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RESEARCH ARTICLE

Effects of Methionine and Lysine on Metabolic Profile in Dairy Cattle During Periparturient Period [1]

Turan CİVELEK * Fatih BİRDANE * Mustafa KABU * C. Çağrı CINGI * Abuzer ACAR *

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* University of Afyon Kocatepe, Faculty of Veterinary Medicine, Department of Internal Medicine, TR-03200 ANS Campus, Afyonkarahisar - TÜRKİYE

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Summary

Periparturient period is highly important in terms of health. For feeding in this stage substances providing high energy should be selected and aimed at reducing the mobilization of reserve fats. For this purpose; various feed additives such as glycogene, liquid fats, glycerol, propilene glycole, propionats, monensin, *methionine, lysine,* colin, niasin, biotin, sodium borate, conjugated linoleic acid ve xylitol can be used. Several studies showed that methionine and lysine are the two most important amino acids. In this study, effects of methionine and lysine on metabolic profile was comparatively investigated in similar age, feeding and productive traits of dairy cattle. The applications of methionine caused significant changes at the concentrations of cholesterol, triglyceride, blood urea nitrogen, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein, glucose, beta-hydroxybutyric acid and non-esterified fatty acid; applications of lysine at serum levels of total bilirubin, direct bilirubin, total protein, albumin, glucose, triglyceride, blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, low-density lipoprotein, very low-density lipoprotein and non-esterified fatty acid; and applications of methionine and lysine together at the levels of blood urea nitrogen, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, glucose, total bilirubin, direct bilirubin, cholesterol, triglyceride and non-esterified fatty acid. Addition of methionine and lysine to the ration in this study had partial effects on serum values of animals however findings showed that the additive materials are not necessary for dairy cattle fed by balanced and adequate rations.

Keywords: *Negative energy balance, Transition period, Milk yield, Fatty liver, Cow*

Periparturient Dönem Sütçü Sığırlarda Metionin ve Lizinin Metabolik Profil Üzerine Etkileri

Özet

Periparturient dönem sağlık açısından son derece önemlidir. Bu dönem beslemede yüksek enerji sağlayan besin maddeleri tercih edilmeli ve rezerv yağların mobilizasyonunun azaltılması amaçlanmalıdır. Bu amaçla; glikojen, likid yağlar, gliserol, propilen glikol, propionatlar, monensin, *metionin, lizin,* kolin, niasin, biotin, sodyum borat, konjuke linoleik asit ve ksilitol gibi çeşitli yem katkı maddeleri kullanılabilir. Birçok araştırma sonucu metionin ve lizinin bu amaçla kullanılabilecek en önemli iki aminoasit olduğunu göstermiştir. Sunulan çalışmada, metionin ve lizinin metabolik profil üzerine etkileri benzer yaş, besleme ve verim özelliklerine sahip süt sığırları üzerinde karşılaştırmalı olarak araştırıldı. Metionin uygulamaların, kolesterol, trigliserit, kan üre nitrojen, yüksek dansiteli lipoprotein, düşük dansiteli lipoprotein, çok düşük dansiteli lipoprotein, glukoz, beta hidroksi bütirik asit ve esterleşmemiş yağ asidi serum konsantrasyonlarında, lizin uygulamalarının total bilirubin, direkt bilirubin, total protein, albümin, glukoz, trigliserit, kan üre nitrojen, aspartat aminotrasferaz, alanin aminotransferaz, düşük dansiteli lipoprotein, çok düşük dansiteli lipoprotein ve esterleşmemiş yağ asiti serum düzeylerinde ve metionin ve lizin birlikte uygulamasının ise kan üre nitrojen, yüksek dansiteli lipoprotein, düşük dansiteli lipoprotein, çok düşük dansiteli lipoprotein, gamma glutamil tranferaz, aspartat aminotrasferaz, alanin aminotransferaz, glukoz, total bilirubin, direkt bilirubin, kolesterol, trigliserit ve esterleşmemiş yağ asidi serum seviyelerinde önemli değişikliklere neden olduğu tespit edildi. Sunulan araştırmada, rasyona metionin ve lizin ilavesinin hayvanların serum değerleri üzerine kısmi etkisinin olduğu belirlendi. Bununla birlikte, elde edilen çalışma bulguları, dengeli ve yeterli rasyonla beslenen süt sığırlarında katkı maddelerinin gerekli olmadığını gösterdi.

Anahtar sözcükler: *Negatif enerji balansı, Geçiş periyodu, Süt verimi, Karaciğer yağlanması, Sığır*

İletişim (Correspondence)
+90 272 2281312/16202
tcivelek@aku.edu.tr

INTRODUCTION

Dairy cattle should be provided with main dietary needs to help them endure the periparturient period without complications [1]. For this purpose, different nutritional additives such as glycogen, liquid fats, glycerol, propylene glycol, propionates, monencine, *methionine, lysine*, choline, niacin, biotin, sodium borate, conjugated linoleic acid, or xylitol can be added to rations of the animals [2].

Among the nutritional additives, methionine is the one of the most essential amino acids of dairy cow's metabolism. Methionine is a precursor substance and required for the hepatic synthesis of apolipoproteins needed for production of very low-density lipoprotein (VLDL) [1].

Furthermore, lysine is required in all species for protein synthesis and forms L-carnitine by combining with methionine; therefore, its deficit impairs protein biosynthesis [3]. Addition of just methionine or methionine plus lysine at higher amounts to rations of the cows at transition periods is shown to protect the animals against liver lipid accumulation [4], increase milk production and milk protein rates [5]. Numerous studies point out that methionine and lysine are main confining amino acids [6].

