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The effects of supplemental niacin and methionine on serum glucose, beta-hydroxybutyric acid, and non-esterified fatty acid levels during late gestation and early postpartum period in Damascus dairy goats

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Abstract: The objective of this study was to assess the changes in serum glucose, beta-hydroxybutyric acid (β-HBA), and nonesterified fatty acid (NEFA) levels in Damascus dairy goats fed niacin (Nia) and methionine (Met) during late gestation and early postpartum period. A total of 75 dairy goats at 105th day of gestation were divided into 3 groups as control (no supplementation), Nia (20 g/kg Nia), and Met (2.5 g/kg Met) groups. The dietary treatments continued until parturition. Dairy goats fed dietary Nia had greater serum glucose levels on day 125 of gestation compared to the other dietary treatments ($P < 0.05$). On day 135 of pregnancy, serum glucose levels were higher in Met group than control group ($P < 0.05$). Serum β-HBA levels decreased on days 135 and 145 in dairy goats fed Nia than those in control group. Dietary Nia and Met lowered serum β-HBA levels on day 10 postpartum compared to control group ($P < 0.05$). Serum NEFA levels decreased ($P < 0.05$) on days 135 and 145 in dairy goats fed Nia supplemented ration than those in control group. Serum NEFA levels d 10 postpartum were different among the groups ($P < 0.05$). In conclusion, dietary Nia and Met supplementation improved serum glucose levels on day 125 of gestation while decreasing β-HBA and NEFA levels during late gestation and early postpartum period in Damascus dairy goats under the conditions of present study.

Key words: Glucose, goat, methionine, NEFA, niacin, β-HBA

1. Introduction

Pregnancy advancement in ruminants smoothly increases the nutritional requirements of these animals. However, an important increase in the nutritional requirements of small ruminants occurs during late gestation due to the acquisition of 80% birth weight by conceptus in the last 2 months of pregnancy [1]. INRA [2] reported that the energy requirement of a pregnant goat in late gestation is 2.5 times higher than in early gestation, and even much higher in the event of multiple pregnancy. This means that energy requirement of pregnant goats increases towards the end of pregnancy in parallel with the growth of conceptus. This would require more intake to meet the nutrient requirements of growing fetuses, however, intake during advancing gestation is usually lowered due to rumen compression by the conceptus, loss of ruminal papillae development, and reduction in absorption rate of fermentation products [3]. In other words, carbohydrates fail to fulfill the energy or glucose requirements for fetal growth. Consequently, small ruminants start mobilizing their body reserves (fat tissues) so as to meet the energy demand of conceptus. The use of fat deposits generates nonesterified fatty acids (NEFAs) that are accumulated in the liver. It implies that the blood NEFA levels increase as the energy requirement increases. NEFAs accumulated in the liver are partly used as energy resources, albeit remaining portion is converted into toxic ketone bodies (β-hydroxybutyric acid (β-HBA), acetoacetic acid and acetone) causing an increase in the concentration of ketone bodies in blood, milk, and urine in addition to hypoglycemia [4–7]. Such a condition in small ruminants is referred to as pregnancy toxemia or ketosis. It is a common metabolic disorder in pregnant goats occurring as a consequence of derangement in carbohydrate and fat metabolism. The animals having poor (≤2) or very high (≤4) body condition scores (BCS), pregnant with multiple

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fetuses, high milk yields, and aged from 2 to 4 years are more prone to this disorder [8–10].

