The Effect of Borax on Some Energy Metabolites in Dairy Cows during the Transition Period

Metin ÖĞÜN 1 * Oğuz MERHAN 1 Abdulsamed KÜKÜRT 1 Mushap KURU 2 Mahmut KARAPÊHLIVAN 3

1 Department of Biochemistry, Faculty of Veterinary Medicine, University of Kafkas, TR-36100 Kars - TURKEY
2 Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Kafkas, TR-36100 Kars - TURKEY
3 Department of Medical Biochemistry, Faculty of Medicine, University of Kafkas, TR-36100 Kars - TURKEY

Abstract

The purpose of this study is to investigate the effects of sodium borate (Na₂B₄O₇·5H₂O) addition to dairy cow rations starting from prepartum period on serum cortisol, glucose, β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglyceride (TG), blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels. Clinically healthy eighty pregnant cows were randomly divided into two groups. Sodium borate (30 g/day) was added to the rations of the borax group (n=40) until day 21 postpartum. Blood samples were taken from all cows (n=40) on days 21, 14 and 7 before parturition, at parturition and on days 7, 14 and 21 after parturition. Serum cortisol levels in the borax group were lower (P<0.05) than those in the control group, there was a decrease (P<0.05) in serum BHB, NEFA and TG levels before, at parturition and after parturition, serum BUN concentrations increased (P<0.05) in prepartum and postpartum samples in the borax group, except for prepartum days 21 and 14, AST concentrations were higher (P>0.05), on all other sampling days, and ALT levels were not affected (P>0.05). It was concluded that adding sodium borate to rations especially in the transition period in highly productive dairy breeds might be an alternative to protect against negative energy imbalances.

Keywords: Borax, Cortisol, Glucose, NEFA, BHB, Dairy Cows

Geçiş Dönemi Süt İneklerinde Bazı Enerji Metabolitlerinin Üzerine Boraksın Etkisi

Özet

Bu çalışmanın amacı, prepartum döneminde başlayan sütçü sığırların rasyonlarına sodyum borat (Na₂B₄O₇·5H₂O) ilavesinin serum kortizol, glukoz, β-hidroksibütirat (BHB), esterleşmemiş yağ asitleri (NEFA), triglyserid (TG), kan üre nitrojen (BUN), aspartat aminotransferaz (AST), alanin aminotransferaz (ALT) düzeylerine etkisi araştırılmasıdır. Klinik olarak sağlıklı 80 adet gebe inek rastgele iki gruba ayrıldı. Borax grubuna (n=40) doımundan 30 gün önce prepartum döneminden başlarak postpartum 21. gün kadar rasyonlarına ek olarak sodyum borat (30 g/gün) eklendi. Hem çalışma hem de kontrol grubundan (n=40) doımundan 21, 14 ve 7. gün parturitinde, parturitinde ve parturitin ardından, serum BUN konsantrasyonu prepartum ve postpartum örneklerinde borax grupuna olduğu gibi prepartum günlerinde 21 ve 14. günlerinde artışa uğradı. AST konsantrasyonlarının doım öncesi 21 ve 14. günlerinde arttığı ve ALT düzeyinin etkilenmediği (P>0.05) belirlendi. Sonuç olarak, yüksek süt verimi ırklarda özellikle geçiş döneminde rasyon sodyum borat ilavesinin negatif enerji dengesizliklerinden korunarak bir seçeneğe olabileceğini belirledik.

Anahtar sözcükler: Boraks, Kortizol, Glukoz, NEFA, BHB, Sütçü İnek

INTRODUCTION

The periodic table of elements represents Boron as B. It has both metal and nonmetal characteristics. 1-3 This dynamic trace element enters the body in small amounts via food and drink. 4-5 Boron can affect at least 26 different enzyme activities required for energy substrate metabolism. 6,7 After inorganic borates are absorbed through mucosal membranes, they are transformed into boric acid. 8 Boric acid is an essential element with biological significance in animal and human nutrition, metabolic, hormonal and physiological events. 9 Its effect
on mineral metabolism, the endocrine system and immune response is also recognized\textsuperscript{[3,10,11]}. Even though studies have been conducted, the effects of boric acid in metabolic events in animals have not been explained in detail\textsuperscript{[3]}

