Kafkas Univ Vet Fak Derg
24 (1): 117-122, 2018
DOI: 10.9775/kvfd.2017.18479

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Investigation of Changes in Metabolic Parameters and Paraoxonase-1 During the Transition Period in Turkish Saanen Goats [1]

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[1] The study was presented at 12th International Conference on Goats, 25-30th September 2016, Antalya, Turkey

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Article Code: KVFD-2017-18479 Received: 21.07.2017 Accepted: 01.10.2017 Published Online: 01.10.2017

Citation of This Article

Salar S, Baştan İ, Baştan A, Pekcan M, Sel T: Investigation of changes in metabolic parameters and paraoxonase-1 during the transition period in Turkish Saanen soats. *Kafkas Univ Vet Fak Derg,* 24 (1): 117-122, 2018. DOI: 10.9775/kvfd.2017.18479

Abstract

The aim of this study was to evaluate the metabolic changes and PON-1 levels in Turkish Saanen goats during the transition period. In the study, 60 Turkish Saanen goats were used and blood samples were taken from the jugular vein on days -30, -15, 0, +15, and +30 from parturition. In order to monitor metabolic profile changes, alkaline phosphatase (ALP), cholesterol (CHO), glucose (GLU), calcium (CA), phosphorus (PHO), total protein (TOP), triglyceride (TG), paraoxonase-1 (PON-1), β-hydroxybutyric acid (BHBA) and non-esterified fatty acid (NEFA) levels were determined. Serum alkaline phosphatase levels were lower on days -30, 0 and +15, and higher on day +30 ($P<0.001$). Serum cholesterol and total protein levels were lower on days -30 and 0, and higher on day +30 ($P<0.001$). Serum levels of glucose, calcium and phosphorus were lower on day 0, and higher on day +30 ($P<0.001$). Serum triglyceride levels were lower on days -15 and 0, and higher on days -30 and +30 ($P<0.05$). Serum paraoxonase-1 levels were lower on day 0, and higher on day -30 ($P<0.05$). Serum β-hydroxybutyric acid levels were lower on day -30, and higher on day +15 ($P<0.05$). Serum non-esterified fatty acid levels were lower on days -30 and +30, and were higher on days -15 and 0 ($P<0.001$). In conclusion, the results of our study showed that clear changes in levels of metabolic parameters and PON-1 were seen during the transition period in Turkish Saanen goats.

Keywords: *Metabolic parameter, PON-1, Transition period, Turkish Saanen goat*

Türk Saanen Keçilerinde Geçiş Dönemi Boyunca Metabolik Parametreler ve Paraoksonaz-1 Değişiminin İncelenmesi

Özet

Bu çalışmanın amacı, Türk Saanen keçilerinde geçiş dönemi boyunca metabolik parametreler ve paraoksonaz-1 düzeylerindeki değişimi incelenmekti. Çalışmada 60 adet Türk Saanen keçisi ve bu keçilerin juguler venasından doğumdan 30 ve 15 gün önce, doğumda ve doğumdan 15 ve 30 gün sonra alınan kan örnekleri kullanıldı. Metabolik profilde meydana gelen değişimleri belirlemek için alkalen fosfataz (ALP), kolesterol (CHO), glukoz (GLU), kalsiyum (CA), fosfor (PHO), total protein (TOP), trigliserit (TG), paraoksonaz-1 (PON-1), β-hidroksibütirik asit (BHBA) ve non-esterifiye yağ asidi (NEFA) düzeyleri belirlendi. Serum alkalen fosfataz düzeyleri -30, 0 ve +15. günlerde en düşük, +30. günde en yüksekti ($P<0.001$). Serum kolesterol ve total protein düzeyleri -30 ve 0. günlerde en düşük, +30. günde en yüksekti ($P<0.001$). Serum glukoz, kalsiyum ve fosfor düzeyleri 0. günde en düşük, +30. günde en yüksekti ($P<0.001$). Serum trigliserit düzeyleri -15 ve 0. günlerde en düşük, -30 ve +30. günde en yüksekti ($P<0.05$). Serum paraoksonaz-1 düzeyleri 0. günde en düşük, -30. günde en yüksekti ($P<0.05$). Serum β-hidroksibütirik asit düzeyleri -30. günde en düşük, +15. günde en yüksekti ($P<0.05$). Serum non-esterifiye yağ asidi düzeyleri -30 ve +30. günlerde en düşük, -15 ve 0. günlerde en yüksekti ($P<0.001$). Sonuç olarak, Türk Saanen keçilerinde geçiş dönemi boyunca metabolic parametreler ve paraoksonaz-1 düzeylerinde belirgin değişimlerin meydana geldiği belirlendi.