In the present study, we aimed to comparatively study the effect of methionine and lysine on the metabolic features of dairy cattle with similar age, feedings, and production traits during periparturient period.

MATERIAL and METHODS

Animals and Treatments

In the current study, 3-5 years old, healthy, pregnant, and multiparous 24 Holstein dairy cows were used. The dairy cattle in the study groups were selected among the healthy animals with no history of enduring diseases of the periparturient period. All of the cows were clinically and systemically examined prior to their inclusion to the current study. Study groups were formed among the similar cows according to their milk production traits (liter/day), live body weight, and body condition score (3.250±0.112) [7] *(Table 1)*. The animals were obtained from a private enterprise operating in Bolvadin, Afyonkarahisar, Turkey (Korel Agriculture and Animal Husbandry Enterprises). The dairy cattle were divided into four groups of six. The groups were designated as follows: Control group (Group C), methionine group (Group M), lysine group (Group L), and lysine plus methionine group (Group LM). The groups were followed on a daily basis during the periparturient period for 30 days (15 prenatal and postnatal 15 days). The animals in Group M were orally provided with 15 g/day Mepron M85- equivalent of 12.75 g rumen-protected methionine (Degussa AG, Hanau, Germany), 50 g/day AminoShure-L-equivalent of 18 g rumen-protected lysine (Balchem, Animal Nutrition & Health Co.) was given orally to the cows in Group L. Finally, the animals in Group LM were orally provided with 15 g/day Mepron M85 and 50 g/day AminoShure-L. All of the cows included in the present study were fed with the same type of ration throughout the study. The contents of the ration are illustrated in *Table 2*.

Table 1. *Initial values of MY and BW in terms of comparison groups (mean ±SE)*

Tablo 1. *Süt verimi ve canlı ağırlık başlangıç verilerinin gruplarası karşılaştırılması*

Parameter	Grup M	Grup L	Grup LM	p
BW (kg)	697.500±17.953	697.667±17.383	697.00±9.145	NS
MY (L)	31.167±1.222	30.667±0.882	30.500±0.579	NS

BW: *live weight,* ***MY:*** *milk yield,* ***NS:*** *Not significant*

Table 2. *Ingredient and nutrient composition of prepartum and postpartum diets*

Tablo 2. *Prepartum ve postpartum rasyonun içerik ve besin kompozisyonu*

Ingredient	Prepartum	Postpartum
% DM		
Corn silage	30.1	26.0
Wet brewers grains	4.9	5.2
Alfalfa hay	14.0	18.2
Barley hay	18.5	10.3
Wheat Bran	2.9	9.2
Barley	13.4	6.8
Corn	8.4	11.0
Cottonseed meal (32%)	2.2	7.9
Cottonseed meal (48%)	4.3	2.6
Bypass fat [1]	1.0	0.6
Bypass protein [2]	0.0	0.6
Salt	0.14	0.30
Minerals and vitamins [3]	0.16	0.04
Sodium bicarbonate [4]	0.0	0.5
Yeast [5]	0.000	0.004
Marble dust ($CaCO_3$ source)	0.0	0.7
Chemical composition		
DM	60	60
CP (% DM)	12.7	17.0
Rumen Degradable Protein (% DM)	7.7	11.8
Bypass protein (% DM)	5.0	5.2
NEL (cal/g)	1.47	1.58
NDF (% DM)	45.94	39.89
ADF (% DM)	27.28	23.42
Ca (% DM)	0.46	0.72
P (% DM)	0.27	0.42

[1] Megalac (Church & Dwight Co., Inc., Rinceton. NJ); [2] Soy Pass (Borregaard LignoTech); [3] Rovimix 302-FM/20 providing by kg 15.000.000 IU vitamin A, 3.000.000 IU vitamin D_3, 20.000 mg vitamin E, 10.000 mg manganese, 10.000 mg iron, 10.000 mg zinc, 5.000 mg copper, 100 mg cobalt, 100 mg iodine; [4] $NaHCO_3$ (99%, Şişecam Chemicals Group); [5] Yeast (Beta Agriculture, Yüreğir/Adana); ***DM:*** *Dry matter;* ***CP:*** *Crude protein;* ***NEL:*** *Net energy lactation;* ***NDF:*** *Neutral detergent fiber;* ***ADF:*** *Acid detergen fiber*

Blood Collection and Analysis

Biochemical analyses were performed weekly before and after the diet application. The analyses were carried out at five separate times as described: 1st measurement was done at prepartum 2nd week prior to the diet application (0 time), 2nd measurement was obtained at prepartum 1st week prior to the diet application (1 week), 3rd measurement was taken at calving day 4th measurement was acquired at postpartum 1st week, and 5th measurement was done at postpartum 2nd week. The blood samples were collected from the jugular vein, put into dry tubes, centrifuged, and obtained serums were removed and kept at -20°C until use.

Commercially available test kits were used to measure levels of biochemical parameters (Roche Diagnostics, Germany). The kits were used according to the instructions of manufacturers using an autoanalyzer (Roche Cobas C111). The value of VLDL was calculated using the formula triglyceride/5 [8]. Moreover, beta-hydroxybutyric acid (BHBA) (Randox Laboratories Ltd. United Kingdom, Kat. No: RB 1008) and non-esterified fatty acid (NEFA) (Randox Laboratories Ltd. United Kingdom, Kat.No: FA 115) measurements were done spectrophotometrically (Shimadzu UV-1601).

Statistical Method

Statistical analyses of the data were performed using the program SPSS 10.0 (SPSS Inc, for Windows). One way ANOVA test was used to compare the groups. The control of significance within and between the groups was checked with Tukey test.

