

## Effect of L-Carnitine Administration on Energy Metabolism During Periparturient Period in Ewes

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Yayın Kodu: 2007/26-A

### Summary

Two experiments were conducted to investigate the effect of L-Carnitine administration on energy metabolism during periparturient period in fat tailed ewes. In Experiment I, L-Carnitine (1 g/50 kg, Treatment I, n=8) and physiologic saline (Treatment II; n=8) were administered subcutaneously weekly until lambing for seven or eight weeks. In Experiment II, L-Carnitine (0.5 g/50 kg, Treatment I, n=6) and physiologic saline (Treatment II; n=5) administered subcutaneously twice a week until lambing for at least three weeks. Blood samples were collected during treatments and one week after lambing to determine serum non esterified fatty acid (NEFA),  $\beta$ -hydroxybutyric acid (BHBA), total triglyceride and glucose concentrations. In experiment I, NEFA concentrations significantly ( $P<0.01$ ) increased until parturition followed by sudden decrease, and the concentrations were significantly lower ( $P<0.01$ ) in L-Carnitine group. In Experiment II, serum NEFA concentrations were significantly ( $P<0.01$ ) lower at and one week prior to lambing in ewes treated with more than four weeks compared to those treated with four or less than four weeks prepartum. Serum concentrations gradually ( $P<0.01$ ) increased until parturition followed by sudden decrease in all groups. However, serum NEFA concentrations did not differ in Experiment II. In conclusion, L-Carnitine administration during periparturient period decreased serum NEFA concentrations without any changes in serum BHBA, triglyceride and glucose concentrations.

**Keywords:** Ewes, L-Carnitine, NEFA, Pregnancy

### Koyunlarda Periparturient Dönemde L-Karnitin Uygulamasının Enerji Metabolizması Üzerine Etkisi

#### Özet

Peripartum dönemdeki yağlı kuyruklu koyunlarda L-Karnitin'in enerji metabolizması üzerine etkisi iki çalışmayla araştırıldı. Birinci çalışmadaki gebe koyunlara L-Karnitin (1 g/50 kg, uygulama I, n=8) veya fizyolojik tuzlu su (uygulama II; n=8) subkutan yolla haftada bir kez doğuma kadar toplam yedi-sekiz hafta süresince enjekte edildi. İkinci çalışmadaki gebe koyunlara L-Karnitin (0.5 g/50 kg, uygulama I, n=6) veya fizyolojik tuzlu su (uygulama II; n=5) subkutan yolla haftada iki kez doğuma kadar en az üç hafta süreyle enjekte edildi. Uygulamalar süresince ve doğumdan sonraki hafta serumdaki serbest yağ asit (NEFA),  $\beta$ -hidroksibutyrik asit (BHBA), toplam trigliserit ve glikoz konsantrasyonlarını belirlemek amacıyla kan örnekleri alındı. Birinci çalışmada, serum NEFA konsantrasyonu doğuma kadar önemli derecede ( $P<0.01$ ) artarken, bunu ani bir düşüş izledi ve NEFA konsantrasyonu L-Karnitin grubunda önemli derecede ( $P<0.01$ ) düşük bulundu. İkinci çalışmada, kuzulamadan bir hafta önceki ve doğumdaki serum NEFA konsantrasyonları prepartum dönemde dört haftadan fazla süreyle L-Karnitin uygulananlarda, daha kısa süreyle L-Karnitin uygulananlara göre önemli derecede ( $P<0.01$ ) düşük bulundu. İkinci çalışmanın tüm gruplarında NEFA konsantrasyonlarındaki doğuma kadar kademeli olarak artışı ( $P<0.01$ ) ani düşüş izledi. Fakat, uygulama ve kontrol grupları arasında NEFA konsantrasyonları bakımından bir farklılık bulunamadı. Sonuç olarak, periparturient dönemde L-Karnitin uygulamasının serum BHBA, trigliserit ve glikoz konsantrasyonları üzerine etkisi olmaksızın, serum NEFA konsantrasyonunda azalmaya neden oldu.