Basoglu et al.\textsuperscript{[11]} have determined that supplementing rations with sodium borate can be effective in preventing fatty liver in cow. Another study found that sodium borate added to the rations of dairy cow during the transition period did not affect blood urea nitrogen (BUN) and alanine aminotransferase (ALT) levels, but that aspartate aminotransferase (AST) and β-hydroxybutyrate (BHB), triglyceride (TG) and non-esterified fatty acids (NEFA) levels decreased and glucose levels increased\textsuperscript{[12]}. The transition period is a period of approximately 6 weeks encompassing the three weeks before parturition and three weeks after parturition, during which important endocrine and metabolic changes take place in dairy cows. During this time there is a greater need for nutrients for the fetus, development of the mammary glands and synthesis of milk. In this situation, the body is unable to meet the demand for glucose required for energy metabolism, and so it satisfies the need for energy by metabolizing NEFA from adipose tissues. The energy deficit results in insulin sensitivity and loss of appetite by causing an increase in circulating NEFA. All of these metabolic and hormonal changes give rise to metabolic syndromes such as fatty liver in dairy cows that produce large quantities of milk\textsuperscript{[13-19]}. Compensating mechanisms play an important role in minimizing the changes that occur in metabolic activities during the transition period in cows. These changes in particularly energy metabolism cause a certain amount of stress in the body. All types of environmental and care conditions that could create stress on the animal need to be removed\textsuperscript{[16,20-23]}. Therefore, substances that provide energy in addition to rations or that reduce the mobilization of triglycerides can be provided for nutrition during this time\textsuperscript{[12,19,24]}.

The purpose of this study was to investigate the effects of sodium borate on cortisol levels that can occur due to the negative energy balance and the stress of parturition, and its effects on the consequently varying energy metabolites such as serum glucose, BHB, NEFA, TG, BUN, AST and ALT when added to rations 30 days prepartum and 21 days postpartum in Red Holstein cows.

**MATERIAL and METHODS**

This study was conducted after obtaining approval from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ HADYEK - Study code: 2015/111, Meeting number: 2015/13, Edition no: 2015/133).

The study was conducted at the Niğtaş Farm in the province of Niğde, which practices intensive farming. The study material consisted of 80 pregnant Red Holstein cows ranging from 3-5 years of age. The cows’ body condition scores were between 3.00-3.50 before parturition and 2.50-3.00 after parturition on a 5-point scale with increments of 0.25\textsuperscript{[25,26]}. Postpartum milk production varied between 24-28 liters per day. The study included cows which had at least one normal parturition. Cows that had difficult parturitions were not included in the study.

Eighty cows that were clinically healthy and which had been given anti-parasite medications and vaccinations prior to pregnancy were randomly divided into two groups. Beginning 30 days before parturition, sodium borate (Na$_2$B$_4$O$_7$·5H$_2$O, 30 g/day, Merck) was added to the rations of the experimental group (Borax group, n=40) until day 21 postpartum. It has been shown that ration contain were given to animals at the Table 1. Blood samples were taken from both the experimental and the control group (n=40) on days 21, 14 and 7 prepartum, at parturition and on days 7, 14 and 21 postpartum. Blood was taken from the vena coccygea and placed in 10-ml vacuum vials (BD Vakutainer®, Tipkimsan, Turkey) using sterile holder needles. The blood was brought to a laboratory on the farm within one hour of being drawn and centrifuged for 10 min at 3.000 rpm (Hettich Universal 320®, Hettich, Germany). The sera were stored at -20°C until biochemical tests were performed.

The serum samples from the study were analyzed for cortisol, glucose, BHB, NEFA, TG, BUN, AST and ALT. Cortisol measurements were performed using the Radio-immunoassay (RIA, Beckman Coulter®, USA) method with a commercial kit (Access® Cortisol, Unicel Dxl 600, Beckman Coulter, USA). Spectrophotometric measurements (Epoch®, Biotek, USA) were performed using commercial kits for glucose, TG, BUN, ALT and AST levels (DDS, Turkey), BHB (Ranbut®, Randox, UK) and NEFA (Wako Diagnostics, VA).

