

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

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Abstract

The purpose of this study is to investigate the effects of sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 5\text{H}_2\text{O}$) 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 dönemden başlanarak sütçü sığırların rasyonlarına sodyum borat ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 5\text{H}_2\text{O}$) ilavesinin serum kortizol, glukoz, β -hidroksibütirat (BHB), esterleşmemiş yağ asitleri (NEFA), trigliserid (TG), kan üre nitrojen (BUN), aspartat aminotransferaz (AST), alanin aminotransferaz (ALT) düzeylerine etkisinin araştırılmasıdır. Klinik olarak sağlıklı 80 adet gebe inek rastgele iki gruba ayrıldı. Borax grubuna (n=40) doğumdan 30 gün önce başlanarak postpartum 21. güne kadar rasyonlarına ek olarak sodyum borat (30 g/gün) eklendi. Hem çalışma hem de kontrol grubundan (n=40) doğum öncesi 21, 14 ve 7. gün, doğumda ve doğum sonrası 7, 14 ve 21. günlerde kan örnekleri alındı. Analizler sonrasında borax grubu serum kortizol düzeyinin kontrol grubuna göre daha düşme ($P<0.05$), doğum öncesi, doğum ve doğum sonrasında serum BHB, NEFA ve TG düzeyinde azalma ($P<0.05$), serum BUN konsantrasyonunda doğum öncesi ve doğum sonrası artış ($P<0.05$), AST konsantrasyonunun doğum öncesi 21 ve 14. günleri hariç diğer günlerde daha yüksek ($P>0.05$) olduğu ve ALT düzeyinin etkilenmediği ($P>0.05$) belirlendi. Sonuç olarak, yüksek süt verimli ırklarda özellikle geçiş döneminde rasyona sodyum borat ilavesinin negatif enerji dengesizliklerinden korunmada bir seçenek olabileceği belirlendi.

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



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on mineral metabolism, the endocrine system and immune response is also recognized [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 [3].

Basoglu et al.^[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 [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 [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 [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 [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 [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 ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 5\text{H}_2\text{O}$, 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®, Tıpkinsan, 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 serums 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, ALT and AST. 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. Ingredient and nutrient composition of prepartum and postpartum diets

Tablo 1. Prepartum ve postpartum diyetlerin 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 \pm 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 were 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 [12,33].

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**Tablo 2.** Sodyum borat eklenen rasyonla beslenen geçiş dönemi süt ineklerinde serum kortizol, glukoz, BHB, NEFA, TG, BUN, ALT ve AST düzeylerinin değişimi

Parameters	Groups	Days							P value
		-21	-14	-7	Parturition	+7	+14	+21	
Cortisol (nmol/L)	C	9.8±0.75 ^b	12.3±0.21 ^b	18.8±0.54 ^c	42.8±11.6 ^d	4.18±0.34 ^a	2.68±0.06 ^a	2.45±0.14 ^a	**
	B	8.65±0.6 ^b	11.9±0.34 ^b	17.6±0.07 ^c	35.9±7.5 ^d	3.96±0.23 ^a	2.56±0.37 ^a	2.44±0.06 ^a	**
	P value	NS	NS	NS	*	NS	NS	NS	
Glucose (mg/dL)	C	58.2±1.2 ^c	56.4±0.9 ^{abc}	53.7±1.3 ^{abc}	51.2±0.9 ^a	52.7±0.6 ^{ab}	56.4±0.8 ^{abc}	56.9±1.1 ^{bc}	**
	B	62.7±1.2 ^d	59.5±1.1 ^{bcd}	56.7±0.7 ^{ab}	54.8±1.1 ^a	57.9±1.3 ^{abc}	61.6±0.9 ^{cd}	63.8±1.1 ^d	**
	P value	*	*	*	*	*	*	*	
BHB (mmol/L)	C	0.52±0.21 ^a	0.58±0.18 ^b	0.67±0.25 ^c	0.84±0.32 ^e	0.75±0.27 ^d	0.72±0.12 ^d	0.66±0.18 ^c	**
	B	0.47±0.24 ^a	0.53±0.31 ^b	0.61±0.15 ^c	0.76±0.09 ^e	0.69±0.14 ^d	0.68±0.12 ^d	0.60±0.08 ^c	**
	P value	*	*	*	*	*	*	*	
NEFA (mmol/L)	C	0.27±0.05 ^a	0.33±0.04 ^a	0.45±0.12 ^b	0.82±0.23 ^e	0.61±0.15 ^d	0.58±0.28 ^{cd}	0.52±0.14 ^c	**
	B	0.21±0.18 ^a	0.28±0.09 ^b	0.36±0.28 ^c	0.76±0.18 ^f	0.55±0.19 ^e	0.54±0.16 ^e	0.46±0.31 ^d	**
	P value	*	*	*	*	*	*	*	
TG (mg/dL)	C	19.4±2.1 ^a	21.8±3.4 ^{ab}	22.2±1.9 ^{ab}	26.8±1.5 ^c	25.8±1.7 ^{bc}	23.9±2.8 ^{abc}	19.8±2.7 ^a	**
	B	18.2±2.3	19.2±1.8	20.2±2.3	21.6±1.9	20.11±1.6	19.7±1.8	17.6±0.8	NS
	P value	NS	NS	NS	*	*	*	NS	
BUN (mg/L)	C	98.7±14.7 ^a	105.6±21.3 ^{bc}	118.3±15.6 ^d	153.9±21.6 ^e	121.6±12.4 ^d	109.8±19.7 ^c	102.8±10.5 ^{ab}	**
	B	109.6±21.2 ^a	118.7±13.6 ^c	125.8±12.2 ^d	160.4±21.5 ^f	136.8±17.5 ^e	123.9±20.7 ^d	114.6±18.9 ^b	**
	P value	*	*	NS	NS	*	*	*	
ALT (U/L)	C	28.6±3.2 ^c	24.7±4.26 ^{abc}	22.8±8.7 ^{ab}	20.9±3.9 ^a	22.5±5.4 ^a	23.7±6.2 ^{ab}	26.8±3.6 ^{bc}	**
	B	27.6±4.6 ^b	23.9±5.2 ^{ab}	21.8±3.7 ^a	19.8±6.4 ^a	21.6±2.8 ^a	22.7±2.7 ^a	23.8±4.7 ^{ab}	**
	P value	NS	NS	NS	NS	NS	NS	*	
AST (U/L)	C	71.3±5.6 ^a	75.9±11.6 ^b	78.4±12.4 ^{bc}	80.6±5.6 ^c	98.4±8.7 ^e	89.6±6.8 ^d	80.7±3.6 ^c	**
	B	69.5±7.4 ^a	78.6±9.4 ^b	84.6±11.8 ^c	93.8±15.6 ^d	102.6±13.8 ^e	95.3±8.6 ^d	86.7±7.2 ^c	**
	P value	NS	NS	*	*	*	*	*	

^{a,b,c,d,e,f} 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

In this study, BHB concentrations changed in both groups during the peripartum period. It was particularly remarkable that BHB levels increased in both group 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

during calving ^[12,33]. One study demonstrated that BUN values reach their peak on the 21st day postpartum ^[35]. 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 ^[33]. There are reports of a significant correlation between AST activity and the concentration of glucose, NEFA and BHB ^[35]. 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.

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