

Short Term Effects of Dietary Boron on Mineral Status in Dairy Cows*

Nuri Baspinar¹, Abdullah Basoglu², Ahmet Semacan³, Erdem Gulersoy²

¹Department of Biochemistry, Faculty of Veterinary Medicine, Selcuk University, Turkey

²Department of Internal Medicine, Faculty of Veterinary Medicine, Selcuk University, Turkey

³Department of Gynecology, Faculty of Veterinary Medicine, Selcuk University, Turkey

Abstract— Aim of this study was to obtain knowledge on boron supplemented diet to mineral status of body fluids and feces in short term nutrition of dairy cattle. A total of 24 healthy Holstein dairy cows were used. The animals were fed with standard ration, and boron at three different doses was added to experimental' diets as boron compound: borax, for 10 days. Boron and other macro and trace minerals were determined in serum, milk, urine and feces samples taken on 0 and 11st days. In this study, there were no overt signs of toxicosis, and a pivotal knowledge was obtained in dairy cattle fed with boron supplemented diet on boron absorption, excretion, and its interaction with other minerals. Boron could not completely absorb from gastrointestinal tract. Urine was the most important excretion way of boron. More less boron was also eliminated by milk. Boron levels in body fluids (serum and milk, $p < 0.000$) were increasingly changed based on the dose. Boron, among minerals, provided a striking increase for Ca ($p < 0.003$) and Mg ($p < 0.028$) levels in serum by increasing absorption of these minerals. This topic is worth evaluating as an alternative approach in the prevention of hypocalcemia in transition cows.

Keywords— Boron, Macro minerals, Micro minerals, Cattle.

I. INTRODUCTION

Today, most nutritionists do not consider a trace element essential unless it has a defined biochemical function in higher animals or humans (Nielsen, 2014a). Trace minerals have critical roles in the key interrelated systems of immune function, oxidative metabolism, and energy metabolism in ruminants. To date, the primary trace elements of interest in diets for dairy cattle have included Zn, Cu, Mn, and Se although data also support potentially important roles of Cr, Co, and Fe in diets (Overton and Yasui, 2014). In vitro, animal, and human experiments have shown that boron is a bioactive element in nutritional amounts whereas boron has received little attention in ruminant nutrition (Nielsen, 2014b; Fry *et al.*, 2011). Boron appears to be of relatively low toxicity to animals. As in previous studies (Owen, 1944; Green and Weeth, 1977), inorganic boron is of low toxicity to cattle. There are many studies on energy, mineral and vitamin supplementation in transition cows in order to prevent production diseases. The objective of the study presented was to determine how dietary boron at different doses affects the distribution of macro and trace minerals in body fluids of dairy cattle.

II. MATERIAL AND METHOD

The experimental design was approved by the Committee on Use of Animals in Research of the Selcuk University, Faculty of Veterinary Medicine. A total of 24 pregnant, multiparous Holstein cows in late lactation were enrolled; mean age was 3.5 years, mean 305-day milk production was 6,500 kg, and mean body weight was 650 kg at the start of the experiment. Cows were fed individually and had free access to tap water.

The cows were randomly allocated to 4 groups: 1 control and 3 experimental, each 6 animals which were offered drinking water (0.032 mg B/L) and a basal diet (0.55 mg B/kg). Ingredient and daily consumption ratios were corn silage (12 kg), sugar beet pulp (10 kg), wheat straw (4.5 kg) and concentrate (8.5 kg). The concentrate consisted of 35% barley, 19.85% wheat, 15%, wheat bran, 25% cotton seed meal, 3% limestone, 0.3% salt, and 0.35% vitamin-mineral mixture. It contained 21.5% crude protein and 2,850 kcal/kg metabolizable energy. Three experimental groups received 5g, 10g and 15g of borax (567, 1134 and 1701 mg of boron), respectively as an oral bolus. The bolus administration schedule was chosen to fit within the normal management activities of large dairies, and it required cows to be restrained in headlocks only once daily.