Statistical analyses of the serum cortisol, glucose, BHB,

| Table 1. Prepartum ve Postparantium Dijüterin bileşen ve besin kompozisyonu |
|---------------------------------|-----------------|-----------------|
| **Ingredients (%DM)**            | **Prepartum**   | **Postpartum**  |
| Corn silage                      | 11.17           | 19.64           |
| Hay                              | 28.49           | 2.78            |
| Alfalfa hay                      | 23.46           | 17.67           |
| Barley                           | 0.00            | 5.89            |
| Dairy cattle feed                | 0.00            | 25.92           |
| Heifer feed                      | 16.39           | 0.00            |
| Barley pulp                      | 20.11           | 23.79           |
| Soypass                          | 0.00            | 4.13            |
| Yeast                            | 0.372           | 0.181           |
| NEL (cal/g)                      | 1.28            | 1.52            |

**DM:** Dry matter; **NEL:** Net energy lactation
NEFA, TG, BUN, ALT and AST levels were performed using the SPSS® (SPSS 20, IL, USA). The change in biochemical parameter levels in the groups by days was analyzed with the Anova, Tukey HSD test. Statistical comparison of the groups by days was performed using the Student-t test. The results are provided as mean ± SD (SD: Standard deviation). Values of \( P < 0.05 \) were considered statistically significant.

**RESULTS**

Prepartum and postpartum serum cortisol levels did not change (\( P > 0.05 \)). During parturition, however, serum cortisol concentrations increased in both the control and the borax group (\( P < 0.001 \)). When the groups were compared, serum cortisol levels in the Borax group were significantly lower (\( P < 0.05 \)) than those of the control group.

Serum glucose concentrations were lower (\( P < 0.001 \)) during parturition than in both prepartum and postpartum samples in both groups. However, the administration of sodium borate affected the serum glucose levels. The glucose concentration in the borax group was significantly higher than that in the control group on the sampling days (\( P < 0.05 \)).

It was determined that serum BHB levels were affected after parturition and that there was a trend higher (\( P < 0.001 \)) in both groups after parturition. In addition, supplementing rations with sodium borate resulted in a statistically significant decline (\( P < 0.05 \)) in serum BHB levels prepartum, at parturition and postpartum compared to the control group.

The NEFA concentration increased in both groups in the time leading up to parturition and fell postpartum (\( P < 0.001 \)). NEFA levels on all sampling days in the borax group were significantly lower than in the control group (\( P < 0.05 \)).

Serum TG levels were higher until close to the time of parturition in the control group and later the serum concentrations fell again (\( P < 0.001 \)). However, serum TG levels in the borax group were similar on sampling days (\( P > 0.05 \)). Furthermore, TG levels during parturition and on postpartum days 7 and 14 were significantly lower than those of the control group (\( P < 0.05 \)).

Serum BUN concentrations increased in the borax group compared to the control group (\( P < 0.05 \)). However, administration of sodium borate in the first week prepartum and during parturition did not affect serum BUN levels (\( P > 0.05 \)). Furthermore, there was a tendency for the BUN levels to rise in both groups as it came closer to time to give parturition (\( P < 0.001 \)).

Measurements showed that serum ALT levels were similar (\( P > 0.05 \)) in both groups, but that concentrations were significantly lower (\( P < 0.05 \)) in serum samples from the borax group taken on postpartum day 21.

AST concentrations in serum samples on days -21 and -14 prepartum were similar between the two groups (\( P > 0.05 \)). On the other days, however, the AST concentration in the borax group was significantly higher. In addition, AST levels were highest in both groups in the first postpartum week (\( P < 0.001 \)).

Changes in levels of serum cortisol, glucose, BHB, NEFA, TG, BUN, ALT and AST in blood samples taken at days -21, -14, -7, parturition, 7, 14 and 21 after administration of sodium borate for both the control group and the borax group are summarized in Table 2.

**DISCUSSION**

The changes that occur in connection with energy metabolism in the transition period in cows cause a certain amount of stress on the body [16,17,27-29]. The increasing cortisol level in particular plays a role in the beginning of labor during parturition. Some diseases that may occur during this period are also thought to be an indicator of stress [30-32]. Serum cortisol levels in cows during a normal parturitions are higher than in the prepartum period [29]. Similarly, we found that serum cortisol levels are higher during parturition than they are in the prepartum or postpartum period (\( P < 0.001 \)). However, serum cortisol was lower in the group whose rations where supplemented with sodium borate (\( P < 0.05 \)). This suggests that adding sodium borate to rations could be a way to reduce stress during parturition. It is thought that this might be due to the positive effect of sodium borate on energy metabolism.