Anahtar sözcükler: *Geçiş dönemi, Metabolik parametre, PON-1, Türk Saanen keçisi*

INTRODUCTION

Turkish Saanen goat originated by crossing of Saanen breed and is commonly used in goat husbandry in Turkey according to higher milk production and fertility efficiency [1]. The average lactation duration and yield of Turkish Saanen goat are between 270-290 days and 500-600 kg (can reach up to 805 kg), respectively [1,2].

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Transition period defines the 3-week period before and after the parturition and it is mostly used for dairy cows. Important hormonal and metabolic changes occur during the transition period of cows. Immunosuppression, infections such as metritis, mastitis and fertility problems can be observed depending on these changes [3]. The transition period is the most critical period among lactation periods in terms of health, fertility, milk yield and thus the profit, especially in high-yielding animals [4].

Nutritional requirements for milk production and fetal growth prominently increase in the late pregnancy and early lactation period [4,5]. The energy requirement also increases in line with the rapidly increasing milk yield immediately after the calving. However, the feed consumption of cows is unable to meet their energy needs and thus negative energy balance (NED) occurs [4,6,7]. Negative energy balance is characterized with the fat tissue mobilization and rise in blood ketone bodies in order to meet energy requirements [7]. Immune system functions are negatively affected by this metabolic stress condition [8]. Physiological stress and an increase in oxygen requirements and energy demands occur depending on the onset of milk production and physiological changes in mammary glands. Oxidative stress occurs as a result of the excess accumulation of reactive oxygen species (ROS) due to the rise in oxygen consumptions [9,10]. There are metabolic changes in addition to the endocrine profile changes in goats in the periparturient period [11]. Even though lipid mobilizations [12] and oxidative stress [13,14] are specified in goats as in the cows, there are limited numbers of studies on the metabolic changes in high-yielding dairy goats.

Paraoxonase-1 is synthesized in liver and it is an enzyme associated with high-density lipoprotein (HDL) [15] and it is also specified as the negative acute phase protein [16]. PON-1 is used as oxidative stress biomarkers due to their protective roles on HDL and low density lipoproteins (LDL) [17]. According to the literature, even though there are studies about PON-1 in cattle [18-22], dogs [15,23,24] and horses [25], there is no study conducted on this enzyme in any goat breed.

To our knowledge, there are no published paper on changes of metabolic parameters during the transition period in Turkish Saanen does. We hypothesized that important changes at levels of metabolic parameters and PON-1 can occur during the transition period in Turkish Saanen goats. For this, we aimed to evaluate the metabolic changes in Turkish Saanen goats does during the transition period.

MATERIAL and METHODS

Animal, Housing and Management

The study was conducted on a commercial farm in Ankara, Turkey (39°53'E; 32°45'N) in the period from January to March 2014. Sixty Turkish Saanen goats (75% Saanen × 25% Kilis (B_1) crossbreed), aged 2 to 4 years, clinical healthy, with similar body weight (range of body weight was 50-55 kg) and body condition score (BCS of 3.0) [26] were included in the study. The goats with disorders related to calving or any metabolic and infectious diseases during study period were excluded from the study. All animals were housed in an outdoor paddock under natural conditions. The animals were fed with wheat straw, alfalfa hay and commercial compound (feed regimen (per doe) during dry period; 500 g commercial compound + 500 g alfalfa hay and *ad libitum* wheat straw; feed regimen (per doe) during early lactation period: 1000 g commercial compound + 1000 g alfalfa hay and ad libitum wheat straw). All animals had unlimited access to fresh water and mineralized salt blocks. In the farm, all animals were regularly vaccinated against brucellosis, peste des petits ruminants and foot and mouth disease and were routinely received worming treatment. After the parturition, kids were kept together with their does for suckling during 45 days in milk. After the suckling, all animals were milked once a day using a milking machine in the morning and the average milk yield was about 3 liter/day per doe. The average litter size at birth was 1.97±0.04 per doe (mean±SE).