Several approaches have been suggested to prevent the development of pregnancy toxaemia. Prophylactic measures are one of these strategies [11,12]. Feed supplements like L-carnitine, chrome, propylene glycol, sodium propionate, ionophores, niacin (Nia), and methionine (Met) have been proposed to prevent this metabolic disorder [13,14]. Nia and Met are known for their roles in the suppression of lipolysis in transition dairy cows to regulate the hepatic lipid metabolism [15,16]. However, there is no known study available in the literature that describes the effects of using supplemental Nia and Met on pregnancy toxicity in the late gestation and early postpartum period of the goats. In practice, blood glucose, β-HBA and NEFA levels are used as primary biochemical parameters to assess the energy status of pregnant animals [17]. We hypothesized that dietary Nia and Met may regulate the lipid metabolism in goats during late pregnancy and early lactation that, in turn, may help to protect goats from the development of pregnancy toxemia. Therefore, the present study was conducted to evaluate the changes in serum glucose, β-HBA and NEFA levels of goats fed Nia and Met during late gestation and early postpartum period.

2. Material and methods

The present study was conducted at a commercial goat farm located in the Nurdağı district of Gaziantep province in Turkey, as the coordinates of latitude: 37°10′45″N and longitude: 36°44′10″E. All the experimental procedures followed in this study were approved by the Animal Care and Use Committee of Mustafa Kemal University, Hatay, Turkey via letter no. 2016/4/6.

2.1. Study design and treatments

A total of 75 Damascus goats aging from 2 to 5 years, weighing from 45 to 60 kg having given birth at least once were randomly allocated to 3 groups, each group comprising of 25 animals. In order to synchronize the start of the research on day 105 of the pregnancy, all goats in their breeding season were treated with intravaginal sponges impregnated with 20 mg of chronolone (Flugestone acetate, Chronogest CR, Intervet, İstanbul, Turkey). The intravaginal sponges were retained for a period of 9 days. Later, sponges were removed and 500 IU pregnant mare serum gonadotropin (PMSG, Chronogest/PMSG, 6000 IU, Intervet, İstanbul, Turkey) and 0.075 mg of d-cloprostenol (Senkrodin, Vetaş, İstanbul, Turkey) were injected intramuscular. The estrous was detected in goats and mated with a fertility-proven buck. Pregnancy was diagnosed transabdominally 50 days postmating using a 6–8 MHz linear probe and a real time ultrasound device (Falco, PieMedical, Maastricht, the Netherlands). The goats confirmed to be gravid upon pregnancy diagnosis were assigned into 3 equal groups each consisting of 25 pregnant goats on 105th day of gestation. The animals included in control group were fed diets without any supplementation. The animals in Nia and Met groups received feed concentrates supplemented with 20 g/kg of Nia and 2.5 g/kg of Met, respectively, until parturition. Throughout the study period, all animals were provided with a feed concentrate composed of 16% crude protein, 2800 kcal/kg metabolizable energy (ME), 12% crude fiber, 3% crude fat, 10% crude ash, 0.5% sodium, 3.5% calcium, and 1.5% phosphorus along with good quality alfalfa hay as roughage. Water and mineral blocks were supplied ad libitum.

2.2. Serum Glucose, β-HBA, and NEFA levels

Ten goats were randomly selected from each group. Blood samples were collected before feeding (at 07:00 AM) by venipuncture from these animals 6 times, on days of gestation 105, 115, 125, 135 and 145, and on the 10th day postpartum. Serum samples were obtained by centrifugation of the blood samples at 5000 rpm for 10 min immediately after clotting. Sera were stored at –20 °C until further analysis. Tests were conducted spectrophotometrically with the aid of an automatic biochemistry analyzer (RX Monaco, Randox Laboratories Ltd., UK). Commercial kits were used for the analysis of glucose (GL8319, Randox Laboratories Ltd., UK), β-HBA (Ranbut RB1008, Randox Laboratories Ltd., UK), and NEFA (FA 115, Randox Laboratories Ltd., UK) levels in sera.

2.3. Statistical analysis

All the statistical procedures were performed in a computer based statistical software package version 17.0 (SPSS Inc., Chicago, IL, USA). Data were analyzed using one-way analysis of variance (ANOVA) to assess the changes in serum glucose, β-HBA, and NEFA levels of goats in response of dietary Nia and Met supplementation during late gestation and early postpartum period. Duncan's multiple range test was used as post hoc test to compare the means. Birth type and prolificacy rates were compared using chi-square analysis.