RESULTS

Methionine caused significant changes (P=0.000) at the concentrations of cholesterol, triglyceride, blood urea nitrogen, low-density lipoprotein, very low-density lipoprotein and glucose. Furthermore, serum beta-hydroxybutyric acid (P=0.004) and non-esterified fatty acid (P=0.005) levels were considerably different in Group M when compared within the groups.

We measured statistically significant at serum levels of total bilirubin (P=0.014), direct bilirubin (P=0.011), total protein (P=0.002), albümin (P=0.000), glucose (P=0.001), triglyceride (P=0.000), blood urea nitrogen (P=0.025), aspartate aminotransferase (p=0.001), alanine aminotransferase (P=0.024), low-density lipoprotein (P=0.011), very low-density lipoprotein (P=0.000) and non-esterified fatty acid (P=0.000) for applications of lysine.

Also, we measured considerable changes at the levels of blood urea nitrogen (P=0.023), high-density lipoprotein (P=0.015), low-density lipoprotein (P=0.001), very low-density lipoprotein (P=0.000), gamma-glutamyl transferase (P=0.038), alanine aminotransferase (P=0.024), aspartate aminotransferase (P=0.020), glucose (P=0.000), total bilirubin (P=0.003), direct bilirubin (P=0.005), cholesterol (P=0.012), triglyceride (P=0.000) and non-esterified fatty acid (P=0.004) in group LM.

The study results as a summary is given in the *Tables 3, 4, 5, 6,* and *7*.

Table 3. *Serum biochemical parameters (TP, ALB and BUN) in dairy cattle during periparturient period (2 weeks in prepartum and 2 weeks in postpartum) according to oral treatments (Group M: methionine (12.75 g/day); group L: lysine (18 g/day); group ML: methionine (12.75 g/day) + lysine (18 g/day); group C: negative controls) (n = 6 in each group). Results are expressed as means ± standard deviations*

Tablo 3. *Serum TP, ALB ve BUN konsantrasyonlarının gruplararası ve grupiçi değişimi*

Parameter	Group	-2 weeks	-1 week	Calving	+1 week	+2 weeks	P
TP (g/L)	C	7.883±0.101	6.917±0.150	7.467±0.199	7.917±0.305	8.102±0.551	0.096
	M	7.917±0.408	7.250±0.589	6.783±0.380	7.617±0.430	7.383±0.355	0.466
	L	6.783±0.119^{b}	7.200±0.121^{ab}	7.133±0.136^{ab}	7.567±0.279^{a}	7.800±0.089^{a}	**0.002**
	LM	6.867±0.201	7.500±0.236	7.133±0.297	7.217±0.368	7.950±0.319	0.124
	P	**0.003**	0.673	0.385	0.580	0.623	
ALB (g/dL)	C	3.450±0.763	3.550±0.342	3.617±0.703	3.400±0.816	3.317±0.167	0.223
	M	3.350±0.106	3.233±0.095	3.117±0.107	3.267±0.128	3.217±0.046	0.469
	L	3.567±0.033^{a}	3.666±0.033^{a}	3.667±0.076^{a}	3.283±0.083^{b}	3.217±0.087^{b}	**0.000**
	LM	3.350±0.067	3.483±0.031	3.550±0.095	3.133±0.108	3.366±0.051	0.073
	P	0.166	0.000	0.001	0.357	0.666	
BUN (mg/dL)	C	10.208±0.633^{b}	11.667±0.791^{ab}	14.700±1.504^{a}	13.633±0.843^{ab}	13.367±0.747^{ab}	**0.022**
	M	7.417±0.853^{c}	8.967±1.060^{bc}	13.517±2.134^{ab}	15.083±1.190^{a}	15.383±0.527^{a}	**0.000**
	L	12.600±0.865^{b}	15.100±1.114^{ab}	17.683±1.482^{a}	15.450±0.471^{ab}	13.767±1.029^{ab}	**0.025**
	LM	11.533±0.853^{a}	12.133±0.592^{ab}	10.633±1.047^{ab}	14.133±1.545^{ab}	15.783±1.347^{a}	**0.023**
	P	**0.001**	**0.001**	**0.039**	0.627	0.240	

BUN: *Blood urea nitrogen,* ***ALB:*** *Albumin,* ***TP:*** *Total protein, Different superscripts a,b in the same row indicate significant differences (P<0.05 or more) according to time during the periparturient period for a given group*

Table 4. *Serum biochemical parameters (TG, CHOL, NEFA, BHBA and GLU) in dairy cattle during periparturient period (2 weeks in prepartum and 2 weeks in postpartum) according to oral treatments (Group M: methionine (12.75 g/day); group L: lysine (18 g/day); group ML: methionine (12.75 g/day) + lysine (18 g/day); group C: negative controls) (n = 6 in each group). Results are expressed as means ± standard deviations*

Tablo 4. *Serum TG, CHOL, NEFA, BHBA ve GLU konsantrasyonlarının gruplararası ve grupiçi değişimi*