Study Design, Blood Sampling and Biochemical Analyses

All does were subjected to controlled mating with Turkish Saanen bucks on natural oestrus. To determine pregnant does, transrectal ultrasonography was done using 7.5 megahertz (MHz) linear probe connected to a portable B-mode scanner (Hasvet 838®, Hasvet, Turkey) by the same observer up to 25 to 30 days after mating. Expected delivery dates were determined for each doe according to pregnancy diagnosis for blood sampling.

In the study, blood samples were collected from the jugular vein into Vacutainer tubes containing clot activator 4 h after the feeding on days of -30, -15, 0, +15 and +30 from expected parturition. These blood samples were centrifuged at 3.000 rpm for 20 min at 4°C within 1 h after the sampling and serum were kept in a freezer at –20°C until biochemical analyses were performed.

Levels of alkaline phosphatase (ALP), cholesterol (CHO), glucose (GLU), calcium (CA), phosphorus (PHO), total protein (TOP), triglyceride (TG), β-hydroxybutyric acid (BHBA), non-esterified fatty acid (NEFA) and paraoxonase-1 (PON-1) were measured in the samples. All the parameters except paraoxanase activity were measured using Erba XL 600 autoanalyzer and its accompanied commercial reagents (Erba-Diagnostics, Mannheim). Serum paraoxonase activitity was measured in a 37°C ambient temperature as the rate of hydrolysis of paraoxone at 412 nm in 0.05 mmol/L glycine buffer (pH 10.5) with 1 mmol/L $CaCl_2$. All biochemical analyses were conducted on Biochemistry Laboratory at Ankara University, Faculty of Veterinary Medicine.

The experimental procedures were approved by the Local

Ethical Committee on Animal Experiments of Ankara University in Ankara (Turkey) (Approval no; 2013-14-106).

Statistical Analyses

Before analyses, data were checked for normality and homogeneity of variance using the Kolmogorov-Smirnov and Levene test, respectively. General linear model (GLM) for repeated measures procedure was used to evaluate the differences of blood serum metabolite parameters between period. A multiple comparison test of least-squares means was performed using the Bonferroni correction. P<0.05 was used as the criterion for significance. All results were presented as the means ± standard error of means (mean ± SEM). All data analysis was carried out by using the SPSS 14.01 (Licence No: 98692604) statistical program.

RESULTS

Serum ALP levels were lower on days -30, 0 and +15, and were higher on day +30 (P<0.001). Serum CHO and TOP levels were lower on days -30 and 0, and were higher on day +30 (P<0.001). Serum levels of GLU, CA and PHO were lower on day 0, and were higher on day +30 (P<0.001). Serum TG levels were lower on days -15 and 0, and were higher on days -30 and +30 (P<0.05). Serum PON-1 levels were lower on day 0, and were higher on day -30 (P<0.05). Serum BHBA levels were lower on day -30, and were higher on day +15 (P<0.05). Serum NEFA levels were lower on days -30 and +30, and were higher on days -15 and 0 (P<0.001). All results were presented in *Table 1* and *Table 2*.

DISCUSSION

In our study, serum ALP levels were similar in -30, -15, 0, and +15 days whereas it reached to its maximum amounts on the +30 day. ALP is an enzyme which is defined as the physical stress indicator [27]. Hepatic diseases, intestinal disorders, drug use, pregnancy, and various diseases are the factors which increase serum ALP levels [28]. Bogin et al.[29] stated that serum ALP levels prominently increased in cows with fatty liver. The maximum serum ALP levels on the +30 day can be related to the effect of the hyperketonemia on the fatty liver (it was detected that BHBA levels prominently increased in the postpartum period). In case of the negative energy balance, the glucose need is met by lipolysis by using body fat stores. In case fatty acids excessively accumulated in the liver, the liver functions are deteriorated and hepatic lipidosis occurs [30,31].

We detected that the maximum levels of TG on the 30 d postpartum and this finding can be supported with serum ALP outcome. The fat accumulation in the liver of cows with fatty liver syndrome occurs primarily as triglycerides [32]. Kalaitzakis et al.[33] specified that TG levels were high in cows died due to the fatty liver syndrome. They further claimed that the high TG levels were associated with high liver damage and poor prognosis.

Serum TOP levels were lower on the 30 d prepartum and 0 day whereas it was gradually increased and reached the higher levels on 30 d postpartum. This can be associated with the onset of colostrum production. Transition of immunoglobulins and serum proteins from blood to colostrum occurs with the onset of colostrum production in udder at prepartum period. These findings were in line with results of Sadjadian et al.[11] obtained from Saanen goats.