3. Results

The study revealed that total birth rate was 90% (27/30) whereas birth type and multiple birth rate were not different among the groups (Table 1).

Serum glucose levels remained unaffected irrespective of the diets on days 105, 115, 145 of gestation, and day 10 postpartum (Figure 1). However, dairy goats fed dietary Nia had greater serum glucose levels on day 125 of gestation in comparison with other dietary treatments ($P < 0.05$). On day 135 of pregnancy, serum glucose levels were higher in Met fed dairy goats compared to those fed diets without any supplementation ($P < 0.05$).

Table 1. Birth type and multiple birth rate in dairy goats fed dietary niacin and methionine during late gestation and early postpartum period.

Birth type	Control	Niacin	Methionine
Single (n)	1	1	1
Twin (n)	8	9	8
Triplet (n)	1	-	1
Multiple birth rate (%)	90	90	90

No difference was noted in serum β-HBA levels among the treatments on days 105, 115, and 125 of gestation that declined ($P < 0.05$) on days 135 and 145 in dairy goats fed Nia supplemented ration than those in control group (Figure 2). On day 10 postpartum, dairy goats receiving dietary Nia and Met had lower serum β-HBA levels than those fed ration without any supplementation ($P < 0.05$).

Serum NEFA levels were not different among the treatments on days 105, 115, and 125 of pregnancy. However, these levels decreased ($P < 0.05$) on days 135 and 145 in dairy goats fed Nia supplemented ration than those in control group (Figure 3). Serum NEFA levels day 10 postpartum were different among the groups ($P < 0.05$), lowest in dietary Met fed dairy goats followed by dietary Nia supplemented group and control group.

4. Discussion

In view of previous report by Bani Ismail et al. [9], the present study was carried out in animals with a high risk of developing pregnancy toxemia, which were 2 to 5-year-old Damascus goats with a multiple birth rate of 90% (27/30).

In late gestation, the increased volume of the uterus associated with reduced feed consumption, increased levels of progesterone and prolactin, and reduced levels of insulin elevate the predisposition to the development of hypoglycemia in ruminants [18,19]. For normal physiological functions, goats require a blood glucose concentration of 30–60 mg/dL. A blood glucose concentration below this level creates a risk for the development of pregnancy toxemia [12]. Throughout the study period, the blood glucose concentrations of Damascus goats did not fall below the level required for normal physiological processes (30–60 mg/dL) in any of the groups.

Blood β-HBA level is important in terms of diagnosis of pregnancy toxemia in pregnant goats. Serum β-HBA level lower than 0.8 mmol/L in pregnant goats is an indicator of pregnancy toxemia developed [9,20]. β-HBA is the final product of fat metabolism and constitutes almost 85% of the ketone bodies, the remaining 15% is composed of acetone and acetoacetate [5,21]. NEFA concentration is positively correlated with the negative energy balance, an indicator of lipolysis. NEFAs, generated as a result of the mobilization of body fat stores, accumulate in the liver. As the fetus cannot use NEFAs as an energy source, the increased energy requirement is accompanied by an increased NEFA level [6,9,10]. A free fatty acid level of ≥0.6 mmol/L is considered as an important sign of pregnancy toxemia [22]. In the present study, it was determined that while the β-HBA and NEFA levels increased with the