**Anahtar sözcükler:** Koyun, L-Karnitin, NEFA, Gebelik

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## INTRODUCTION

L-Carnitine is a vitally important, vitamin-like quaternary ammonium compound and it is endogenously synthesized from lysine and methionine in liver and kidneys<sup>1</sup>. It plays an important role in the production of energy through mitochondrial  $\beta$ -oxidation in cells<sup>2,3</sup>. It has been shown that Carnitine has important functions in some metabolic processes including oxidation of long-chain fatty acids<sup>4</sup>, regulation of ketosis<sup>5</sup> and stimulation of immune system<sup>6</sup>.

It has been reported that L-Carnitine serves as acetyl buffer in muscle cells during anaerobic motor activations, and it regulates metabolic processes in high yielding lactating cows and ewes with advanced stage of pregnancy<sup>7</sup>. Recent studies<sup>4,8-10</sup> indicate that supplemental L-Carnitine in diet is not required. However, its use in pigs<sup>4</sup>, dogs<sup>8</sup> and cattle<sup>9,10</sup> is recommended to increase performance and support medical treatment. It has also been demonstrated that the addition of L-Carnitine to the ruminant feed increased its amount in plasma, liver and milk<sup>9,10</sup>.

Majority of studies on the effect of supplemental Carnitine focused on ketosis in ruminants<sup>10-14</sup>. However, a limited number of studies dealing with the effect of supplemental Carnitine on metabolism and performance parameters were published in healthy ruminants<sup>9,15-20</sup> and other domestic species such as pigs<sup>4</sup> and dogs<sup>8</sup>. Therefore, the objective of this study was to investigate the effect of L-Carnitine administration on energy metabolism during periparturient period in pregnant ewes as a treatment of choice for pregnancy toxemia in ewes.

## MATERIALS and METHODS

Experiment I was conducted at the Kafkas University, Faculty of Veterinary Medicine, Research and Training Farm, Kars-TURKEY during winter 2004-2005. The Animal Handling and Ethical Board approved all the experimental procedures. Following pregnancy diagnosis with transabdominal ultrasonography (Pie Medical, 100 Falko Vet Model equipped with 6 MHz probe) at 90-100 days after breeding, pregnant fat tailed (2 to 5 years old) ewes were randomly assigned

into one of the treatment groups. Ewes were housed in flock barn with access to a feeding lot and were fed with grass hay *ad libitum*. Grass hay (%93.3 dry matter, %8.8 crude protein, total fiber %31.1, 2000 kcal/kg ME as fed basis) was given twice a day. L-Carnitine (Sigma Tau, Industrie Farmaceutiche Riunite S.p.A., Pomezia-Italy, 1 g/50 kg; Treatment I, n=8) and physiologic saline (Treatment II; n=8) injections were administered subcutaneously weekly prior to feeding until lambing. In treatment and control groups, 7/8 and 5/8 received injections for eight weeks before lambing; respectively. Rest of the ewes received seven injections in each group.

Experiment II was conducted at the same institution during winter 2005-2006. Following pregnancy diagnosis as in Experiment I, ewes (2 to 5 years old) were randomly assigned into one of the treatment groups. Ewes were housed and fed with grass hay as in Experiment I. Grass hay (%92.4 dry matter, %8.4 crude protein, total fiber %31.1, 2000 kcal/kg ME as fed basis) was given twice a day. However, eleven ewes were completed all the experimental protocols. Total amount of L-Carnitine (1 g/50 kg; Treatment I, n=6) and physiologic saline (Treatment II; n=5) were split into two doses and administered subcutaneously 3-4 days apart prior to feeding until lambing. In Treatment I, 3-7 injections were administered to each of 6 ewes while in the treatment II, 3-8 injections were administered to each of 5 ewes.

Blood samples were collected weekly during treatments and one week after lambing prior to feeding via jugular vein. Once transferring to laboratory, blood samples were kept at room temperature for 20 min. They were then centrifuged at 3000 rpm for 10 min and serum samples were stored at  $-25^{\circ}\text{C}$  until being assayed.

Commercial kits (Randox Laboratories Ltd. U.K., for serum NEFA and BHBA, and bioMerieux, Marcy l'Étoile, France for serum total triglyceride and glucose) were used. All these parameters were measured with a spectrophotometer (UV-1201, Shimadzu, Japan).

Data were analyzed according to repeated

measures using mixed procedures of SAS <sup>21</sup>. In Experiment I, the statistical model included treatment, week, and interaction of treatment by week. In Experiment II, the model included treatment, week, total week of drug administration, interactions of treatment by week, treatment by total week of drug administration, and treatment by week by total week of drug administration.