Vannucchi et al. [29] found an inverse relationship between the level of serum cortisol and serum glucose concentrations during parturition. In our study, serum glucose concentrations showed a tendency to decline as parturition approached, which was inversely proportional to serum cortisol. However, it was found that administration of sodium borate caused an increase in serum glucose levels compared to the control group (\( P > 0.05 \)). Studies have reported that adding borax to rations causes an increase in serum glucose levels during parturition compared to prepartum and postpartum periods [12,23]. In our study, on the other hand, glucose was significantly higher in the control group, but tended to decline at parturition compared to the prepartum and postpartum periods. The results in some studies report variations depending on the rate of borax administered to the cows, the source from which the borax was obtained and borax absorption in the body.

It has been reported that BHB levels, which are characterized as a response to the negative energy balance during peripartum, generally rise during parturition [12,23].
In this study, BHB concentrations changed in both groups during the peripartum period. It was particularly remarkable that BHB levels increased in both groups during parturition. However, administration of sodium borate caused a decrease in serum BHB levels compared to the control group. Kabu and Civelek [12] argued in their study that administration of borax did not affect BHB levels in prepartum and postpartum weeks. In the same study, they reported that serum NEFA levels rise during parturition and that the administration of borax did not affect NEFA concentration during parturition. In the present study, however, we found a statistically significant rise in NEFA levels during parturition in both the control group and the borax group. Furthermore, the administration of borax reduced the formation of NEFA compared to the control group. The rise in epinephrine and norepinephrine levels during parturition in cows contributes to the rise in plasma NEFA and TG concentrations. In particular, it reportedly increases the rate of adipose tissue lipolysis through adrenergic stimulation in the transition from the dry period to lactation in primiparous cows [13,34]. In our study, TG levels in the control group increased (P<0.001) in the period leading up to parturition, which is consistent with the literature. However, there was no statistically significant increase (P>0.05) in TG levels in the borax group in the period leading up to parturition. In fact, TG levels in the control group were significantly higher (P<0.05) than those in the borax group. These data suggest that borax could be used to protect dairy cows from the formation of TG in the periparturient period. It is thought that borax might lower serum BHB, NEFA and TG concentrations because it raised serum glucagon levels [12], and our study did find this occurred in connection with its effect of raising glucose levels, which might mean that it would mitigate liver damage.

It has been reported that BUN concentrations in cow can vary between 78-250 mg/L and reach peak values

### Table 2. Changes in levels of serum cortisol, glucose, BHB, NEFA, TG, BUN, ALT and AST at days -21, -14, -7, Parturition, 7, 14 and 21 for the control group and the borax group