Cholesterol levels in goats are changeable depending on nutritional and physiological (pregnancy, lactation) conditions [34]. The serum CHO level was higher on the 15 d prepartum whereas it was in its minimum level on

Table 2. *Mean values (± SEM) of PON-1 and keton bodies in Turkish Saanen goats during the preparturient period*

Days	Paraoxonase-1	β-hydroxybutyric Acid	Non-esterified Fatty Acid
-30	168.30±9.05 a	0.62±0.04 b	0.30±0.03 b
-15	164.37±8.72 a	0.70±0.04 ab	0.54±0.05 a
0	138.43±7.02 b	0.73±0.06 ab	0.53±0.07 a
+15	161.17± 7.53 a	0.85±0.05 a	0.40±0.04 ab
+30	151.33±7.20 ab	0.70±0.06 ab	0.23±0.02 b
p	*	*	**

-30: *Prepartum 30;* ***-15:*** *Prepartum 15;* ***0:*** *Parturition;* ***+15:*** *Postpartum 15;* ***+30:*** *Postpartum 30; ** P<0.001; * P<0.05; abc The same superscript letters in the same column indicate no significant differences statistically*

Table 1. *Mean values (±SEM) of metabolic parameters and macrominerals in Turkish Saanen goats during the preparturient period*

Days	Alkaline Phosphatase	Cholesterol	Glucose	Calcium	Phosphorus	Total protein	Triglyceride
-30	27.77±3.57 b	65.78±2.92 bc	47.50±2.18 b	4.68±0.27 b	4.95±0.27 b	3.96±0.35 bc	88.94±2.52 ab
-15	33.50±3.81 b	76.06±3.40 b	50.78±3.89 b	5.06±0.30 b	5.04±0.26 b	5.19±0.36 b	80.10±1.96 c
0	23.77±5.53 b	59.24±2.50 c	33.18±2.39 c	3.54±0.22 c	4.89±0.34 b	3.24±0.27 c	80.60±2.24 bc
+15	29.05±4.30 b	74.60±2.85 b	48.22±2.12 b	5.30±0.21 b	5.88±0.39 b	5.08±0.29 b	85.04±2.32 abc
+30	59.05±7.90 a	108.86±2.99 a	67.93±1.81 a	7.96±0.14 a	9.44±0.35 a	8.62±0.16 a	90.33±2.03 a
P	**	**	**	**	**	**	*

-30: *Prepartum 30;* ***-15:*** *Prepartum 15;* ***0:*** *Parturition;* ***+15:*** *Postpartum 15;* ***+30:*** *Postpartum 30; ** P<0.001; * P<0.05; abc The same superscript letters in the same column indicate no significant differences statistically* the 0 day. Higher serum CHO level was determined on the 30 d postpartum as a result of the gradual increase after kidding. These findings were in line with results of Sadjadian et al.[11] obtained from Saanen goats. According to results of previous study [11], stated that the low CHO levels in the last week of the pregnancy can be associated with the rise of needs for the fetal growth and increase in the steroid hormone synthesis [35]. It was also stated that the increase of CHO in the lactation period can also be related to the lipid immobilization [36]. Additionally, it can also be associated with prepartum and postpartum nutritional differences because animals were fed with energy rich feeds after the kidding compared to the dry period.

Serum glucose levels were similar on 30 d and 15 d prepartum. Even though we observed the minimum serum glucose level at the time of the kidding, it gradually increased and became maximum on the 30 d postpartum. Similarly, Sadjadian et al.[11] and Radin et al.[37] reported that serum glucose levels were higher in the early lactation period compared to prepartal period. This was associated with the decrease in the severity of the negative energy balance that was observed after the 15 d postpartum. This thought is verified with the rise in the BHBA levels on 15 d postpartum and the reduction of BHBA levels on 30 d postpartum day. Sadjadian et al.[11] detected that glucose levels were minimum in the two weeks before from kidding and it reached its peak level during the parturition. The researchers claim that endocrine changes during the parturition can be associated with the induction of the gluconeogenesis. In our study, we detected that serum glucose level was the lowest on the day of parturition. The decline may be associated with decreased food intake due to physiological stress of parturition. Another possible explanation is that this finding can be associated with the high incidence of the pregnancy toxemia (when evaluate the both glucose and BHBA levels together) which can occur due to the nutritional deficiencies.