## RESULTS

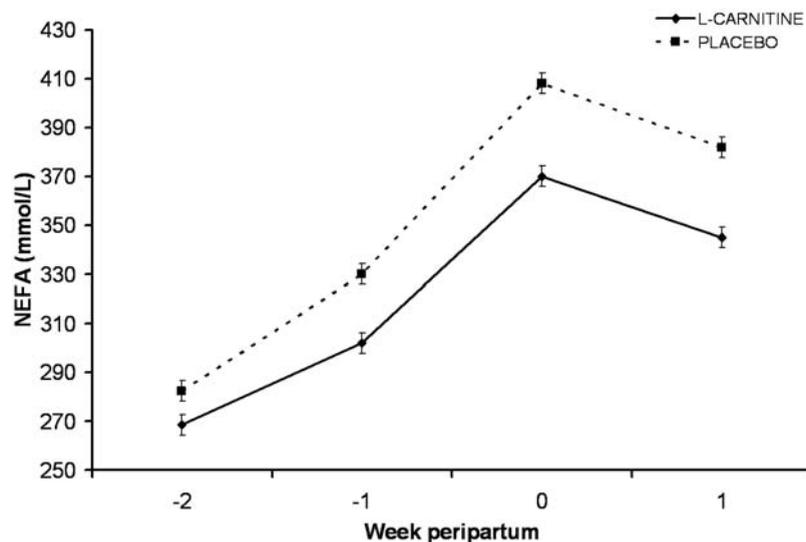
### Experiment I

Frequency of twinning was 1/8 and 2/8 in treatment and control groups, respectively. No clinical signs of ketosis were observed during the experiment. There were significant effects of treatment ( $P<0.01$ ), experimental week ( $P<0.01$ ) and treatment by experimental week interaction ( $P<0.05$ ) on serum NEFA concentrations. NEFA concentrations gradually increased until parturition followed by sudden decreases one week after lambing in both groups (*Figure 1, Table 1*). However, the concentrations were significantly lower ( $P<0.01$ ) in treatment group during periparturient period (*Figure 1*).

lambing to one week postpartum ( $P<0.01$ ). Triglyceride concentrations at lambing and postpartum were significantly ( $P<0.01$ ) lower than those at prepartum. Similarly, glucose concentrations gradually decreased towards lambing, and the concentrations at lambing and postpartum were significantly ( $P<0.01$ ) lower than those at prepartum.

### Experiment II

Neither twinning nor clinical signs of ketosis were observed. There were effects of total week of drug administration ( $P<0.01$ ) and week ( $P<0.01$ ) on serum NEFA concentrations. NEFA concentrations were significantly ( $P<0.01$ ) lower at and one week prior to lambing in ewes treated with more than four weeks compared to those treated with less than or equal to four weeks. It gradually increased until parturition followed by sudden decrease one week after lambing in all groups (*Figure 2, Table 2*). Moreover, the interaction of treatment by total week of drug administration by week was tended to be significant ( $P<0.08$ ). However, the concentrations did not differ between treatment and control groups during periparturient period (*Figure 2*).



**Fig 1.** Effects of treatment ( $P<0.01$ ), week ( $P<0.01$ ) and treatment by week ( $P<0.05$ ) interaction on serum NEFA concentrations were significant

**Şekil 1.** L-Karnitin uygulamasının ( $P<0.01$ ), haftanın ( $P<0.01$ ) ve uygulama ile hafta arasındaki etkileşimin ( $P<0.05$ ) serum NEFA konsantrasyonu üzerine etkisi