Parameter	Group	-2 weeks	-1 week	Calving	+1 week	+2 weeks	p
TG (mg/dL)	C	25.833±2.197^{a}	21.833±4.222^{a}	7.333±1.085^{b}	6.500±0.885^{b}	6.167±1.222^{b}	**0.000**
	M	23.000±3.022^{a}	24.500±0.957^{a}	10.500±2.376^{b}	10.333±1.647^{b}	10.167±1.667^{b}	**0.000**
	L	22.167±2.495^{a}	20.833±1.579^{a}	9.167±1.302^{b}	8.500±1.204^{b}	7.000±0.577^{b}	**0.000**
	LM	22.167±1.600^{a}	20.000±1.732^{a}	12.667±2.641^{b}	7.667±0.803^{b}	7.833±0.792^{b}	**0.000**
	P	0.666	0.604	0.301	0.169	**0.049**	
CHOL (mg/dL)	C	115.500±5.264^{b}	113.500±3.566^{b}	100.000±3.670^{b}	113.500±5.383^{b}	144.500±7.379^{a}	**0.000**
	M	124.667±7.154^{ab}	107.833±4.643^{bc}	85.833±3.953^{c}	114.000±9.402^{abc}	139.333±8.204^{a}	**0.000**
	L	72.333±7.112	79.333±7.356	63.000±6.061	65.833±8.268	77.167±6.710	0.439
	LM	77.500±4.072^{b}	85.000±4.823^{ab}	72.833±5.192^{b}	78.667±7.237^{b}	105.833±9.488^{a}	**0.012**
	P	0.000	0.000	0.000	0.000	0.000	
NEFA (mmol/L)	C	0.214±0.036^{a}	0.291±0.032^{a}	0.506±0.114^{a}	0.503±0.048^{a}	0.470±0.089^{a}	**0.018**
	M	0.303±0.009^{b}	0.316±0.012^{b}	0.633±0.060^{ab}	0.865±0.222^{a}	0.640±0.073^{ab}	**0.005**
	L	0.195±0.029^{b}	0.147±0.020^{b}	0.682±0.172^{a}	0.617±0.082^{a}	0.460±0.042^{ab}	**0.000**
	LM	0.163±0.028^{b}	0.145±0.026^{b}	0.518±0.101^{a}	0.368±0.053^{ab}	0.375±0.099^{ab}	**0.004**
	P	**0.014**	**0.000**	0.668	0.061	0.142	
BHBA (mmol/L)	C	0.109±0.041	0.212±0.072	0.067±0.026	0.119±0.049	0.079±0.023	0.220
	M	0.049±0.006^{b}	0.047±0.007^{b}	0.046±0.004^{b}	0.111±0.022^{a}	0.065±0.013^{ab}	**0.004**
	L	0.390±0.089	0.557±0.145	0.527±0.151	0.302±0.072	0.563±0.107	0.432
	LM	0.099±0.017	0.210±0.060	0.242±0.152	0.573±0.295	0.223±0.116	0.316
	P	**0.000**	**0.004**	**0.018**	0.151	**0.001**	
GLU (mg/dl)	C	52.000±3.864^{ab}	46.667±3.442^{ab}	58.000±7.127^{a}	34.833±8.308^{ab}	30.667±4.702^{b}	**0.013**
	M	50.500±2.487^{a}	46.167±1.939^{a}	59.333±12.068^{a}	25.000±2.757^{a}	21.167±2.725^{b}	**0.000**
	L	48.167±2.257^{ab}	55.833±2.072^{a}	46.167±5.186^{abc}	36.667±3.242^{bc}	31.000±5.228^{c}	**0.001**
	LM	53.333±3.333^{a}	53.500±2.232^{ab}	52.500±3.019^{a}	42.667±4.372^{bc}	24.833±5.890^{c}	**0.000**
	P	0.669	**0.026**	0.610	0.143	0.417	

TG: *Triglycerides,* ***NEFA:*** *Non esterified fatty acids,* ***BHBA:*** *Beta-hydroxy butyric acid,* ***GLU:*** *Glucose,* ***CHOL:*** *Cholesterol, Different superscripts a,b in the same row indicate significant differences (P<0.05 or more) according to time during the periparturient period for a given group*

DISCUSSION

The effect of adding methionine or/and lysine supplements in dairy cow diets on animals' performance and efficiency traits has been extensively studied for last 30 years [6]. Although several studies regarding the contribution of methionine or/and lysine in rations to metabolic profiles of dairy cattle during periparturient period are available [9,10], the number of the studies comparing the effects of methionine and lysine on animals' metabolic profiles are limited.

In the present study, we noticed statistically significant change in total protein (TP), only in Group L (P=0.002). Studies report no marked change in TP concentrations during prepartum and postpartum periods. Nevertheless, TP level is shown to be increased in postpartum period [11,12] but it is reduced to its lowest concentrations at calving day [13]. In the current study, although we determined no marked change in TP concentrations in control and methionine groups, we measured considerable increase in TP levels in Group L at postpartum 1 w and 2 w. At their study Chibisa et al.[14] similarly demonstrate no momentous alterations in TP concentrations during prepartum and postpartum periods. Likewise, TP concentrations in the current study were similar and within the reference range (6.2-8.2 mg/dl) [15] in all groups during prepartum and postpartum periods.

Likewise, within group comparisons albumine (ALB) concentration was noted to be considerably changed in only Group L. This observation may be associated with the initiation of lactation. Krober et al.[16] show that methionine and lysine does not affect serum albumin levels during postpartum period.