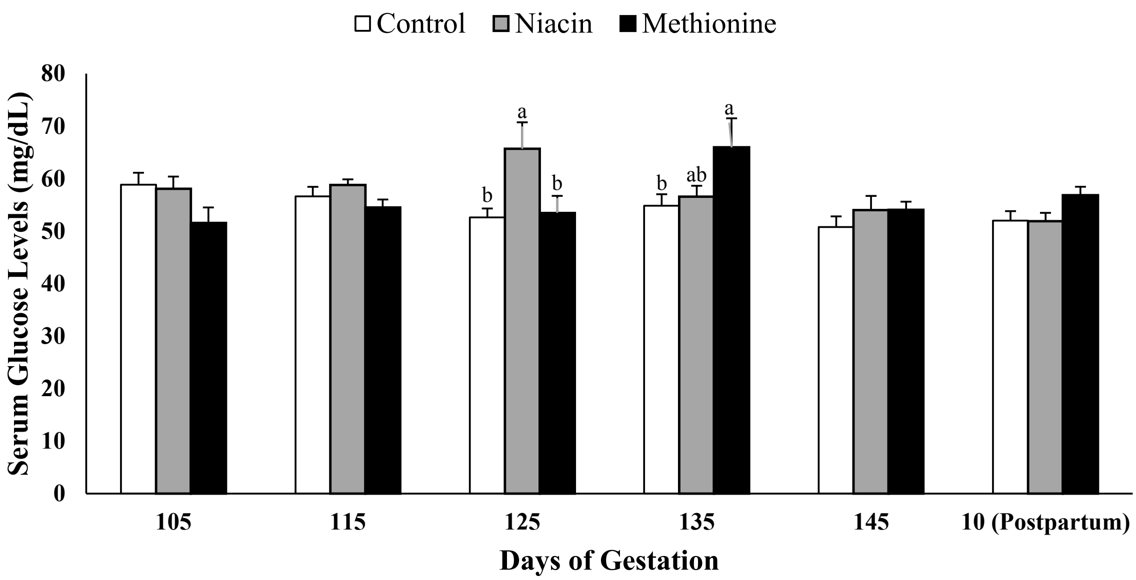

Figure 1. Changes in serum glucose levels in dairy goats fed niacin and methionine during late gestation and early postpartum period. Bars bearing different superscripts on the same day differ significantly ($P < 0.05$).

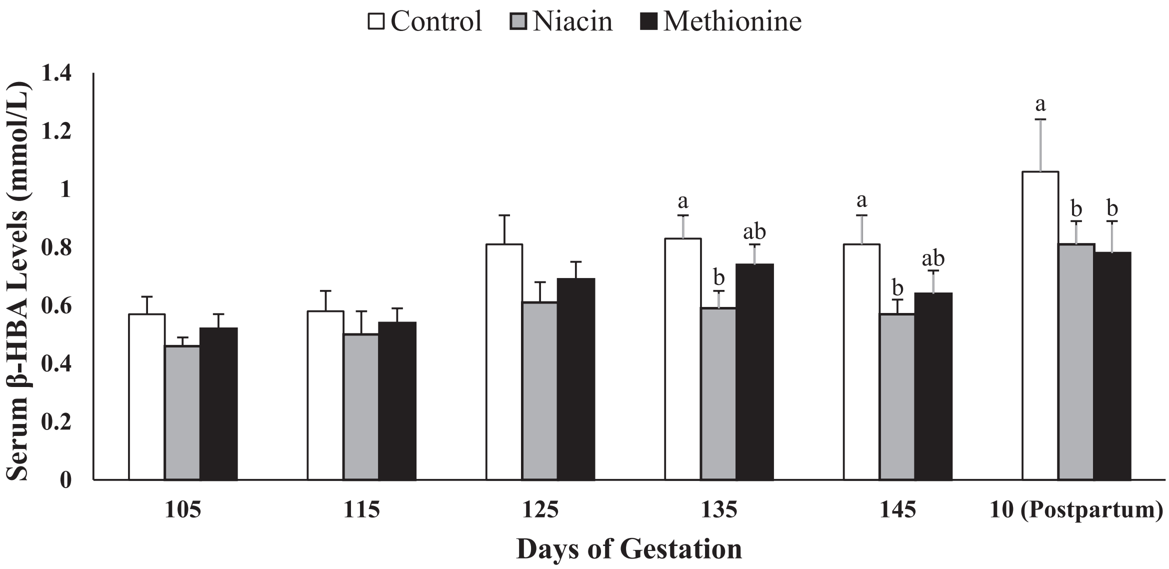

Figure 2. Changes in serum β-HBA levels in dairy goats fed niacin and methionine during late gestation and early postpartum period. Bars bearing different superscripts on the same day differ significantly ($P < 0.05$).