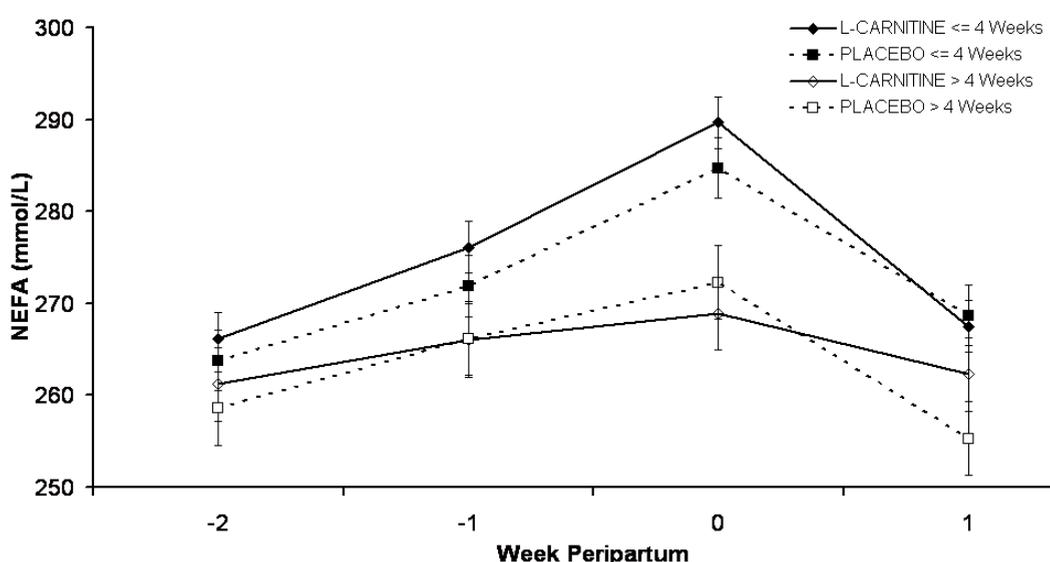
There was a week effect on serum NEFA, BHBA, triglyceride and glucose concentrations ( $P<0.01$ ; *Table 1*). BHBA concentrations gradually increased from two weeks before

There was a week effect on serum NEFA, BHBA, triglyceride and glucose concentrations ( $P<0.01$ ) (*Table 2*). BHBA concentrations gradually increased from two weeks before

**Table 1.** Serum NEFA, BHBA, triglyceride and glucose concentrations in Experiment I  
**Tablo 1.** Deney I'deki serum NEFA, BHBA, trigliserit ve glikoz konsantrasyonları

Week Peripartum	NEFA (mmol/L)	BHBA (mmol/L)	Triglyceride (mg/dl)	Glucose (mg/dl)
Two weeks before lambing	275.5 ± 3.0 <sup>a</sup>	0.59 ± 0.02 <sup>a</sup>	20.8 ± 0.42 <sup>b</sup>	65.0 ± 0.8 <sup>c</sup>
One week before lambing	316.1 ± 3.0 <sup>b</sup>	0.61 ± 0.02 <sup>ab</sup>	21.3 ± 0.42 <sup>b</sup>	62.0 ± 0.8 <sup>b</sup>
Week of lambing	389.1 ± 3.0 <sup>d</sup>	0.65 ± 0.02 <sup>b</sup>	16.1 ± 0.42 <sup>a</sup>	55.9 ± 0.8 <sup>a</sup>
One week after lambing	363.5 ± 3.0 <sup>c</sup>	0.78 ± 0.02 <sup>c</sup>	16.4 ± 0.42 <sup>a</sup>	56.7 ± 0.8 <sup>a</sup>

<sup>a-d</sup> Different superscripts differ in the same column. There was a week effect on NEFA, BHBA, triglyceride and glucose concentrations ( $P < 0.01$ ). \*Values were referred as the least square means with standard error of means (SEM).



**Fig 2.** Effects of total week of drug administration ( $P < 0.01$ ) and experimental week ( $P < 0.01$ ) on serum NEFA concentrations

**Şekil 2.** Toplam L-Karnitin uygulanan hafta ( $P < 0.01$ ) ve haftanın ( $P < 0.01$ ) serum NEFA konsantrasyonu üzerine etkisi

**Table 2.** Serum NEFA, BHBA, triglyceride and glucose concentrations in Experiment II  
**Tablo 2.** Deney II'deki serum NEFA, BHBA, trigliserit ve glikoz konsantrasyonları

Week Peripartum	NEFA (mmol/L)	BHBA (mmol/L)	Triglyceride (mg/dl)	Glucose (mg/dl)
Two weeks before lambing	262.5 ± 1.8 <sup>a</sup>	0.61 ± 0.02 <sup>a</sup>	21.1 ± 0.5 <sup>b</sup>	62.0 ± 0.9 <sup>c</sup>
One week before lambing	270.0 ± 1.8 <sup>b</sup>	0.68 ± 0.02 <sup>b</sup>	21.8 ± 0.5 <sup>b</sup>	57.7 ± 0.9 <sup>b</sup>
Week of lambing	278.9 ± 1.8 <sup>c</sup>	0.75 ± 0.02 <sup>c</sup>	14.9 ± 0.5 <sup>a</sup>	53.4 ± 0.9 <sup>a</sup>
One week after lambing	263.4 ± 1.8 <sup>a</sup>	0.85 ± 0.02 <sup>d</sup>	16.0 ± 0.5 <sup>a</sup>	54.8 ± 0.9 <sup>a</sup>