Blood, urine, milk and feces samples were collected in just before and after the 10-day period. Blood samples were taken from the coccygeal vein. Serum was harvested within an hour by centrifugation for 15 minutes at 3,000 rpm. All the samples were stored at -20°C till analysis. They were analyzed for macro and micro minerals (B, Ca, P, Mg, Na, K, Mo, Mn, Cr, Cu, Fe, Ni, S, Zn) by ICP-AES (VARIAN VISTA AX CCD) using Reference Material 8414 (National Institute of Standards and Technology) on day 0 and day 11th. Only boron levels were measured on three consecutive days after dietary boron exposure.

All laboratory data were presented as the mean \pm SD. Statistical significance between groups' first and last samples was determined with Paired *t* test. One Way Anova Posthoc Tukey was applied for statistical significance between groups' last samples' values. Values of $P < 0.05$ were considered significant. SPSS 21.0 program was used for statistical analyses.

III. RESULTS

All animals appeared healthy during the boron-diet treatment period. Feed and water consumption were unaffected by treatments. There were no overt signs of toxicosis. Increased boron ingestion increased serum, urine, milk and feces boron concentrations (Table 2). The percentage of boron was increased with the 60, 120 and 180 mg of B/kg of diet treatments.

Apart from boron Ca, P, Mg, Na, K, Mo, Mn, Cr, Cu, Fe, Ni, S, Zn concentrations were also measured in all the samples. Compared to the samples in the beginning of the boron exposure, some changes in macro and trace minerals' level at the end of the experiment were as following: for Ca ($p < 0.003$) and Mg ($p < 0.028$) concentrations significant increases in serum and significant decreases (Ca: $p < 0.001$, Mg: $p < 0.001$) in feces were observed in association with boron intake. This data indicates that boron increases Ca and Mg absorption. The same thing may be expressed for Na mineral, because Na was increased in serum ($p < 0.007$) and decreased ($p < 0.000$) in feces. It was also decreased in urine. P, K and S concentrations tended to be decreased in serum samples. Ca and K concentrations, and Mg and P concentrations (only in experimental 3) were significantly decreased in milk samples (Tables 3, 4, 5 and 6). Although these changes are outstanding, it seems to be difficult interpreting them properly.

TABLE 1
BASAL DIET ANALYSIS

TSBM^f, %	61,39
SE-1X, Mcal/kg ^f	2,78
ME-3X, Mcal/kg ^f	2,12
NEL-3X, Mcal/kg ^f	1,30
NEL-4X, Mcal/kg ^f	1,22
NEM-3X, Mcal/kg ^f	1,30
NEG-3X, Mcal/kg ^f	0,71
Dry matter, %	60,96
Crude Protein, %	12,82
NDICP, %	1,36
ADICP, %	0,70
Crude fat, %	4,63
NDF, %	37,31
ADF, %	23,42
NFC, %	31,93
Lignin, %	5,31
Crude ash, %	13,30
N fraction A, % HP	17,62
N fraction B, % HP	76,94
N fraction C, % HP	5,44
RUP, CP %, %2, (Bypass Protein) ^f	33,45
RUP, CP%, %4, (Bypass Protein) ^f	37,64
RUP Sind, % ^f	67
^f :calculated by formula	