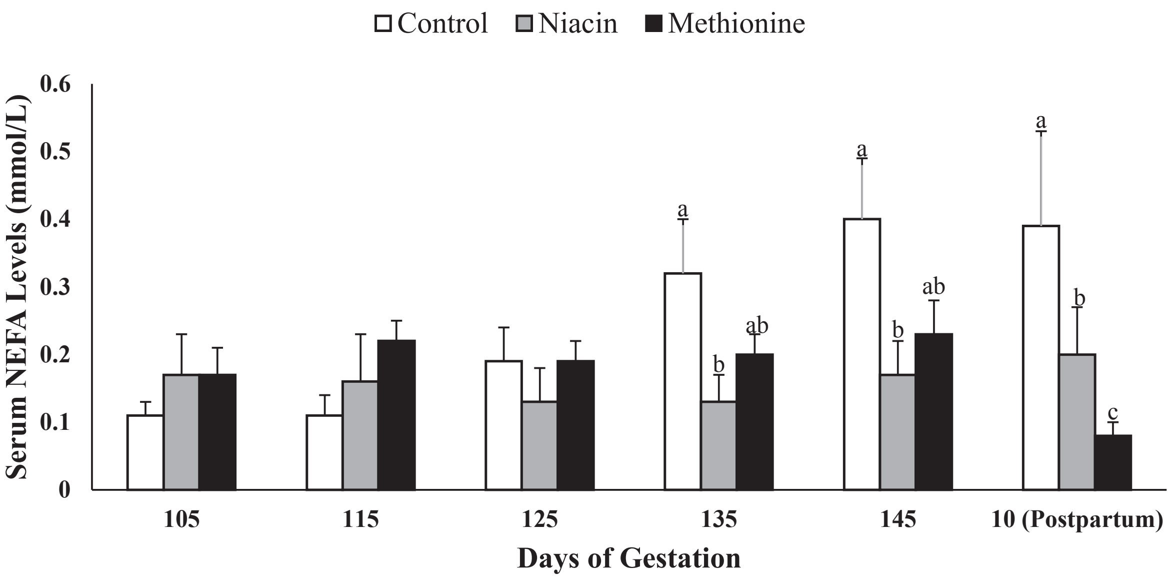

Figure 3. Changes in serum NEFA levels in dairy goats fed niacin and methionine during late gestation and early postpartum period. Bars bearing different superscripts on the same day differ significantly ($P < 0.05$).

advancing gestation in the control group, they showed a fluctuating course in the study groups and did not exceed the pregnancy toxemia threshold. It is considered that the concentrate having ME 2800 kcal/kg fed during this period to all the animals was effective from this perspective.

Nia, also known as vitamin B3, is a water-soluble vitamin that has an important role in the functioning of co-enzymes involved in energy metabolism [23,24]. In the rumen, Nia is converted into nicotinamide and nicotinic acid, and a large part of nicotinamide is converted into nicotinic acid in the acidic environment of the abomasum. Nicotinic acid, which is known to be involved in the carbohydrate metabolism, elevates blood glucose and insulin levels either by increasing gluconeogenesis or by preventing the mobilization of extrahepatic glucose. Nicotinic acid also depresses lipolysis in the fat tissue thereby reducing NEFA levels. Decreased NEFA concentrations, on the other hand, reduce triglyceride accumulation and ketone formation in the liver [15,23,25]. Keeping in view these effects, it is suggested that dietary supplementation of Nia might have decreased blood NEFA and β-HBA levels while increasing blood glucose levels [23,24]. In the present study, the pregnant goats fed Nia supplemented feed had glucose level increased to 65.68 mg/dL on day 125 of gestation, this level decreased on day 135, and continued to decrease on day 145, and in the postpartum period. Furthermore, the β-HBA level increased until day 125 of gestation, followed by a decrease until parturition ($P > 0.05$), and later increased once again in the postpartum period. On the other hand, the level of free fatty acids decreased until parturition and increased in the postpartum period. Based on these results, it was concluded that Nia supplementation did not increase glucose levels, but reduced β-HBA and NEFA levels until parturition.