| Parameters | Groups | Days | P value |
|------------|--------|------|---------|
|            |        |      |         |
| Cortisol (mmol/L) |        |      |         |
| C         | 9.8±0.75a | 12.3±0.21b | 18.8±0.54c | 42.8±11.6d | 4.18±0.34e | 2.68±0.06f | 2.45±0.14g | ** |
| B         | 8.65±0.6a | 11.9±0.34b | 17.6±0.07c | 35.9±7.5d | 3.96±0.23e | 2.56±0.37f | 2.44±0.06g | ** |
| P value   | NS     | NS   | NS      | NS      | NS      | NS      | NS      | ** |
| Glucose (mg/dL) |        |      |         |
| C         | 58.2±1.2a | 56.4±0.9bc | 53.7±1.3ac | 51.2±0.9a | 52.7±0.6ab | 56.4±0.8bc | 56.9±1.1ac | ** |
| B         | 62.7±1.2a | 59.5±1.1bcd | 56.7±0.7ab | 54.8±1.1a | 57.9±1.3ac | 61.6±0.9ab | 63.8±1.4ac | ** |
| P value   | NS     | NS   | NS      | NS      | NS      | NS      | NS      | ** |
| BHB (mmol/L) |        |      |         |
| C         | 0.52±0.21a | 0.58±0.18c | 0.67±0.25c | 0.84±0.32c | 0.75±0.27c | 0.72±0.12c | 0.66±0.18c | ** |
| B         | 0.47±0.24a | 0.53±0.31a | 0.61±0.15c | 0.76±0.09a | 0.69±0.14b | 0.68±0.12c | 0.60±0.08c | ** |
| P value   | NS     | NS   | NS      | NS      | NS      | NS      | NS      | ** |
| NEFA (mmol/L) |        |      |         |
| C         | 0.27±0.05a | 0.33±0.04b | 0.45±0.12c | 0.82±0.23c | 0.61±0.15c | 0.58±0.28c | 0.52±0.14c | ** |
| B         | 0.21±0.18a | 0.28±0.09a | 0.36±0.28c | 0.76±0.18c | 0.55±0.19c | 0.54±0.16c | 0.46±0.31c | ** |
| P value   | NS     | NS   | NS      | NS      | NS      | NS      | NS      | ** |
| TG (mg/dL) |        |      |         |
| C         | 19.4±2.1a | 21.8±3.4a | 22.2±1.9c | 26.8±1.5c | 25.8±1.7ab | 23.9±2.8ab | 19.8±2.7ab | ** |
| B         | 18.2±2.3a | 19.2±1.8a | 20.2±2.3a | 21.6±1.9a | 20.1±1.6a | 19.7±1.8a | 17.6±0.8a | NS |
| P value   | NS     | NS   | NS      | NS      | NS      | NS      | NS      | NS |
| BUN (mg/L) |        |      |         |
| C         | 98.7±14.7a | 105.6±21.3ab | 118.3±15.6c | 153.9±21.6a | 121.6±12.4ab | 109.8±19.7bc | 102.8±10.5ac | ** |
| B         | 109.6±21.2a | 118.7±13.6a | 125.8±12.2a | 160.4±21.5c | 136.8±17.5d | 123.9±20.7bc | 114.6±18.9d | ** |
| P value   | NS     | NS   | NS      | NS      | NS      | NS      | NS      | NS |
| ALT (U/L) |        |      |         |
| C         | 28.6±3.2a | 24.7±4.24a | 22.8±8.7a | 20.9±3.9a | 22.5±5.4a | 23.7±6.2a | 26.8±3.6a | ** |
| B         | 27.6±4.6a | 23.9±5.2a | 21.8±3.7a | 19.8±6.4a | 21.6±2.8a | 22.7±2.7a | 23.8±4.7a | ** |
| P value   | NS     | NS   | NS      | NS      | NS      | NS      | NS      | NS |
| AST (U/L) |        |      |         |
| C         | 71.3±5.6a | 75.9±11.6a | 78.4±12.4a | 80.6±5.6a | 98.4±8.7a | 89.6±6.8a | 80.7±3.6a | ** |
| B         | 69.5±7.4a | 78.6±9.4a | 84.6±11.8a | 93.8±15.6a | 102.6±13.8a | 95.3±8.6a | 86.7±7.2a | ** |
| P value   | NS     | NS   | NS      | NS      | NS      | NS      | NS      | NS |

*The difference between values with different letters on the same line is significant at the P value, * P<0.05, ** P<0.001, NS: Not significant, C: Control group, B: Borax group, BHB: β-Hydroxybutyrate, NEFA: Non-Esterified Fatty Acids, TG: Triglyceride, BUN: Blood Urea Nitrogen, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase.
during calving \[12,13\]. One study demonstrated that BUN values reach their peak on the 21st day postpartum \[13\]. This study found that serum BUN levels varied between 98 to 160 mg/L and the peak BUN level was measured during calving. Serum BUN levels were significantly higher during parturition in the group given sodium borate. In the study conducted by Kabu and Civelek \[12\], they found that serum BUN levels were highest on day 7 postpartum in the group given borax. It is thought that the reason for the increase in BUN levels, especially postpartum, may be due to the fact that sodium borate reduces lipid infiltration and increases protein anabolism. 

In studies conducted during the periparturient period, researchers have reported that ALT and AST level may rise during calving \[12\] or remain the same \[13\]. There are reports of a significant correlation between AST activity and the concentration of glucose, NEFA and BHB \[13\]. Using borax supplements in rations not affect to the ALT and AST levels. AST levels are reportedly affected by parturition in both the borax and control group and rise in the postpartum period \[12\]. In our study, AST levels increased in both groups during parturition and the postpartum period. It is thought that these increases may be due to cellular damage that can occur in the liver due to lipid mobilization that happens in connection with the negative energy balance that occurs the closer the cows get to having parturition.