TABLE 2
BORON LEVELS (mg/L).BETWEEN GROUPS' IN THE BEGINNING AND AT THE END OF THE EXPERIMENT

Body fluids	Experimental 1			Experimental 2			Experimental 3		
	Beginning Mean±SD	End Mean±SD	P	Beginning Mean±SD	End Mean±SD	P	Beginning Mean±SD	End Mean±SD	P
Serum	0,052 ± 0,033	0,114 ± 0,017 ^b	0.018	0,061 ± 0,025	0,136 ± 0,064 ^{ab}	0.068	0,08 ± 0,038	0,23 ± 0,080 ^a	0.008
Milk	0,051 ± 0,011	0,131 ± 0,037	0.001	0,038 ± 0,012	0,114 ± 0,021	0.000	0,003 ± 0,025	0,089 ± 0,042	0.001
Urine	0,689 ± 0,272	2,081 ± 1,543	0.015	0,893 ± 0,347	2,132 ± 1,840	0.032	0,494 ± 0,225	2,721 ± 1,034	0.000
Feces	0,085 ± 0,021	0,144 ± 0,049 ^b	0.014	0,086 ± 0,019	0,155 ± 0,027 ^b	0.000	0,093 ± 0,010	0,208 ± 0,018 ^a	0.000
Percentage rate of boron (%) between groups' at the end of the experiment (mg/L).									
Body fluids	Experimental 1			Experimental 2			Experimental 3		
	Beginnin g Mean	End Mean	Percentage rate %	Beginning Mean	End Mean	Percentage rate %	Beginnin g Mean	End Mean	Percentage rate %
Serum	0,052	0,105	101,9	0,061	0,136 ^b	121,8	0,07	0,214	202,1
Milk	0,051	0,131	161,2	0,038	0,114	184,7	0,003	0,089	206,4
Urine	0,689	2,681	276,7	0,893	3,132	286,0	0,494	2,721	450,6
Feces	0,085	0,144	71,4	0,086	0,155	81,9	0,093	0,208 ^a	118,2

Data are expressed as the mean±SD.

TABLE 3
MINERAL LEVELS (PPM) IN SERUM SAMPLES IN THE BEGINNING AND AT THE END OF THE EXPERIMENT

Minerals	Experimental 1			Experimental 2			Experimental 3		
	Beginning Mean±SD	End Mean±SD	P	Beginning Mean±SD	End Mean±SD	P	Beginning Mean±SD	End Mean±SD	P
Co	0.000±0.000	0.005±0.002	0.008	0.000±0.000	0.005±0.004	0.014	0.000±0.000	0.004±0.003	0.024
Mo	0.001±0.001	0.002±0.001	0.233	0.001±0.001	0.002±0.002	0.683	0.002±0.001	0.002±0.002	0.960
Ca	5.751±1.372	13.810±1.700 ^a	0.013	6.991±2.626	13.525±1.556 ^a	0.003	8.205±3.904	18.859±2.787 ^a	0.003
Cr	0.000±0.000	0.003±0.001 ^a	0.000	0.000±0.000	0.003±0.002 ^a	0.002	0.000±0.000	0.003±0.001 ^a	0.002
Cu	0.033±0.023	0.031±0.011	0.832	0.036±0.016	0.025±0.007	0.164	0.040±0.019	0.042±0.013	0.591
Fe	0.225±0.181	0.116±0.034	0.228	0.147±0.048	0.098±0.044	0.139	0.139±0.011	0.122±0.038	0.264
K	12.91±11.53	12.43±3.18	0.930	19.68±8.55	11.62±4.93 ^a	0.131	22.60±10.48	19.26±6.87	0.412
Mg	1.116±1.168	1.867±.433 ^a	0.245	1.581±.812	1.742±0.557	0.739	1.815±1.099	2.992±1.105 ^b	0.028
Mn	0.002±0.002	0.000±0.000	0.072	0.001±0.000	0.000±0.000	0.000	0.000±0.000	0.000±0.000	0.005
Na	71.43±23.01	109.84±8.29 ^a	0.022	82.34±19.91	107.40±12.06 ^a	0.053	84.79±12.49	124.57±14.70 ^a	0.007
Ni	0.000±0.000	0.005±0.002	0.013	0.000±0.000	0.005±0.003	0.002	0.000±0.000	0.005±0.002	0.006
P	6.998±6.212	6.530±1.708	0.865	8.918±3.684	6.205±2.661	0.249	11.374±6.014	10.978±4.596	0.855
S	55.454±40.016	48.310±13.113	0.697	68.891±24.963	47.461±15.207	0.174	84.572±37.287	73.812±23.209	0.419
Zn	0.042±0.038	0.056±0.021	0.436	0.063±0.037	0.050±0.024	0.488	0.0631±0.034	0.114±0.040	0.115

Data are expressed as the mean±SD

TABLE 4
MINERAL LEVELS (PPM) IN URINE SAMPLES IN THE BEGINNING AND AT THE END OF THE EXPERIMENT.