Jaster and Ward [26] reported reduced blood NEFA and β-HBA levels and increased glucose levels in dairy cows given 6 g of Nia on daily basis during early lactation. In a similar study, Dufva et al. [27] reported reduced blood NEFA and β-HBA levels and increased glucose levels in transition dairy cows fed 12 g of Nia daily. In the present study, it was observed that the pregnant goats fed Nia supplemented feed had higher blood glucose levels than those of the C group on 125th and 135th days of gestation and did not differ from those of the C group on the other measurement days. The β-HBA levels of the C group were greater on 135th and 145th days of gestation and 10 days postpartum compared to Nia group.

Met, an essential amino acid for ruminants, is reported to serve for protection from fatty liver syndrome and ketosis [7,28]. As feed intake decreases in animals suffering from pregnancy toxemia, increased NEFAs that are not likely to be entirely consumed are eventually accumulated in the liver in the form of triglyceride resulting in the development of fatty liver syndrome. Triglycerides stored in the liver need to be converted into very low-density lipoproteins (VLDL). Met acts as a methyl donor in phospholipid synthesis and aids as a precursor in the synthesis of apolipoproteins, which are required for VLDL synthesis in the liver. In view of these facts, it is suggested that the supplementation of the feed ration with Met would be of benefit as it would accelerate the conversion of triglycerides into VLDL [29,30].

Waterman and Schultz [31] reported that oral administration of 40 g Met hydroxy analogue (MHA) gradually increased the glucose and reduced the NEFA and β-HBA levels in cows suffering from ketosis on days 0, 2, 7, 14, and 21 postpartum. In the present study, the pregnant goats fed Met supplemented feed had mean blood glucose level of 51.55 mg/dL on day 105 of gestation and increased to 65.95 mg/dL on day 135. It was ascertained that Met supplementation could increase glucose levels, while β-HBA and NEFA levels were also observed to have increased throughout the present study, no statistically significant alteration was determined to have occurred in these parameters.

In the present study, compared to C group, animals fed Met supplemented diets had higher glucose levels on days 135 and 145 of gestation and during the postpartum period whereas β-HBA levels were lower on days 135 and 145 of gestation and during the postpartum period. Furthermore, NEFA levels were lower in Met fed animals on days 135 and 145 of gestation and during the postpartum period.

Schlumbohm and Harmeyer [32] demonstrated that elevated β-HBA concentrations significantly suppressed endogenous glucose production, however, had no impact on the use of glucose. These researchers suggested that this particular effect of hyperketonemia could trigger the development of hypoglycemia. In cases of multiple pregnancy, the reduction observed in glucose levels could also be associated with other factors. For instance, rather than the suppression of glucose synthesis, the processes involved in such cases could be an increased fetal intake of glucose associated with the lack of the use of β-HBA as an energy resource by the fetus [33]. In their research on induced hyperketonemia in sheep, Harmeyer and Schlumbohm [17] and Heitmann and Fernandez [34] detected an increase in β-HBA concentrations, whereas NEFA concentrations decreased. On the other hand, Moallem et al. [33] ascertained that, plasma NEFA concentrations increased in sheep in parallel with an increase in β-HBA concentrations. Similar results were observed in C group in the present study. On the other hand, Nia and Met supplementation did not affect the glucose, β-HBA and NEFA levels measured at the beginning of the study.

In conclusion, Nia and Met supplementation to rations of pregnant Damascus goats during late pregnancy may play a protective role on pregnancy toxemia. Thus, it is considered that the impact of different doses of Met and Nia and their roles in pregnancy toxemia should be further elucidated in future studies.

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