Original study

Affects of boron administration on serum Ca, Mg and P ofperipartum Cows

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Abstract

The aim of this study is to evaluate the effects of sodium borate on the concentrations of serum calcium (Ca), magnesium (Mg) and phosphorus (P) in dairy cattle in the peripartum period. In the study, 14 healthy Holstein cows in the periparturient period (four weeks before and three weeks after calving) were divided into two equal groups according to oral treatments with sodium borate (30 g/day, group B), while some cows of the group were not treated (group C). Blood samples were obtained weekly from the prepartum four weeks until postpartum three weeks. At calving, changes were observed for the concentrations of the serum Ca, Mg and P in groups B and C. Calcium (P>0.05) and Mg (P<0.001) concentrations were higher in group B than in group C at calving. During the postpartum period serum Ca and Mg concentrations increased P<0.05) in group B compared to group C. Serum P concentrations were not affected by boron. The results suggest that sodium borate may be useful for sustaining metabolic balance and perhaps for preventing metabolic disorders such as milk fever and hypomagnesemia in dairy cattle during the periparturient period.

Keywords: boron, calcium, magnesium transition period, dairy cattle

Abbreviations: BCS: body condition score, Ca: calcium, Mg: magnesium, P: phosphorus, PTH: parathyroid hormone

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Introduction

Recent studies about the biological significance of boron for various metabolic, nutritional, hormonal and physiological processes indicated that it is essential for plants (Blevins & Lukaszewski 1998), humans and animals (Nielsen 1997, Hunt 1998, Kabu *et al.* 2008, Hunt 2012, Kabu & Civelek 2012 and Kabu & Akosman 2013). Some studies suggest beneficial effects of boron also for humans. Boron appears to be required for bone and joint functions, possibly via effects on the balance and absorption of calcium (Ca), magnesium (Mg) and phosphorus (P) (Kabu & Akosman 2013). Unfortunately, the detailed mechanism by which boron functions in animals has not yet been fully determined.

Kabu & Civelek (2012) studied the effects of sodium borate orally administrated to 12 pregnant cattle at 30 g/day over a 28-day period that included two-week prepartum and two-week postpartum exposures. In this study, the effects of sodium borate on selected hormone levels and serum metabolites were investigated in both treated and control animals. Blood samples were obtained weekly. There were no differences recorded in blood for the concentration of total protein (TP), albumin (ALB), blood urea nitrogen (BUN), alanine aminotransferase (ALT), total bilirubin (TBil), aspartate aminotransferase (AST) and gammaglutamyltransferase (GGT) compared to the control. Glucose levels were higher during the prepartum period and the postpartum glucagon and β -hydroxybutyric acid (BHBA) serum levels were higher in the control group. At the end of sodium borate administration, concentrations of total cholesterol (TChol), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), glucose, insulin and nonesterified fatty acids (NEFA) in blood were decreased. In summary, administering sodium borate may improve the metabolic situation during the periparturient period (Kabu & Civelek 2012). Another study was performed on 36 Angus and Angus-Simmental cattle that were divided into three groups. A control group received no supplementary B in their diet, a second group was fed a diet containing a 5 mg/kg supplement of B and the third group was fed a 15 mg/kg supplement of B for 47 days to determine the effect on disease resistance to bovine herpesvirus type-1 (BHV-1). The cattle were inoculated with BHV-1 intranasally on the 34th day. On the second day following inoculation rectal temperatures of the cattle and plasma tumor necrosis factor- α concentrations had increased (P<0.05). On the fourth day after inoculation, the plasma acute phase proteins had multiplied (P<0.01) and plasma interferon-y levels were beginning to decline (P < 0.05). The plasma B concentrations had increased slightly after the addition of B (P<0.001), whereas the dietary levels of B fed showed no significant effect on BHV-1 symptoms and had a little influence on plasma acute phase proteins and cytokines (Fry et al. 2010).

The periparturient period of four weeks before and four weeks after calving is characterised by an increased risk of disease (Shanks *et al.* 1981, Curtis *et al.* 1985, Stevenson & Lean 1998), including hypocalcaemia, hypomagnesaemia, ketosis, the downer cow syndrome, abomasal displacement, metritis and poor fertility. During this period, there are many important changes in cows' mineral metabolism (Goff 2008). Important diseases such as hypocalcemia and hypomagnesemia may be related to mineral deficiency during this time period (Horst *et al.* 1994, Larsen *et al.* 2001). The physiological factors that cause hypocalcemia and strategies to prevent hypocalcemia are discussed, focusing on tissue sensitivity to the parathyroid hormone (PTH). Another major risk factor for hypocalcemia is hypomagnesemia, which is observed when animals are fed inadequate amounts of Mg or some factors are present in the diet that prevent adequate absorption of Mg (Reinhardt *et al.* 1988, Lean *et al.* 2006, DeGaris & Lean 2009). Moderate hypomagnesemia impairs a cow's ability to maintain Ca homeostasis and hypocalcemia occurs (Goff 2008). Phosphorus contents during the periparturient period may play an important role in the incidence of hypocalcemia (Leclerc & Block 1989, Goff *et al.* 1991, Goff 2008, DeGaris & Lean 2009).