Our results showed that serum levels of CA were lower on the 0 day and higher on day +30. Similarly, PHO were lower on the 0 day, and were higher on day +30 in serum samples. Ivanov et al.[38] determined that calcium need increased with the initiation of milk production and serum calcium levels decreased in dairy cows. Similarly, we also observed these changes in Turkish Saanen goats. Ca and P levels in goats in the late pregnancy and early lactation periods are associated with the Ca and P levels obtained by rations and Ca/P homeostasis [39]. We detected minimum levels of these parameters at kidding and they gradually reached their maximum levels on the 30 d postpartum. These findings can be associated with nutritional changes after the kidding. The amount of alfalfa hay that is a Ca-rich source is prominently increased in the postpartum period.

PON-1 is synthesized in the liver and it is a Ca-dependent and HDL associated enzyme [40]. PON-1 decreases the oxidative stress in transgenic mice [41], and there is an increase in the gene expressions related with oxidative stress in PON-1-knockout mice. Therefore, PON-1 is defined as the negative acute phase protein [16]. In our study, it was found that serum PON-1 levels were minimum on 0 day (138.43±7.02 U/ml) and maximum on -30 day (168.30±9.05 U/mL; $P<0.05$). Furthermore, PON-1 levels of -15 (164.37± 8.72 U/mL) and +15 (161.17± .53 U/mL) day were similar with levels of -30 day (168.30±9.05 U/mL). According to the changes in the PON-1 levels, it is possible to claim that the minimum oxidative stress level was detected on -30 day, it reaches its maximum level at kidding, and oxidative stress continues during the late pregnancy and early lactation period. Similarly, Turk et al.[18] reported that PON-1 level is lower in late pregnancy and early lactation in cows compared to other periods.

NEFAs are precursors of ketone bodies and reflect the mobilization from fatty storage. Fatty tissues are mobilized when glucose supply cannot meet requirements for pregnancy and milk production. This condition results in a decrease and an increase of glucose and NEFA concentrations in blood stream, respectively [42]. Serum NEFA levels were lower on days -30 (0.30±0.03 mmol/L) and +30 (0.23±0.02 mmol/L), and were higher on days -15 (0.54±0.05 mmol/L) and 0 (0.53±0.07 mmol/L; $P<0.001$). The higher levels of NEFA from 15 d prepartum to parturition can be supported by serum glucose outcome. Serum NEFA threshold for cows was defined as <0.4 mEq/L for prepartal period. However, there are no published data on the cutoff levels for NEFA in Turkish Saanen goats.

In the diagnosis of pregnancy toxemia in goats, BHBA threshold level should be accepted as 0.8 mmol/L in case the numbers of fetuses are not known. In case the numbers of fetuses are known, we need to consider the possible diseases in multiple pregnancies and BHBA measurements should be done and the threshold levels should be accepted as 1.1 mmol/L [43]. Bani Ismail et al.[44] stated that the BHBA level is accepted as 0.86-1.6 mmol/L for the subclinical toxemia of pregnancy in their study. However, there is no determined threshold level for BHBA for Turkish Saanen goats. We detected the minimum BHBA level on -30 day (0.62±0.04 mmol/L) and maximum on +15 day (0.85±0.05 mmol/L; $P<0.05$). BHBA level showed a linear increase from -30 day to +15 day (0.62±0.04 mmol/L; 0.70±0.04 mmol/L; 0.73±0.06 mmol/L; 0.85±0.05 mmol/L) and it decreased on the +30 day (0.70±0.06 mmol/L). Our findings are in line with the results of Sadjadian et al.[11]. These results indicate the gradual rise in the lipid mobilization till +15 day. Meanwhile, it was found that BHBA levels were higher than 0.8 mmol/L in 14 out of 60 animals (data were not presented) according to the cut-off BHBA levels, that is, there was a high hyper-ketonemia incidence.

In conclusion, this paper reports first results of changes in metabolic parameters and PON-1 levels during late pregnancy and early postpartum period in Turkish Saanen goats. Conclusively, clear changes in levels of metabolic parameters and lipid mobilization were seen during the transition period. Significant changes in levels of PON-1 were occurred in Turkish Saanen goats and PON-1 may be used as oxidative stress biomarker. Further studies are required to determine the availability of PON-1 as a marker of oxidative stress and to confirm these findings. It should be taken into consideration that implementations of preventive strategies against oxidative stress and hyperketonemia could be useful to maintain health and production.

Acknowledgments

We want to express our thanks to farm's staff and its owner Aslan Saracoglu for help to perform this study.

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