2±1.7a</td>
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<td>Chol</td>
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<td>88.4±1.94a</td>
<td>83.8±2.06b</td>
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<td>36.0±2.6a</td>
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<td>Ca</td>
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<td>8.98±0.14a</td>
<td>9.15±0.09b</td>
<td>8.68±0.14b</td>
<td>8.7±0.12a</td>
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<td>P</td>
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<td>6.29±0.4b</td>
<td>4.59±0.3b</td>
<td>4.66±0.3b</td>
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<td>6.59±0.4b</td>
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<tr>
<td>Mg</td>
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<td>2.21±0.1a</td>
<td>1.94±0.1b</td>
<td>2.08±0.1a</td>
<td>1.92±0.1b</td>
<td>2.74±0.1a</td>
<td>2.56±0.1a</td>
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</table>

a,b: There is statistical significance between values expressed in different letters on the same line (p <0.001).

The total protein concentrations between days of the study were found to be very close to each other and these values were also found at the reference range (TP reference range = 50-75 gr/dL). It was determined that the TP levels peaked (6.92 g/dL) at the first week postpartum and was lowest (6.52 g/dL) at the second week prepartum (Table 2). Again serum albumin levels were detected within reference range during the study period (albumin reference range = 0.5-5 g/dL), lowest albumin levels were detected at the week before parturition (3.47 g / dL) and the highest level was detected at postpartum 3rd week (Table 2).

BUN levels were detected to decrease till the delivery and steadily increased after parturition. BUN levels were lowest (9 mmol/L) at the week before parturition and highest in postpartum week 2 (16.4 mmol/L), respectively (Table 2). The level of creatinine gradually increased until the day of delivery, reached the peak level at the time of delivery (0.68 mg/dL), and gradually decreased in the postpartum period and remained within the reference range (Table 2). Serum AST values were found to be within normal limits throughout the study period (AST reference range = 2- 75 IU/L). AST levels were lowest birth 1 week before the parturition (42 IU/L) and highest at week 3 postpartum (69.8 IU/L) (Table 2).

It is known that the serum GGT in goats is 0-30 IU/L (Batmaz, 2013). According to that reference value, GGT levels were found to be slightly higher in our study especially at 2. and 3 weeks after parturition (Table 2). Serum SDH values gradually increased until the birth, reached to peak level during delivery (29.9 IU/L) and gradually decreased after birth. Serum glucose values of the animals used in the study were at the peak level (62.8 mmol/L) at the 7th day prepartum and the lowest levels (45.5 mmol/L) were detected three weeks before parturition (Table 2). It was also detected that the lowest level value is slightly below the reference values (Glucose reference range = 50-75 mg/dL).

The serum cholesterol levels were within reference range (cholesterol reference range = 17-210 mg/dL) during the study period. Lowest cholesterol levels...
(83.8 mg/dL) were detected at the day of parturition and the highest (97.7 mg/dL) levels were detected on the third week before parturition (Table 2). Triglyceride levels were significantly higher in prepartum period when compared to postparum period. Along with that triglyceride levels reach the lowest point (9.4 mg/dL) which is very close to the lowest reference value (triglyceride reference range = 6 - 200 mg/dL) at time of parturition. Serum calcium levels were also detected within reference range in all samplings during the study period (reference = 8.9 -11.7 mg/dL) (Batmaz, 2013). Calcium levels were highest at week 3 prepurum (9.38 mg/dL) and lowest on the second week after parturition (8.33 mg/dL)(Table 2).

Serum phosphorus levels in our study were found to be lowest (4.59 mg/dL) in the week before parturition (Table 2), and peaked (6.59 mg/dL) in the second week after delivery. Along with that phosphorus levels were detected within reference values in all samplings during the study period (Phosphorus reference range = 4.2-9.1 mg/dL). Magnesium levels were lowest on day 7 postpartum and highest on day 14 postpartum (Table 2).

**DISCUSSION**

This study was conducted in order to determine whether some significant biochemical blood parameters in Saanen goats vary between prepartum and postpartum periods.