Lysine is required for protein synthesis [3]. Nevertheless, serum TP concentrations were not significantly changed in Group M and Group LM when the obtained results were

Table 5. *Serum biochemical parameters (HDL, LDL and VLDL) in dairy cattle during periparturient period (2 weeks in prepartum and 2 weeks in postpartum) according to oral treatments (Group M: methionine (12.75 g/day); group L: lysine (18 g/day); group ML: methionine (12.75 g/day) + lysine (18 g/day); group C: negative controls) (n = 6 in each group). Results are expressed as means ± standard deviations*

Tablo 5. *Serum HDL, LDL ve VLDL konsantrasyonlarının gruplararası ve grupiçi değişimi*

Parameter	Group	-2 weeks	-1 week	Calving	+1 week	+2 weeks	p
HDL (mg/dL)	C	97.333±3.750^{b}	95.283±2.985^{b}	91.283±3.287^{b}	99.183±4.899^{b}	123.066±7.725^{a}	**0.001**
	M	105.467±6.577^{ab}	90.183±2.887^{ab}	75.250±7.627^{b}	97.950±9.711^{ab}	119.850±8.410^{a}	**0.004**
	L	64.483±5.949	68.967±5.877	60.467±4.817	61.867±7.105	70.483±5.883	0.712
	LM	67.717±3.451^{ab}	70.767±3.989^{ab}	66.833±4.456^{b}	71.533±6.654^{ab}	92.150±7.200^{a}	**0.015**
	P	**0.000**	**0.000**	**0.003**	**0.003**	**0.000**	
LDL (mg/dL)	C	20.000±2.557^{a}	16.317±1.445^{ab}	11.167±0.758^{b}	13.248±1.669^{ab}	20.000±2.155^{a}	**0.006**
	M	25.083±1.724^{a}	17.033±0.697^{bc}	11.867±0.776^{c}	15.900±0.917^{c}	22.017±2.267^{ab}	**0.000**
	L	7.150±1.044^{ab}	9.617±1.590^{a}	3.450±0.742^{b}	5.533±1.114^{ab}	6.950±0.955^{ab}	**0.011**
	LM	10.150±0.907^{ab}	13.433±1.263^{a}	4.767±1.190^{b}	6.750±1.080^{b}	13.233±2.510^{a}	**0.001**
	P	NS	NS	NS	NS	NS	
VLDL (mg/dL)	C	5.167±0.439^{a}	4.367±0.844^{ab}	1.466±0.217^{c}	2.300±0.867^{bc}	1.233±0.244^{c}	**0.000**
	M	4.600±0.604^{a}	4.900±0.191^{a}	2.100±0.475^{b}	2.067±0.329^{b}	2.033±0.233^{b}	**0.000**
	L	4.433±0.499^{a}	4.167±0.316^{a}	1.833±0.260^{b}	1.700±0.241^{b}	2.701±0.276^{b}	**0.000**
	LM	4.433±0.320^{a}	4.267±0.204^{a}	2.100±0.422^{b}	1.600±0.179^{b}	2.100±0.634^{b}	**0.000**
	P	NS	NS	NS	NS	NS	

HDL: *high density lipoprotein,* ***LDL:*** *low density lipoprotein,* ***VLDL:*** *very low density lipoprotein, Different superscripts a,b in the same row indicate significant differences (P<0.05 or more) according to time during the periparturient period for a given group, NS: Not significant*

Table 6. *Serum biochemical parameters (TBIL and DBIL) in dairy cattle during periparturient period (2 weeks in prepartum and 2 weeks in postpartum) according to oral treatments (Group M: methionine (12.75 g/day); group L: lysine (18 g/day); group ML: methionine (12.75 g/day) + lysine (18 g/day); group C: negative controls) (n = 6 in each group). Results are expressed as means ± standard deviations*

Tablo 6. *Serum TBIL ve DBIL konsantrasyonlarının gruplararası ve grupiçi değişimi*

Parameter	Group	-2 weeks	-1 week	Calving	+1 week	+2 weeks	p
TBIL (mg/dL)	C	0.135±0.015	0.160±0.023	0.350±0.116	0.275±0.052	0.223±0.049	0.130
	M	0.120±0.007	0.115±0.011	0.240±0.024	0.270±0.121	0.168±0.027	0.232
	L	0.196±0.036^{b}	0.155±0.014^{b}	0.870±0.305^{a}	0.550±0.149^{ab}	0.262±0.025^{b}	**0.014**
	LM	0.173±0.019^{b}	0.143±0.016^{b}	0.597±0.142^{a}	0.360±0.092^{b}	0.233±0.051^{b}	**0.003**
	P	NS	NS	NS	NS	NS	
DBIL (mg/dL)	C	0.025±0.003	0.045±0.011	0.073±0.027	0.065±0.003	0.052±0.019	0.276
	M	0.020±0.008^{a}	0.025±0.006^{a}	0.082±0.017^{a}	0.063±0.028^{a}	0.032±0.006^{a}	**0.045**
	L	0.013±0.005^{b}	0.022±0.007^{b}	0.190±0.067^{a}	0.148±0.005^{ab}	0.083±0.008^{ab}	**0.011**
	LM	0.017±0.002^{b}	0.016±0.007^{b}	0.135±0.042^{a}	0.100±0.028^{ab}	0.055±0.016^{ab}	**0.005**
	P	NS	NS	NS	NS	NS	

TBIL: *total bilirubin,* ***DBIL:*** *direct bilirubin, Different superscripts a,b in the same row indicate significant differences (P<0.05 or more) according to time during the periparturient period for a given group,* ***NS:*** *Not significant*

compared within the groups and according to sampling times. By contrast, even though TP concentration was altered considerably in Group L, the levels of serum TP were still between the reference ranges. This situation may be accounted for the fact that all the animals used in the present study were fed with intuitionally well balanced ration and possessed similar performance and production traits.

Moreover, no marked difference in activity of creatine kinase (CK) within group evaluations may reflect no protein mobilization primarily from the muscle tissue and other body reservoirs in all present groups [17].