Minerals	Experimental 1			Experimental 2			Experimental 3		
	Beginning Mean±SD	End Mean±SD	P	Beginning Mean±SD	End Mean±SD	P	Beginning Mean±SD	End Mean±SD	P
Co	0.000±0.000	0.005±0.002	0.001	0.000±0.000	0.007±0.003	0.003	0.000±0.000	0.005±0.003	0.001
Mo	0.002±0.001	0.004±0.004 ^{ab}	0.264	0.001±0.001	0.009±0.006 ^a	0.022	0.002±0.001	0.003±0.002 ^b	0.312
Ca	4.498±5.136	10.294±.903 ^a	0.020	5.210±5.649	12.173±3.021 ^a	0.022	1.716±0.967	10.939±1.988 ^a	0.000
Cr	0.000±0.000	0.003±0.001 ^a	0.000	0.000±0.000	0.003±0.001 ^a	0.000	0.000±0.000	0.002±0.001 ^b	0.002
Cu	0.002±0.005	0.000±0.000	0.291	0.001±0.001	0.000±0.000	0.048	0.002±0.003	0.000±0.000	0.039
Fe	0.025±0.020	0.070±0.046	0.076	0.0321±0.008	0.083±0.055	0.083	0.083±0.130	0.049±0.022	0.413
K	188.38±88.56	155.51±62.50 ^a	0.377	247.51±85.5	184.41±77.89 ^a	0.201	116.12±50.42	106.87±48.52 ^b	0.095
Mg	21.374±10.632	27.069±19.618 ^a	0.494	30.182±13.290	32.042±17.395	0.853	15.630±7.598	23.362±7.327 ^a	0.055
Mn	0.000±0.000	0.000±0.000	0.000	0.001±0.001	0.000±0.000	0.012	0.005±0.007	0.000±0.000	0.090
Na	4.584±5.240	15.56±15.214 ^a	0.105	14.826±12.229	17.190±7.652 ^a	0.167	6.647±6.057	18.680±33.566 ^b	0.324
Ni	0.000±0.000	0.007±0.003	0.001	0.000±0.000	0.004±0.002	0.005	0.000±0.000	0.005±0.002	0.000
P	0.352±0.122	0.336±0.210	0.847	0.481±0.119	0.456±0.264	0.793	0.333±0.134	0.428±0.300	0.453
S	14.399±6.76	25.200±15.074 ^a	0.067	19.045±6.047	36.810±28.097 ^a	0.232	9.296±3.913	24.523±8.859 ^a	0.001
Zn	0.000±0.000	0.007±0.007	0.029	0.007±0.013	0.016±0.011	0.179	0.047±0.084	0.039±0.042	0.796

Data are expressed as the mean±SD.

TABLE 5
MINERAL LEVELS (PPM) IN MILK SAMPLES IN THE BEGINNING AND AT THE END OF THE EXPERIMENT.

Minerals	Experimental 1			Experimental 2			Experimental 3		
	Beginning Mean±SD	End Mean±SD	P	Beginning Mean±SD	End Mean±SD	P	Beginning Mean±SD	End Mean±SD	P
Co	0.000±0.000	0.005±0.002	0.000	0.000±0.000	0.005±0.002	0.000	0.000±0.000	0.006±0.001	0.000
Mo	0.003±0.001	0.002±0.001	0.014	0.002±0.001	0.003±0.001	0.438	0.003±0.001	0.002±0.002	0.512
Ca	27.323±4.443	26.156±8.266	0.739	37.580±14.53	29.804±9.546 ^a	0.229	55.575±16.500	25.502±5.692 ^b	0.001
Cr	0.003±0.001	0.006±0.002 ^a	0.001	0.003±0.001	0.006±0.001 ^a	0.003	0.004±0.002	0.006±0.002	0.011
Cu	0.022±0.003	0.028±0.007	0.084	0.021±0.004	0.031±0.009	0.039	0.025±0.009	0.022±0.003	0.369
Fe	0.195±0.022	0.298±0.066 ^{ab}	0.005	0.220±0.102	0.346±0.081 ^a	0.004	0.224±0.057	0.254±0.059	0.274
K	48.096±14.91	39.034±17.514 ^a	0.309	60.171±18.19	49.086±14.540 ^a	0.266	92.119±27.64	36.795±12.61 ^b	0.001
Mg	2.468±0.562	2.667±1.133	0.673	3.579±1.584	3.462±1.444	0.885	4.960±1.306	2.648±.908 ^a	0.001
Mn	0.002±0.001	0.000±0.000	0.000	0.012±0.020	0.000±0.000	0.140	0.004±0.002	0.001±0.002	0.032
Na	12.955±4.241	12.303±7.286	0.839	16.785±8.310	15.631±5.349	0.764	19.754±3.146	11.166±4.382	0.004
Ni	0.000±0.001	0.007±0.003	0.000	0.003±0.002	0.014±0.014	0.058	0.003±0.002	0.014±0.015	0.157
P	19.110±4.033	18.179±8.396	0.773	24.149±8.525	23.327±10.249	0.872	36.206±9.626	16.834±6.500 ^a	0.001
S	7.427±1.558	7.878±3.208	0.753	9.207±2.959	9.649±2.555	0.751	12.184±2.836	7.437±2.483 ^a	0.004
Zn	0.114±0.015	0.129±0.033	0.336	0.148±0.055	0.146±0.044	0.948	0.198±0.062	0.184±0.137	0.715