Although there are many studies on the effects of boron on Ca, Mg and P metabolism on poultry, rats or rabbits, there are no studies about dairy cows. This study evaluated the effects of sodium borate administration on Ca, Mg and P metabolism of dairy cows in the periparturient period.

Material and methods

Animals and experimental design

Fourteen healthy and pregnant Holstein dairy cows, 3-5 years old and multiparous were used. Two groups of seven cows were formed with cattle similar to each other in terms of milk productivity ($26.5 \pm 3.5 L$ /day), body weight ($670.0 \pm 41.3 kg$), and body condition scores (BCS: 3.09 ± 0.4) according to treatment during the periparturient period (prepartum 4 weeks and postpartum 3 weeks): The group B was orally administrated 30 g/day of sodium borate (Na₂B₄0₇5H₂O, Eti Mine Works, Kirka, Turkey) while cows not receiving sodium borate (group C) served as controls.

The same rations (Table 1) were given to both groups. Food substances were sampled by the appropriate methods. Laboratory analyses of the samples were carried out using the method described by the Association of Official Analytical Chemistry (AOAC 1990). Crude protein, dry matter, acid detergent fibre (ADF) and neutral detergent fibre (NDF) analyses were done using the method of Georing & Van Soest (1970). Metabolic energy and net energy levels were calculated (NRC 2001).

Blood collection and mineral analyses

Before and after treatments, blood samples were drawn weekly from the jugular vein into serum dry biochemistry tubes. Blood samples were centrifuged at 3000 g for 10 min. Serums were carefully harvested and kept at -20 °C until analysis. Measurements of serum Mg (Cat. No.: 05401712), P (Cat. No.: 05401780) and Ca (Cat. No.: 04718933) were performed in an autoanalyser (Roche Cobas C111, Roche Deutschland Holding GmbH, Grenzach-Wyhlen, Germany) using commercial kits (Roche Deutschland Holding GmbH, Germany).

Statistical analysis

After applying ANOVA using SPSS software for Windows v. 16.0 (SPSS Inc., Chicago, IL, USA) the statistical difference was determined with an inside group Tukey test. The significance of the difference between the groups was determined via Mann Whitney U tests. Differences were considered as significant when *P*-values were less than 0.05.

	Prepartum	Postpartum
Ingredient, % 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
Yeast⁵	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

Table 1 Ingredient and nutrient composition of prepartum and postpartum diets

¹Megalac (Church & Dwight Co. Inc., Princeton, NJ, USA), ²Soy Pass (Borregaard LignoTech, Sarpsborg, Norway), ³Rovimix 302-FM/20 providing by kg 15.000.000 IU vitamin A, 3.000.000 IU vitamin D₃, 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, ⁴NaHCO₃ (99%, Şişecam Chemicals Group, Instanbul, Turkey), ⁵Yeast (Beta Agriculture, Yüreğir/Adana, Turkey), DM: dry matter, CP: crude protein, NEL: net energy lactation, NDF: neutral detergent fibre, ADF: acid detergen fiber

0.46

0.27

0.72

0.42

Results and discussion

Ca, % DM

P. % DM

There were no significant changes in serum Ca concentrations in the prepartum (-4, -3 and -2 week) between C and B groups (Table 2). However, there was an increase (P=0.001) in serum Ca for the B group over the control in prepartum week 1 ($2.27 \pm 0.11 \text{ mmol/L}$ vs. $1.92 \pm 0.06 \text{ mmol/L}$). Calcium concentrations during calving were lower in both groups (C: $1.51 \pm 0.89 \text{ mmol/L}$ vs. B: $1.84 \pm 0.37 \text{ mmol/L}$). In Group B, while Ca concentrations in postpartum week 1 ($2.22 \pm 0.21 \text{ mmol/L}$) reached their initial values (prepartum week 4 $2.14 \pm 0.80 \text{ mmol/L}$), this level was observed in week 3 in the control group. It was observed that the serum Ca concentration was higher (P<0.01) in prepartum week 1 and postpartum week 1 (P<0.01), 2 (P=0.001) and 3 (P<0.05) than in the control group. While the Ca serum amounts increased quickly in the postpartum B group, it lasted longer in the control group (Table 2).