We noted considerable variation in BUN levels during periparturient period in all study groups. However, the levels of BUN in control, M, L, and LM groups were within the physiological reference ranges (7.8-25 mg/dl) [15]. While Sevinc et al.[11] report statistically important increase in total urea on calving day, Bauchart [18] indicate that TP and urea decrease on the day before calving. Increases during

Table 7. *Serum biochemical parameters (GGT, ALT, AST, ALP and CK) in dairy cattle during periparturient period (2 weeks in prepartum and 2 weeks in postpartum) according to oral treatments (Group M: methionine (12.75 g/day); group L: lysine (18 g/day); group ML: methionine (12.75 g/day) + lysine (18 g/day); group C: negative controls) (n = 6 in each group). Results are expressed as means ± standard deviations*

Tablo 7. *Serum GGT, ALT, AST, ALP ve CK konsantrasyonlarının gruplararası ve grupiçi değişimi*

Parameter	Group	-2 weeks	-1 week	Calving	+1 week	+2 weeks	P
GGT (U/L)	C	20.833±2.845	22.167±3.103	26.166±4.100	26.000±5.215	26.500±4.341	0.785
	M	20.833±2.242	20.833±1.922	19.333±2.788	20.833±1.249	22.667±1.977	0.863
	L	17.833±1.194	17.833±1.166	20.1667±0.872	26.167±5.717	24.000±3.454	0.244
	LM	13.167±1.222	13.500±1.384	16.000±1.460	18.333±2.060	19.333±1.763	**0.038**
	P	**0.043**	**0.033**	0.119	0.754	0.438	
ALT (U/L)	C	28.000±2.503	23.667±2.348	23.167±1.887	22.333±1.584	24.333±2.261	0.403
	M	27.333±2.917	23.666±3.138	20.000±2.294	18.167±2.762	21.000±2.280	0.172
	L	22.167±1.447^{ab}	24.833±2.151^{a}	19.833±1.922^{ab}	19.333±1.382^{ab}	17.167±.0543^{b}	**0.024**
	LM	21.500±1.544^{ab}	22.667±1.837^{a}	19.000±2.000^{ab}	16.333±0.667^{b}	17.333±0.714^{ab}	**0.024**
	P	NS	NS	NS	NS	NS	
AST (U/L)	C	70.000±4.082^{b}	68.667±5.506^{b}	81.500±3.567^{ab}	94.667±4.773^{a}	89.833±8.360^{ab}	**0.008**
	M	71.500±2.693	66.333±2.789	65.500±3.784	73.500±5.649	69.000±5.894	0.669
	L	66.667±4.997^{b}	71.500±4.588^{b}	65.500±9.172^{b}	113.167±13.197^{a}	90.333±5.643^{ab}	**0.001**
	LM	62.167±3.135^{ab}	56.000±7.928^{b}	71.833±4.728^{ab}	87.667±8.252^{a}	79.667±8.293^{ab}	**0.020**
	P	0.347	0.246	0.197	**0.030**	0.149	
ALP (U/L)	C	54.767±7.433	58.100±5.771	64.750±6.015	49.333±8.602	48.033±6.973	NS
	M	32.650±2.883	40.017±5.496	52.517±13.314	30.717±3.218	35.920±3.382	NS
	L	46.317±6.080	48.500±6.566	58.067±11.289	56.117±10.372	42.350±9.619	NS
	LM	48.400±7.024	54.800±4.972	62.450±7.976	50.367±6.033	45.650±5.659	NS
	P	NS	NS	NS	NS	NS	
CK (U/L)	C	118.000±11.778	116.383±17.781	179.916±36.875	231.467±87.509	127.250±12.709	NS
	M	90.116±14.691	89.733±18.775	311.68±203.292	107.317±6.889	166.920±45.212	NS
	L	118.217±35.546	105.983±14.127	105.150±11.640	186.133±35.992	163.117±42.247	NS
	LM	124.250±15.297	114.983±12.270	168.783±39.050	132.367±21.031	132.267±8.743	NS
	P	NS	NS	NS	NS	NS	

GGT: *gamma-glutamyl transferase,* ***ALT:*** *alanine aminotransferase,* ***AST:*** *aspartate aminotransferase,* ***ALP:*** *alkaline phosphatase,* ***CK:*** *creatine kinase, Different superscripts a,b in the same row indicate significant differences (P<0.05 or more) according to time during the periparturient period for a given group,* ***NS:*** *Not significant*

calving day are thought to be associated with calving stress and with the hemodynamic effect of stress on glomerular filtration rate [11]. The cause of reductions is considered to be due to decrease in food intake or colostrums production, and as food intake increases BUN concentration raises. Increase in dry matter taken with diet is reported to regulate blood protein and urea levels [19]. There are studies asserting that urea concentration is associated with dietary protein degradability, amount of energy and non-protein nitrogen [20], and with the number of calving [19]. Blum et al.[21] fed lactating cows with methionine for five days and observed a slight increase at the end of the treatment but the increase was not meaningful. While addition of methionine (12.4 mg/dl) to rations containing %16 proteins was shown to decrease urea levels, methionine plus lysine supplementations were not noted to change its levels [9]. Nevertheless, decrease in serum urea levels in the animals fed with rations containing lysine and methionine could be associated with increase in nitrogen cycle [22]. Krober et al.[16] demonstrated that methionine, lysine, or methionine plus lysine supplementations during early lactation period did not markedly alter serum BUN levels.

In our study, we measured statistically significant dissimilarity in serum triglyceride (TG) concentrations in all the groups among sampling day evaluations (P=0.000). TG levels in all the groups were lower at calving day, postpartum 1 w (the first week in postpartum period), and postpartum 2 w (the second weeks in postpartum period) measurements when compared to prepartum 2 w. We also accomplished considerable difference (P=0.049) at only postpartum 1w TG concentrations at evaluations among the groups, and the lowest TG level was computed in control group. TG is used for making milk fat by mammary gland during lactation period [4]; therefore, TG serum concentration is lower in lactation than dry period [23]. On the other hand,

current TG serum concentrations measured at calving day and postpartum period were between reference limits 0-14 mg/dl. Elevated prepartum TG levels measured here in all groups could be associated with reduced catabolism in dry period and/or with excessive production [24].