Data are expressed as the mean±SD

TABLE 6
MINERAL LEVELS (PPM) IN FECES SAMPLES IN THE BEGINNING AND AT THE END OF THE EXPERIMENT

Minerals	Experimental 1			Experimental 2			Experimental 3		
	Beginning Mean±SD	End Mean±SD	P	Beginning Mean±SD	End Mean±SD	P	Beginning Mean±SD	End Mean±SD	P
Co	0.000±0.000	0.005±0.002 ^a	0.000	0.000±0.000	0.003±0.002 ^b	0.007	0.000±0.000	0.005±0.002 ^{ab}	0.000
Mo	0.005±0.001	0.005±0.001	0.656	0.005±.002	0.006±0.002	0.508	0.006±0.001	0.007±0.003	0.266
Ca	103.35±18.35	54.456±17.27 ^a	0.001	107.18±25.5	48.285±10.092 ^a	0.000	114.74±12.77	71.58±27.65 ^a	0.010
Cr	0.010±0.007	0.012±0.002	0.445	0.008±.0037	0.013±0.003	0.000	0.007±0.002	0.017±0.007	0.008
Cu	0.069±0.010	0.074±0.012	0.465	0.069±.0095	0.069±0.016	0.901	0.077±0.011	0.080±0.022	0.705
Fe	2.156±0.323	2.316±0.689	0.544	2.219±.433	2.143±0.558	0.573	2.349±0.394	3.142±1.008	0.097
K	4.465±2.311	14.155±7.141	0.001	6.584±1.938	12.361±4.925	0.008	7.566±2.101	9.833±4.904	0.243
Mg	46.125±3.837	23.859±7.430 ^{ab}	0.000	46.852±5.839	21.442±5.152 ^b	0.000	48.683±3.249	30.768±4.990 ^a	0.000
Mn	0.233±0.044	0.279±0.102	0.301	0.241±.0536	0.237±0.046	0.845	0.270±0.025	0.385±0.154	0.082
Na	2.454±2.077	4.666±2.919	0.015	7.101±1.436	4.615±3.166	0.069	8.827±2.831	3.544±1.551	0.000
Ni	0.017±0.005	0.017±0.007	0.990	0.016±0.004	0.021±0.008	0.074	0.018±0.001	0.022±0.007	0.123
P	7.430±1.625	8.332±3.834	0.586	8.344±1.892	7.176±2.217	0.058	8.145±1.102	9.133±4.308	0.572
S	5.784±0.614	6.877±1.529	0.115	6.749±1.913	6.410±1.258	0.307	7.313±1.660	7.667±1.905	0.693
Zn	0.223±0.037	0.286±0.096	0.127	0.244±0.056	0.276±0.063	0.079	0.254±0.029	0.356±0.120	0.064

Data are expressed as the mean±SD

IV. DISCUSSION

This is the first study evaluating dietary boron supplementation at different doses on body fluid distribution of boron, and other macro and trace minerals.