Serum miner	el parameters (Ca,Mg and P) in (dairy cows durin	ig periparturient	: period (4 week	s in prepartum a	and 3 weeks in p	ostpartum) acco	ording to oral tre	atments
Parameters	Group (n=7)	-4 Weeks	–3 Weeks	–2 Weeks	-1 Weeks	Calving	+1 Weeks	+2 Weeks	+3 Weeks	Ρ
Ca, mmol/L	Control	2.05 ± 0.12^{abcde}	$1.95 \pm 0,06^{abcde}$	2.00 ± 0.09^{abcde}	1.92 ± 0.06^{bcdeB}	1.51 ± 0.89^{f}	$1.73\pm0.16^{\mathrm{efb}}$	1.90 ± 0.22^{cdeB}	$2.07 \pm 0.34^{\text{abcdB}}$	P=0.0001
	Borax P	2.14 ± 0.80 ^{abcd} 0.091	$\begin{array}{c} 2.11 \pm 0.25^{abcd} \\ 0.072 \end{array}$	2.08 ± 0.12^{abcd} 0.133	2.27±0.11 ^{aA} 0.000	1.84±0.37 ^{cde} 0.230	2.22 ± 0.21^{abcA} 0.000	$2.28 \pm 0,12^{aA}$ 0.001	2.25 ± 0.12^{abA} 0.040	
Mg, mmol/L	Control	0.87 ± 0.09^{abcd}	0.81 ± 0.28^{bcd}	$0.95 \pm 0,07^{\rm abc}$	$0.96\pm0,08^{\rm abc}$	0.79 ± 0.12^{bcdB}	0.56 ± 0.09^{eB}	0.67 ± 0.11^{deB}	0.67 ± 0.10^{deB}	P=0.0001
	Borax P	0.79 ±0.11 ^{bcd} 0.299	$\begin{array}{c} 0.75\pm0.17^{cde}\\ 0.232\end{array}$	0.98 ± 0.05^{ab} 0.315	0.94±0.08 ^{abc} 0.699	1.07±0.12ª ^A 0.000	$\begin{array}{c} 0.90 \pm 0.05^{bcdA} \\ 0.001 \end{array}$	$\begin{array}{c} 0.95\pm0.06^{abcA}\\ 0.000\end{array}$	0.96 ± 0.07^{abcA} 0.001	
P, mmol/L	Control	1.75 ± 0.15^{ab}	1.77 ± 0.32^{abA}	1.77 ± 0.31^{ab}	1.81 ± 0.23^{ab}	$1.38\pm0.34^{\rm bc}$	1.57 ± 0.47^{abc}	1.47 ± 0.29^{abcB}	$1.86\pm0.28^{\rm ab}$	P=0.0001
	Borax P	1.59 ± 0.13^{abc} 0.055	1.45 ± 0.23^{abcB} 0.032	1.67 ± 0.21^{ab} 0.740	1.79 ± 0.10^{ab} 0.962	$1.12 \pm 0.38^{\circ}$ 0.133	1.74±0.47 ^{abc} 0.382	1.91 ± 0.27^{abA} 0.012	1.97 ± 0.17^{a} 0.755	
Down codium	20 20 20 2 12	In Decider and and	succed as moone	- ctondard douit	tions abDifforce	t curouccuinto in	the came with	dicato cicabilicant	difference (D < O	OF or more

Table 2

Borax: sodium borate, 30 g/day, Results are expressed as means \pm standard deviations, ^{ab}Different superscripts in the same row indicate significant differences (P<0.05 or more) according to time during the periparturient period for a given group, ^{AB}Different supercripts in the same column indicate significant difference (P<0.05 or more) according to L treatments for a given time. Serum Mg increased in prepartum week 2 in group B and it was higher than 0.90 mmol/L until postpartum week 3 (Table 2). In the control group, the serum Mg concentration was at its highest of periparturient period in prepartum week 1 ($0.96 \pm 0.08 \text{ mmol/L}$). It decreased statistically (P<0.001) at calving ($0.79 \pm 0.12 \text{ mmol/L}$) and tended to be at its lowest level in postpartum week 1 ($0.56 \pm 0.09 \text{ mmol/L}$). While Mg increased in the control group in postpartum week 2 ($0.67 \pm 0.11 \text{ mmol/L}$), it could not reach prepartum levels. In group B, the serum Mg concentration was at a higher level (P<0.001) starting from calving until postpartum week 3 (Table 2).

The serum P concentration was at the lowest level during calving in the control $(1.38 \pm 0.34 \text{ mmol/L})$ and boron $(1.12 \pm 0.38 \text{ mmol/L})$ groups (Table 2). After calving in group B, while there was a steady increase in serum P concentration, the serum P fluctuated in the control group. In both groups, in postpartum week 3 (C: $1.86 \pm 0.28 \text{ mmol/L}$ vs. B: $1.97 \pm 0.17 \text{ mmol/L})$ was at its highest level during the periparturient period. While serum P in group B prepartum week 3 was lower (*P*<0.05) in the control group, it was higher in postpartum week 2 (Table 2).