At their recent study, Phillips et al.[25] demonstrate that inclusion of methionine into diets of cows drops off their serum TG at postpartum 1st week. By contrast, the studies using methionine during early lactation period report no change in serum TG levels [26,27]. Xu et al.[28] discloses that the addition of rumen-protected amino acids containing methionine (13 g/day) and lysine (40 g/day) into the rations of multiparous Holstein dairy cattle reduces serum levels of TG and NEFA. In the current study, at the comparisons within the groups were calculated the lowest serum TG levels at postpartum 1 w in Group LM but at postpartum 2 w in Group M, Group L, and in control groups. At evaluations among the groups, we obtained numerically the highest TG level on calving day in Group LM, and at postpartum 1 w and 2 w in Group M.

In the present study at evaluations within the groups, serum CHOL concentrations were decreased between prepartum 2 w and calving day but increased between calving day and postpartum 2 w in all groups. CHOL levels at evaluations between the groups were the highest in control group and the lowest in Group L on calving day. When compared to other groups, the lowest serum CHOL levels were measured in Group L. We also noted numerical increase in high-density lipoprotein (HDL) levels in all study groups between calving day and postpartum 2 w. The lowest concentrations of CHOL and HDL were measured on the day of calving [29], an observation that might be associated with stress at the time of calving because cortisol, a steroid hormone and released in response to stress is derived from the cholesterol [30]. CHOL and HDL levels were measured to be lower at postpartum 2nd week in Group L than the other study groups. That 60% of HDL is made of cholesterol might be reason for this reduction [31]. Basoglu et al.[32] reported that the levels of HDL are higher in late lactation than early lactation and dry period. In another study, Yildiz et al.[13] demonstrate that serum HDL concentration is higher in the last month of pregnancy than calving day. The results of the present study are consistent with these studies mentioned here. On the other hand, methionine supplementation during early lactation in cows is shown to have no effect on serum HDL levels [26]. Likewise, methionine addition into the rations of dairy cattle during lactation period is reported not to affect CHOL levels [27]. At their study on dairy cattle with high milk production, Trinacty et al.[10] show that treatment of dairy cattle with rumen-protected methionine, lysine, or both does not influence levels of blood metabolites except for BHBA. Similarly, Ye et al.[22] report that while feeding of dairy cattle with pellet containing methionine and lysine during periparturient decrease serum NEFA and BHBA levels, it does not affect other blood parameters.

It reports that while methionine supplementation during prepartum period does not alter NEFA concentration [25], its inclusion to diets during postpartum period either reduces NEFA level [21], or does not affect its level [9]. Likewise, methionine supplementation during periparturient period in cows is reported not to affect normal concentration of NEFA [25]. In the present study, we observed statistically significant changes in serum NEFA concentrations according to all sampling days in all groups *(Table 4)*. At evaluations within the groups we determined numerically increased NEFA levels on calving day with respect to prepartum 2 w. Serum NEFA concentrations were decreased at postpartum 1 w and 2 w in Group L and Group LM when compared with calving day NEFA measurement values and the main reduction at evaluations among the groups was in Group LM. In addition, at evaluations among the groups serum NEFA concentrations was only significantly diminished at prepartum 1w measurement (P=0.000).

Furthermore, in our study serum BHBA levels were considerably different (P=0.004) in only Group M when compared within the groups. At comparisons among the groups, the changes at BHBA levels were momentous at prepartum 1 w, on calving day, and at postpartum 2 w. When compared with respect to measurement times, a reduction in BHBA levels in Group M was observed till calving day measurement when compared to prepartum 1 w.

Acute increase in NEFA levels during calving is reported to be associated with the initiation of TG infiltration [25,33]. A positive correlation between negative energy balance and NEFA concentration is demonstrated [33]. NEFA concentration is shown to reach its highest concentration one day after calving and begin to decrease till postpartum third week. The reductions in NEFA levels can be evaluated as an indication of decreased body fat mobilization or use of NEFA for synthesizing VLDL in liver [34]. It is shown that at the cases when blood glucose level is appropriate, body does not need fat deposits. In the present study, increase in NEFA concentrations can be related to statistically significant reductions in serum glucose levels at postpartum 2 w in all groups and methionine and lysine appeared to be inadequate to prevent this condition. Methionine supplementation during lactation is reported to have no impact on NEFA concentration [34]. Our present study indicates that lysine seemed to have similar effect on NEFA concentration. Leroy et al.[12] disclose that increase in BHBA in hypoglycemic animals is much higher. There is also statistical correlation between NEFA and BHBA [19]. Cheng et al.[33] determined the highest NEFA and BHBA concentrations at postpartum 1st day and postpartum 2nd week. In the present study at evaluations within the groups, the highest NEFA and BHBA concentrations in Group M were obtained at postpartum 1 w. Possible reason for this might be the use of NEFA in the synthesis of BHBA [33]. In the current study, the highest numerical postpartum BHBA concentrations were measured at postpartum 1w in control group, at postpartum 2 w in Group L, and at postpartum

1 w in Group LM. Even though BHBA concentrations are shown to be associated with negative energy balance and mobilization of stored fats in body, they are also affected by lactation itself [13]. Several studies demonstrate that various doses of methionine supplementations during prepartum and postpartum periods in cows do not change BHBA concentrations [26]. In addition, the level of BHBA in multiparous is reported to be elevated with respect to the cows at their first calving [26]. At their study where they fed the cows with low and normal energy rations, Rulquin and Delaby [5] noted no effect of methionine on serum BHBA levels in both groups. By contrast, there are also studies showing no effect of methionine and lysine supplements during lactation period on serum BHBA concentration [1,28].