<sup>a-d</sup> Different superscripts differ in the same column. There was a week effect on NEFA, BHBA, triglyceride and glucose concentrations ( $P < 0.01$ ). \*Values were referred as the least square means with standard error of means (SEM).

lambing to postpartum week ( $P < 0.01$ ). Triglyceride concentrations at lambing and postpartum were significantly ( $P < 0.01$ ) lower than those at prepartum. Glucose concentrations gradually decreased towards lambing, and serum glucose concentrations were significantly ( $P < 0.01$ ) lower at lambing and postpartum.

## DISCUSSION

The most popular area of carnitine research regarding pregnancy toxemia in sheep and ketosis in cattle has been reported<sup>11,12,14,20,21</sup>. Pregnancy toxemia, the most important metabolic disease that causes important economical losses, can be arisen from disorders of fat metabolism at the last month of the pregnancy, and it can be dangerous for life of ewes and lambs<sup>22</sup>. It has been reported that L-Carnitine supplementation plays a critical role in transforming the fatty acids to the mitochondria for beta oxidation<sup>1,7,14</sup>; therefore, in this study, it was aimed to prevent negative energy balance during early lactation period, to support glukoneogenesis, to prevent existing ketone bodies leading to prevention of ketosis by supplementing L-Carnitine during last trimester of pregnancy.

In both experiments, serum BHBA concentrations gradually increased until parturition and reached the highest level at one week after parturition. In contrast, postpartum triglyceride and glucose concentrations lower than prepartum values in all the ewes used in both experiments. These results reveal the 'glukoneogenesis', and ewes were getting ketotic as pregnancy advances and lactation starts.

L-Carnitine treatment did not have any effect on serum BHBA, triglyceride and glucose concentrations during periparturient period; however, L-Carnitine administration significantly decreased serum NEFA concentrations in this study. These results indicate that subcutaneous administration of L-Carnitine facilitates the energy production by increasing NEFA utilization without any changes in serum BHBA, triglyceride and glucose concentrations. This effect of L-Carnitine could be associated with stimulation of

lipid metabolism through transfer of acyl groups across the mitochondrial membranes<sup>4</sup>. It has been demonstrated that Carnitine supplementation was able to lower free fatty acids and keton bodies in ketotic cows<sup>13</sup>. Furthermore, it has been reported that the administration of 6 g L-Carnitine into the rumen resulted in decreased NEFA levels in healthy cows<sup>9</sup>. Since subcutaneous administration of L-Carnitine did not have any effect on serum BHBA concentrations, it could not be beneficial in the treatment of ovine ketosis. However, it could be used for the prevention of ketosis due to its beneficial effect on NEFA utilization. As no clinical signs of ketosis were observed in this study, the use of L-Carnitine for the treatment of ovine ketosis should be investigated.

No change in serum glucose concentrations was obtained in the present study, as in agreement with previous reports in which supplemental L-Carnitine did not affect blood glucose levels in cows<sup>24-26</sup>. In contrast, it has been reported that the administration of oral Carnitine in lambs resulted in an increased serum glucose concentration<sup>20</sup>. According to previous reports, the supplementation of L-Carnitine resulted in different effects on the metabolism and performance parameters in different animal species such as pigs, dogs, cows and sheep<sup>4,8,9,11-20</sup>. Thus, fat tailed ewes could compensate energy demand via fat mobilization leading to no obvious effect of L-Carnitine supplementation other than decreased NEFA concentrations observed in this study.

In conclusion, L-Carnitine administration during periparturient period studied in sheep decreased serum NEFA concentrations without any changes in other energy metabolites herein. L-Carnitine administration could be an alternative treatment of choice for metabolic disorders during periparturient period, and further research is warranted to investigate its prophylactic and therapeutic use for pregnancy toxemia in sheep. Moreover, practical and economical applicability of L-Carnitine administration should also be clarified in sheep production.

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