In conclusion, the addition of sodium borate to rations starting in the peripartum period caused maternal cortisol levels to fall during parturition compared to the control group, raised serum glucose and BUN levels, reduced BHB, NEFA and TG concentrations, raised AST levels during and after parturition and did not affect ALT levels. It was concluded that adding sodium borate to rations especially in the transition period might be an alternative to protect against negative energy imbalances, especially in highly productive dairy breeds.

**REFERENCES**

1. Murray Fl: A human health risk assessment of boron (boric acid and borax) in drinking water. *Regul Toxicol Pharmacol*, 22, 221-230, 1995. DOI: 10.1006/rtph.1995.0004
2. Zhai HJ, Kiran B, Li J, Wang LS: Hydrocarbon analogues of boron clusters-planarity, aromaticity and antiaromaticity. *Nat Mater*, 2, 827-833, 2003. DOI: 10.1038/nmat1102
3. Kabu M, Akosman MS: Biological effects of boron. *Rev Environ Contam Toxicol*, 225, 57-75, 2013. DOI: 10.1007/978-1-4614-6470-9_2
4. Sabuncuoglu BT, Kocaturk PA, Yaman O, Kavas GO, Tekelioğlu M: Effects of subacute boric acid administration on rat kidney tissue. *Clin Toxicol (Phila)*, 44, 249-253, 2006. DOI: 10.1080/15563650600584386
5. Xu RY, Xing XR, Qian X, Zhang G, Wei FS: Investigations on boron levels in drinking water sources in China. *Environ Monit Assess*, 165, 15-25, 2010. DOI: 10.1007/s10661-009-0923-8
6. Hunt CD: Regulation of enzymatic activity. One possible role of dietary boron in higher animals and humans. *Biochim Biophys Acta*, 66, 205-225, 1998. DOI: 10.1007/BF02783139
7. Bakken NA, Hunt CD: Dietary boron decreases peak pancreatic in situ insulin release in chicks and plasma insulin concentrations in rats regardless of vitamin D or magnesium status. *J Nutr*, 133 (11): 3577-3583, 2003.
8. Bolaños L, Lukaszewski K, Bonilla I, Bleivins D: Why boron? *Plant Physiol Biochem*, 42, 907-912, 2004. DOI: 10.1016/j.plaphy.2004.11.002
9. Bleivins DG, Lukaszewski RM: Boron in plant structure and function. *Annu Rev Plant Physiol Plant Mol Biol*, 49, 481-500, 1998. DOI: 10.1146/annurev.arplant.49.1.481
10. Nielsen FH: Boron in human and animal nutrition. *Plant Soil*, 193, 199-207, 1997. DOI: 10.1023/A:1004276311956
11. Basoglu A, Sevinc M, Birdane FM, Boydak M: Efficacy of sodium borate in the prevention of fatty liver in dairy cows. *J Vet Intern Med*, 16, 732-735, 2002. DOI: 10.1111/j.1939-1676.2002.tb02416.x
12. Kabu M, Civelek T: Effects of propylene glycol, methionine and sodium borate on metabolic profile in dairy cattle during periparturient period. *Rev Méd Vet*, 163, 419-430, 2012.
13. Grummer RR: Etiology of lipid-related metabolic disorders in periparturient dairy cows. *J Dairy Sci*, 76, 3882-3896, 1993. DOI: 10.3168/jds.S0022-0302(93)77729-2
14. Vazquez-Añon M, Bertsic S, Luck M, Grummer RR, Pinheiro J: Periparturient liver triglyceride and plasma metabolites in dairy cows. *J Dairy Sci*, 77, 1521-1528, 1994. DOI: 10.3168/jds.2002-0302(94)77092-2
15. Grummer RR: Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *J Anim Sci*, 73, 2820-2833, 1995.
16. Drackley JK: Biology of dairy cows during the transition period: the final frontier? *J Dairy Sci*, 82, 2259-2273, 1999. DOI: 10.3168/jds.S0022-0302(99)75474-3
17. Drackley JK, Overton TR, Douglas GN: Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. *J Dairy Sci*, 84, E100-112E, 2001. DOI: 10.3168/jds.S0022-0302(01)70204-4
18. Overton TR: Transition cow programs. The good, the bad, and how to keep them from getting ugly. *Adv Dairy Tech*, 13, 17-26, 2001.
19. Kabu M: Bor, propilen glikol ve methioninin süt sığırılardaki metabolik profil üzerinde etkisi. *Kacatepe Vet J*, 3, 37-44, 2012.
20. Knight CH, Beever DE, Sorensen A: Periparturient liver triglyceride and plasma metabolites in dairy cows. *J Dairy Sci*, 82, 2259-2273, 1999. DOI: 10.3168/jds.S0022-0302(99)75474-3
21. Kessel S, Stroehl M, Meyer HHD, Hiss S, Sauerwein H, Schwarz FJ, Bruckmaier RM: Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. *J Anim Sci*, 76, 2903-2912, 2008. DOI: 10.2527/jas.2008-1016
22. Arslan C, Tufan T: Geçiş döneminde süt ineklerinin beslenmesi. Bu döneminde görülen fizyolojik, hormonal, metabolik ve immunolojik dejikliklerle ilieslenen ihtiyaçlar. *Kafkas Univ Vet Fak Derg*, 16, 151-158, 2010. DOI: 10.9775/kvfd.2009.442
23. Sundrum A: Metabolic disorders in the transition period indicate that the dairy cows’ ability to adapt is overstressed. *Animals*, 5, 978-1020, 2015. DOI: 10.3390/ani5040395
24. Stockdale CR, Roche JR: A review of the energy and protein nutrition of dairy cows through their dry period and its impact on early lactation performance. *Aust J Agric Res*, 53, 737-753, 2002. DOI: 10.1071/AR01019
25. Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G: Condition scoring chart for holstein dairy cows. *J Dairy Sci*, 72, 68-78, 1989. DOI: 10.3168/jds.S0022-0302(89)79081-0
26. Kuru M, Merhan O, Kaya S, Oral H, Kukurt A: The effect of short term propargole-releasing intravaginal device treatment on acute inflammation markers for Holstein heifers. *Rev Méd Vet*, 166, 336-340, 2015.
27. Serbester U, Cinar M, Hayrili A: Suçlu ineklerde negatif enerji dengesi ve metabolik indikatörleri. *Kafkas Univ Vet Fak Derg*, 18, 705-711, 2012.
28. Kaçar C, Pancarci ŞM, Karapehlivan M, Kaya S, Kuru M, Çitil M, Gürbulak K: Peripartum dönmende ineklerde subkutan L-karnitin uygulamalarının enerji metabolizmasının bazı biyokimyasal parametrelerine etkisi. *Harran Univ Vet Fak Derg*, 2, 67-74, 2013.
29. Vannucchi CI, Rodrigues JA, Silva LC, Lúcio CF, Veiga GA, Furtado PV, Oliveira CA, Nichi M: Association between parturition conditions and glucose and cortisol profiles of periparturient dairy cows and neonatal calves. *Vet Rec*, 4, 176, 358, 2015. DOI: 10.1136/vr.102862