The biochemical function of boron is still speculative. Lactating cows fed with approximately 620 mg of B/100 kg of body weight/day without toxic effects. Daily intakes of about 765 mg of B/100 kg of body weight (as borax) have deleterious effects (Owen, 1944; Green and Weeth, 1977). The signs of excessive boron ingestion are rather nonspecific (Brook and Boggs 1951). Exposure to large amounts of boron (about 30 g of boric acid) over short periods of time can affect the stomach, intestines, liver, kidney, and brain and can eventually lead to death in humans. Studies of dogs, rats, and mice indicate that the male reproductive organs, especially the testes, are affected if large amounts of boron are ingested for short or long periods of time. The doses that produced these effects in animals are more than 1,800 times higher than the average daily intake of boron in food by adults in the U.S. population (ARSDR, 2010). One outstanding feature in persons is an erythematous skin rash (Polson and Tattersal, 1969). This rash appears to the redness and edema seen in the legs and around the dew claws of cattle (Green and Weeth, 1977). In this presented study here, adverse effects were not seen with daily

intakes of 450, 900 and 1350 mg of boron in these 10 days of boron exposure. In other similar study (Weeth *et al.*, 1981) where beef heifers were given drinking water to which 0, 15, 30, 60 or 120 mg of B/L of water was added for 10 days period, it was found that plasma and urine boron levels were significant increased. Similarly to this, in the present study boron levels increased based on the doses in body fluids. Greatest increase of boron was in urine samples, and lowest in feces samples. Serum and milk boron levels were similar. This indicated dietary boron has been much more eliminated by urine than milk and feces.

Macro and trace minerals are essential components of a feeding program for dairy herds and (Swecker, 2014) have important roles in immune function and may affect health in transition dairy cows (Speer and Weiss, 2008). Deficiencies of calcium, phosphorus, and magnesium result in improper skeletal formation. Deficiencies in other macro minerals (e.g., sodium, chloride, potassium) and trace minerals (e.g., copper, zinc, manganese, cobalt, iron) cause biochemical dysfunctions that lead to inefficient metabolism and growth (Smith, 2015).

Boron seems to affect the metabolism of P as well as Ca in animal and human models (Hunt, 1988). A significant change in serum Zn concentration was reported by Kurtoglu *et al.* (2005) when chicks were given 5 mg/kg boron. Chromium levels' increases in serum and urine samples, and sulfur levels' increases in only urine samples at the end of the experiment became outstanding in the present study. Studies indicated that Cr supplementation may affect health and immune response in ruminants (Spears, 2000). In our previous study (Basoglu *et al.*, 2010), boron (in solution of 1%) 10, 30 and 50 mg/kg body weight/day, given to rabbits by oral gavage at 96 h interval for 7 months, any changes were not observed in serum Ca, P, Mg, Na, K and Cl levels. Dietary combination with boron and phytase did not create a synergism with regard to growth performance and bioavailability of the minerals (Cinat *et al.*, 2015). Boron affects blood P and Mg in humans; serum P concentrations are lower in boron-supplemented subjects than in subjects receiving placebos, and are lower at the end of the study period than during baseline analysis (Meacham *et al.*, 1994).

Effective transition management requires an integrated approach to nutritional and environmental management to provide cows with freedom from rumen disruption, mineral deficiencies, immunosuppression, disorders of lipid metabolism, and other forms of stress (eg, toxic feeds, social disruption) (Sundram, 2015). Clinical milk fever is one of the most recognized diseases of dairy cattle (Oetzel, 2013). Hypocalcaemia around calving is a risk factor for many of these diseases and is an indirect risk factor for increased culling (Goff, 2014). The incidence of clinical hypocalcaemia (milk fever) in the field generally ranges from 0–10%, but may exceed 25% of cows calving (DeGaris and Lean, 2008). In the present study, boron could not be completely absorbed, a part of dietary boron was eliminated by feces. While Ca and Mg levels decreased in feces, their levels in serum increased, associated with boron intake.

V. CONCLUSION

Boron increases Ca and Mg absorption. This topic is worth evaluating as an alternative approach in the prevention of hypocalcemia in transition cows.

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