Increased lipolysis and decreased lipogenesis around parturition causes elevation of blood NEFA levels and BHB levels (Mc Namara, 1994). Sadjadianet al. (2012) reported that serum concentrations of NEFA in Saanen goats gradually increased from day 30 before parturition to the highest level on day of delivery and then gradually decreased until the 35th day postpartum. In our study we detected similar results as NEFA levels were highest at day of parturition and afterwards decreased steadily (Table 2). The increased concentration of NEFA during delivery could be related lipolysis and hormonal changes triggered by both the energy that the animal will spend for labor, the energy needed for milk production after birth, in order to meet the energy required for the development of the fetus and mammary gland in the last period of pregnancy (Vazquez-Anon et al., 1994; Grummer, 1995; Cheng et al., 2007). According to Herdt(1988, 2000), the rise of plasma NEFA in the end of pregnancy may not end with an increase in TG in the liver however but acute NEFA increases at time of delivery may trigger liver TG infiltration. Vazquez-Anon et al.(1994) and Herdt (2000) have concluded that, the concentration of NEFA reaching peak levels during delivery decreased in the first weeks of lactation. During these events, hormonal changes are also very important.Before parturition, the insulin/glucagon ratio is reduced in favor of glyconeogenesis and lipolysis (Vazquez-Anon et al., 1994).In the present study, it was observed that the level of the NEFA gradually began to descend to the basal level in postpartum period.In addition, reductions in NEFA can be interpreted as a sign of reduced fat mobilization or the use of NEFA for VLDL synthesis in the liver. On the other hand, highest plasma concentrations of NEFA were detected at birth and the highest BHBA concentrations were detected at third week postpartum. This lag may be explained by the fact it can be used in the synthesis of BHBA in the liver following the increase of NEFA firstly due to lipolysis (Cheng et al., 2007).

The BHBA is the most important indicator of energy status in the transition period (Duffield, 2003; Inal et al., 2007). Navarre and Pugh (2002) suggested that the concentration of BHBA in the range 0.8 to 1.6 mmol / L was indicative of NEB in sheep. In another study conducted by Moghaddam and Hassanpour (2008) the BHBA concentrations of sheep was higher in the goats in the prepartum period.Sadjadianet al. (2012) reported that the BHBA concentrations increased from 15 days prepartum till the third week postpartum and than tended to decrease in Saanen goats. In our study BHBA levels decreased as parturition approached, increased during labor and gradually increased in the following weeks after birth. The reason for this may be increased energy demand with the onset of lactation.

Similar to other studies (Tanrıtanır et al.,2009; Sadjadianet al. (2012), Serum total protein levels in our study were lower before parturition than the postpartum period. This reduction may be due to the fact that protein synthesis required for the development and growth of the fetus, is carried out using maternal amino acids and that requirement is highest at the end of pregnancy.

As reported by Shetawi et al. (1992), in the present study, albumin levels gradually decreased until parturition and significantly increased 1 week after delivery(Table 2). As in humans (Ogbo do et al., 2012), the decrease in albumin level may be associated with decreased albumin synthesis in pregnancy, also and increased loss in urine in pregnancy.
As Sadjadian et al. (2012) found, BUN concentration in our study were lower in the prepartum period, tended to increase after parturition (Table 2). The reason for lower BUN levels around parturition may be related to reduced feed intake and dry matter consumption, associated with increased stress and hormonal changes.

AST activity is an important indicator for fatty liver disease in cows (Kaneko 1989, Cebra et al., 1997; Herdt, 2000, Seifi et al., 2007, Herdt, 2009). Studies conducted on sheep by Taghipour et al. (2011) was found a significantly higher AST concentration in the postpartum period, and this elevation was explained to be indicative of fat infiltration in the liver due to NEB. Despite the fact that the postpartum AST levels were higher than the prepartum levels in our study, the fact that the AST levels in the pregnancy were within the reference rate could indicate that the animals used in our study were not in the NEB (Table 2).

It has been reported that serum GGT levels are high in negative energy balance associated fatty liver of ruminants, and this parameter may be used as a diagnostic tool (Senturk, 2013). Sevinc et al. (1999) found no statistically significant difference in serum GGT levels between the 7th month of pregnancy and 2nd month after birth in a study conducted in cattle. In the present study, no significant change was observed in GGT levels.

SDH enzyme activity is very important in determining acute hepatocellular damage in ruminants (Senturk, 2013). SDH levels in our study gradually increased until birth and reached peak levels during delivery and then gradually began to fall after birth (Table 2). Although this short-term rise in SDH reaching peak levels at birth strengthens the likelihood of fatty liver, but this elevation also be related to hormonal changes during labor.