Moreover, the lowest low-density lipoprotein (LDL) levels in all the study groups were calculated on calving day at the evaluations within the present study groups. At their study, Basoglu et al.[32] state that prepartum serum LDL levels are higher when compared to late lactation. In this study, the highest serum LDL concentrations were obtained at postpartum 2 w in control group, at prepartum 2 w in Group M, at prepartum 1 w in Group L and Group LM.

The study results established that serum VLDL concentrations in all groups were numerically decreased on calving day, postpartum 1 w and 2 w with regard to prepartum 1 w and 2 w. Prepartum VLDL levels are shown to be higher than those of postpartum 1st week [32]. This condition can be associated with increased VLDL catabolism in mammary glands and excessive fat accumulation in liver [2]. Davidson et al.[26] added methionine to diets of cows at their early lactation period but observed no change in their VLDL concentrations. In the current study at evaluations within the groups, we measured numerically the lowest VLDL concentration in control and methionine groups at postpartum 2 w but at postpartum 2 w in Group L and Group LM. Similarly, Ye et al.[22] fed cows with pellet containing methionine and lysine during periparturient period but noted no change in VLDL concentration when compared to the control group. In the current study at evaluations within the groups we, by contrast, determined significant differences in serum VLDL levels between the study groups (P=0.000).

Furthermore, the highest concentration of glucose (GLU) was obtained on calving day in control and methionine groups and at postpartum 1 w in Group L and Group LM. The lowest glucose level was recorded at postpartum 2w samples in all the groups. Glucocorticoids secreted on the day before calving is known to rise glucose level [35] and the increase in glucose level on calving day is thought to be associated with calving stress [30]. Reduction in postpartum serum glucose level is reported to be associated with decrease in food intake during early lactation [36], and increase in the consumption of glucose by mammary gland [37] and fetus near to calving. Studies state that decline in serum glucose level observed during the first week of lactation can be corrected in later weeks by providing animals with energetically well balanced rations [33,36]. In the present study, serum glucose concentrations at postpartum 2 w were numerically lower than those of calving day and postpartum 1 w. Moreover, postpartum serum GLU concentrations in all groups were determined to be below the reference range (42.1-74.5 mg/dl) [15]. Reductions in serum glucose levels indicate that methionine, lysine, or methionine plus lysine supplementations fail to boost the postpartum serum GLU levels up in the present study groups fed with the same type of ration. Nonetheless, measurement of lower serum NEFA levels and absence of significant differences concerning milk production among the present groups might be an indication of undeveloped postpartum NEB. In the present study, the reduction in GLU level during early lactation can be resulted from increased milk production and the use of glucose for the synthesis of lactose. While some studies show that postpartum serum GLU levels increase [35], the present study reports that postpartum glucose concentrations are reduced [12,32].

Several studies indicate that addition of methionine into diets of dairy cattle does not change serum GLU concentrations [21]. Similarly we noted increase in prepartum GLU levels when compared to postpartum period in the animals provided with methionine. Nonetheless, methionine addition to the diets of lactating cows is shown to decrease blood GLU levels [35]. Likewise, at their study Socha et al.[9] report that addition of methionine in diets of cows reduces serum glucose levels at postpartum first and third weeks and addition of lysine with methionine in rations further diminishes GLU levels, an observation consistent with our present observations.

In the current study, aspartate aminotransferase (AST) values were characterized with insignificant changes in only Group M during prepartum and postpartum periods. By contrast, AST levels were considerable increased, particularly on calving day, and postpartum first week and postpartum second week in control group, Group L, and Group LM. As a result, this can be explained, methionine was observed to prevent excessive cellular activity on calving day and postpartum days but lysine by itself or in combination with methionine was found to be inadequate for suppressing cellular activity. Nevertheless, all the obtained present values were between reference ranges (45-110 U/L) [15].

Liver cells in ruminants do not have high alanine aminotransferase activity (ALT). However, Sevinc et al.[11] report that ALT activity in the first two months of the lactation increases with respect to the calving day and last month of dry period. In our study, while ALT activity was determined to be unimportant in control and methionine groups, it was significantly reduced in Group L and Group LM. Nevertheless, all obtained values for ALT levels in all groups were within the within the reference ranges (6.9-35 U/L) [15]. This observation is consistent with literature and further

supports the concept that ALT activity is not specific in ruminants for evaluating liver functions [2,35,38].

Furthermore, we noted no statistically significant increases in the levels of serum GGT in control, methionine, and lysine groups when compared to prepartum period. The serum GGT levels in these groups were within the reference limits (4.9-26 U/L) [15]. Although there was a meaningful increase in serum GGT levels in Group LM when prepartum and postpartum periods were compared, the values were still within the reference ranges. In the present study, numerical increases in serum GGT levels could be associated with postpartum liver fat infiltration and liver metabolism condition [38].

Metabolic indicators may change depending on composition of the ration [39]. Furthermore, the results of the present study indicated that the use of dietary additives such as methionine and lysine in the rations of dairy cattle that were fed with adequate and nutritionally well balanced rations to provide animals with liver protection and increase their milk production was not essential in order to obtain optimal milk production and protect cows against the development of the periparturient period diseases even though their use affected some parameters of the serum of these animals. On the other hand, the addition of methionine and lysine to the rations of dairy cattle nourished with inadequate and nutritionally unbalanced rations and handled with management problems helps cows recover their specific amino acid needs for gaining liver protection and augmenting milk production.

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