30. Leon JB, Smith BB, Timm KI, LeCren G: Endocrine changes during pregnancy, parturition and the early post-partum period in the llama (*Lama glama*). *J Reprod Fertil*, 88, 503-511, 1990. DOI: 10.1530/jrf.0.0880503

31. Forslund KB, Ljungvall OA, Jones BV: Low cortisol levels in blood from dairy cows with ketosis: A field study. *Acta Vet Scand*, 20, 52, 31, 2010. DOI: 10.1186/1751-0147-52-31

32. Esposito G, Irons PC, Webb EC, Chapwanya A: Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. *Anim Reprod Sci*, 144, 60-71, 2014. DOI: 10.1016/j.anireprosci.2013.11.007

33. Civelek T, Birdane F, Kabu M, Cingi CC, Acar A: Effects of methionine and lysine on metabolic profile in dairy cattle during periparturient period. *Kafkas Univ Vet Fak Derg* 19, 423-432, 2013. DOI: 10.9775/kvfd.2012.7968

34. McNamara JP, Hillers JK: Regulation of bovine adipose tissue metabolism during lactation. 2. Lipolysis response to milk production and energy intake. *J Dairy Sci.*, 69, 3042-3050, 1986. DOI: 10.3168/jds.S0022-0302(86)80767-6

35. Seifi HA, Gorji-Dooz M, Mohri M, Dalir-Naghadeh B, Farzaneh N: Variations of energy-related biochemical metabolites during transition period in dairy cows. *Comp Clin Pathol*, 16, 253-258, 2007. DOI: 10.1007/s00580-007-0682-2