According to Khan et al. (2009), blood glucose concentrations are one of the most important parameters that can reveal the nutritional status of animals during pregnancy. Gurogoze et al. (2009) assessed blood glucose levels in sheep during to transition period, according to it is reported that blood glucose levels of pregnant goats are lower than those of non-pregnant animals. On the other hand, Al-Dewachi (1999) reported that plasma glucose levels are increasing in pregnancy. In our study, blood glucose levels reached peak levels during the parturition, gradually decreased in the postpartum period, and a statistically significant difference between the postpartum glucose level and prepartum glucose level were detected. Decreased glucose levels at the beginning of lactation detected in our study are in accordance with Seifi et al. (2007), and are probably caused by high milk yield.

Cholesterol levels are reported to decrease at the final stages of pregnancy (Ozpinar et al., 1989, Azab and Abdel-Maksoud, 1999; Krajnicakova et al., 2003). Mbassa and Poulsan (1991) reported that plasma cholesterol levels increased during lactation. Similarly in the present study, cholesterol levels decreased during pregnancy and significantly increased after parturition. The cause of decreased cholesterol during to give birth; It is thought that it was caused by mothers giving colostrum and using storage oils for feeding their offspring.

Fatty liver is associated with elevated serum fatty acids and decreased VLDL production. Findings in our study are similar to previous studies (Hamadeh et al., 1996; Rukkwamsuk et al. 1999, Balıkcı et al., 2007) that reported an increase in serum triglyceride levels during the last months of pregnancy in sheep and goats. In contrast, Obidike et al. (2009) reported elevated triglyceride levels after parturition. In the present study, we determined that the triglyceride levels were significantly increased as the parturition approached, reaching peak level in the last week of pregnancy, decreased at birth, and gradually increased again in the postpartum period (Table 2). These changes could be related with increased energy uptake in order to supplement the energy requirement of the fetus by producing glycerol. Increased triglyceride levels after birth may be caused by triglyceride synthesis of mammary glands for milk synthesis.

Krajnikova et al. (2003) reported that serum calcium levels began to fall in the near term and were at the lowest level (1.73 mmol / l) on the third day postpartum and concluded that this decrease was a characteristic of the puerperal period. In the present study, the lowest calcium levels were detected in the second week after birth, in accordance with pervious reports (Krajnikova et al., 2003) this could probably related with the milk yield of the lactating animal (Table 2).

Karapinar et al. (2007) reported a marked hypophosphatemia in ketotic animals, compared to healthy animals and concluded that this could be related with appetite loss due to disease, as well as excessive loss of phosphorus in the milk after parturition. The lowest phosphorus concentration in our study was detected
one week before parturition, slightly elevated at birth, and gradually increased after birth to reach prenatal levels (Table 2). The explanation of the decreased phosphorus levels near term may be due to the fact that as the birth approaches, consumption of dry matter decreases due to stress.

In the present study, the magnesium concentration was found to be lowest in the last week before and the first week after parturition (Table 2). It is possible to relate the decline in the last week near term to the reduction of feed intake due to stress and the postpartum decline with hormonal adaptation or the onset of postpartum hypocalcemia (Senturk, 2017).

Diseases such as pregnancy toxemia, hypocalcemia, infertility, low milk yield cause serious economic loss in Saanen goats. It is necessary to conduct prenatal and postnatal metabolic profile testing in Saanen goats susceptible to metabolic diseases such as pregnancy toxemia and ketosis due to their genetic characteristics. In the present study, we aimed to investigate the presence of these diseases in Saanen goats by metabolic profile test. During the study, the values of NEFA, SDH and CREA increased and CHOL and TG values decreased with parturition. When the values of 3 weeks prepregnant and 3 weeks postpartum were compared, NEFA, CHOL, GLU, TG, Ca levels were higher before and BHBA, Mg, ALB, GGT, AST, TP, P, BUN, SDH and CREA were higher after parturition. Not any of the animals used in the study suffered a metabolic or infectious diseases thus results of our study may be considered as pioneering study for reference biochemical parameters for Saanen goats. On the other hand, in order to clarify metabolic profiles changes during the transitional period in Saanen goats, it is necessary conduct studies with higher study population also evaluating more detailed hormonal and biochemical parameters.

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CONFLICT OF INTEREST
None declared by the authors.

REFERENCES