Boron had positive effects on Ca, Mg and P in the present study. The lowest level of blood calcium concentration was determined in the periparturient period in both groups (Table 2). Serum Ca levels usually remained below 2 mmol/L throughout the study in the group C, while they maintained above 2 mmol/L in the group B except at calving (B: 1.84 ± 0.37 mmol/L) (Table 2). Subclinical hypocalcemia is when serum Ca values are between 1.38-2 and mmol/L (8 and 5.5 mg/dL) during the periparturient period and it occurs in approximately 50% of the cows (Horst *et al.* 2003). These results indicate that subclinical hypocalcemia developed in cows of group C, while boron admistration protected cows from this disease in group B.

Goff (2008) suggests that the nadir in serum Ca concentration occurs between 12 and 24 h after calving and blood samples obtained around this time can reveal the extent of hypocalcemia in dairy cattle. Nearly 25 percent of cows during calving will have a serum Ca concentration <2 mmol/L (8 mg/dL) (Goff 2008). In the present experiment, the serum Ca level (P<0.05) in the boron group was 182 times higher than that of the control group in the postpartum period. However, contrary to these results a study on barrows indicates that supplemental 5 and 10 mg sodium borate decahydrate decreased serum Ca level (Armstrong & Spears 2001).

Magnesium also plays an important role in Ca metabolism of dairy cattle in the periparturient period (DeGaris & Lean 2009). Hypomagnesemia affects Ca metabolism in two ways: first by reducing parathyroid hormone (PTH) secretion in response to hypocalcemia (Littledike *et al.* 1983) and second by reducing tissue sensitivity to PTH (Rude 1998). Goff (2008) states that the cow plasma Mg concentration is usually between 0.75 and 1.0 mmol/L (1.8 and 2.4 mg/dL) and cows with a blood Mg between 0.5 and 0.8 mmol/L (1.15 and 1.8 mg/dL) have few obvious clinical symptoms, although they often are slow to eat and do not produce milk up to their potential. The data show that there was no difference in the serum Mg levels in the boron group were higher (P<0.002) than those of the control group during calving and the postpartum period (Table 2). Moreover serum Mg levels of the cows in the boron group were normal (0.75 and 1.0 mmol/L) in the periparturient period but there was a decrease (P<0.0001) in the control group and continued at hypomagnesemia levels

(0.5 and 0.8 mmol/L) in the postpartum period. Due to the effects of Mg on PTH, the present study observed that the decrease (P<0.001) in the serum Mg level starting during calving and going through the postpartum period in the control group might be the reason for the decrease in the blood Ca level. The increase (P<0.001) in the blood Mg level in the boron administration group might be the reason for the increase in the blood Ca level. Boron may have a direct effect on Mg and this may positively affect Ca and PTH. Some studies reported that boron positively effects blood Mg level (Kurtoğlu *et al.* 2002, Eren *et al.* 2004) in non-ruminants, whereas some of other studies reported it has no effect (Basoglu *et al.* 2010).

It is claimed that P may also play an important role in the pathogenesis of hypocalcemia, with increasing P concentrations the hypocalcemia risk increases (Goff 1999). The normal concentration of serum P is between 1.4 and 2.5 mmol/L (4.3 and 7.8 mg/dL) in cows. In our study, the serum P concentration was at its normal values except during calving in boron and control groups (Table 2). We observed that serum P concentration was higher (P<0.05) in postpartum week 2 in group B than in group C. The data of the study on chickens show that boron supplementation to diets increased the serum P concentration (Kurtoğlu *et al.* 2001, Bozkurt *et al.* 2012), whereas another study on rabbits indicates that boron administration did not change the P concentration (Basoglu *et al.* 2010).

Previously, it was mentioned that boron supplementation affected the metabolism of Ca, Mg and P in human and non-ruminant species (Nielsen *et al.* 1987, Hegsted *et al.* 1991, Hunt *et al.* 1997, Kurtoğlu *et al.* 2001, Bozkurt 2012). However, the experimental subjects were also under nutritional stress (Ca, Mg, P or vitamin D deficiency). In our study, although there were sufficient macrominerals in this diet, serum Ca, Mg and P levels were lower (*P*<0.05) in the control group than in the boron group in the postpartum period.

In conclusion, our study found out that boron administration (30 g/d) positively effects (P<0.0002) the Ca and Mg metabolism of ruminants in the periparturient period. Although the diets of both groups were minerally balanced, serum Ca and Mg concentrations were relatively higher (P<0.002) in the boron group than in the control group in the postpartum period. However, the mechanism of boron contribution to the Ca and Mg concentrations in serum is not clear. Boron might play an important role for sustaining the metabolic balance of Ca, Mg, P and for preventing metabolic disorders such as hypocalcemia and hypomagnesemia in dairy cattles in the periparturient period.

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