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The effects of borax on milk yield and selected metabolic parameters in Austrian Simmental (Fleckvieh) cows

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ABSTRACT: This study was conducted to determine the effects of orally administered borax on milk yield and on several blood variables related to metabolism in early lactation in Austrian Simmental cows (Fleckvieh). Twenty primiparous cows were selected at parturition and then assigned to one of two groups, the control group or the borax group. The study lasted for four weeks. Borax was administered orally at 0.2 mg/kg/day (Boron group) to all treatment cows shortly after the noon milking, whereas cows in the control group were not treated. All cows consumed the same diet. All feeds in the diet were analysed for crude cellulose, protein, ether extract, ash, and dry matter according to the Weende Analysis Systems, in addition to ADF and NDF, according to Van Soest. Blood samples were collected from all cows via the *vena jugularis* on lactation Days 0, 7, 15, 21 and 28 and analysed for the following: serum boron (B), non-esterified fatty acids (NEFA), beta-hydroxybutyric acid (BHBA), total cholesterol (TChol), high density lipids (HDL), total protein (TP), blood urea nitrogen (BUN), albumin (ALB), creatine (CRE), uric acid (UA), glucose (GLU), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) concentrations. Serum B concentration was higher in the borax group than in the control group at Weeks 1, 2, 3, and 4 of the experiment. Serum B concentration did not change in the control group during these weeks, but it gradually increased in the borax group week by week ($P < 0.05$). Borax administration increased serum TP and decreased the serum UA concentration at Week 4, and decreased serum HDL concentration at Week 3 of the experiment. Serum TChol, BHBA, and BUN concentrations increased ($P < 0.05$), while NEFA decreased ($P < 0.05$) after parturition in both groups. The BHBA concentration gradually increased in the control group, but it began to decrease in the borax group during the final week of the experiment. Moreover, milk yield did not differ between the groups for 14 weeks. The results indicate that borax administration did not have any negative effects on the health of Austrian Simmental (Fleckvieh) cows during early lactation. However, studies of longer duration are needed to reveal the effectiveness of borax administration with respect to early lactation in cows.

Keywords: boron; Austrian Simmental (Fleckvieh); lipid profile; milk yield

Boron (B) is primarily a natural product and is generally found in the environment as borates (Howe 1998). Borax, also known as sodium borate, sodium tetraborate, or disodium tetraborate, is an important boron compound, a mineral, and a salt of boric acid. Borax is a commonly found compound of boron, which has been employed in this present study. Borates are boron-oxygen compounds that result from the binding of boron with oxygen (Kabu and Akosman 2013). When administered to animals, borates are biotransformed into boric acids and absorbed from mucosal surfaces. More than 90% of the borate administered to humans or animals is excreted as boric acid (Devirian and Volpe 2003).

Recent studies on the biological significance of boron for various metabolic, nutritional, hormonal, and physiological processes indicate that it may (Blevins and Lukaszewski 1994; Kabu and

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Akosman 2013) or may not (Loomis and Durst 1992) be essential to plants, but B is essential for humans and animals (Nielsen 1997; Hunt 2012; Kabu and Civelek 2012; Kabu and Akosman 2013). Boron influences the activities of at least 26 different enzymes (Hunt 1998), and many of these enzymes are essential in energy substrate metabolism (Bakken and Hunt 2003). We previously reported that boron administration (30 g/day) positively affected Ca and Mg metabolism of ruminants in the periparturient period (Kabu et al. 2013). Although the diets of both groups were balanced in minerals, serum Ca and Mg concentrations were relatively higher in the boron group than in the control group in the postpartum period. We also observed that boron administration had transient effects on several haematological indicators of ruminants in the periparturient period (Kabu et al. 2014).

Dietary boron may also play a role in lowering plasma lipid levels. The administration of two boron-derived hypolipidaemic agents to rats significantly lowered serum low-density lipoprotein (LDL) cholesterol and triglyceride levels after 14 days. Furthermore, both agents inhibited LDL binding and entrance into liver cells, fibroblasts, and aorta cells, and promoted high-density lipoprotein (HDL) binding and deterioration in liver cells (Hall et al. 1989). Naghii and Samman (1997a) and Naghii and Samman (1997b) reported that giving boric acid to rats at the dose of 2 mg/day for two weeks decreased serum total cholesterol, HDL3, TG, and HDL concentrations.

Boron research in ruminants is limited and there is no optimum dose of borax known. There are a few studies describing the use of borax in cattle; however, different doses were used (Fry et al. 2010; Fry et al. 2011; Kabu and Civelek 2012; Kabu et al. 2013; Kabu et al. 2014). This study was conducted to determine the effects of orally administered non-toxic doses of borax on health, milk yield and selected blood indicators related to metabolism in early lactation Austrian Simmental cows (Fleckvieh).

MATERIAL AND METHODS

Animals and experimental design. All procedures involving animals were conducted at the Nigtas Dairy Company, Nigde, Turkey, which breeds approximately 1600 dairy cows, heifers, and calves. Twenty primiparous Austrian Simmental (Fleckvieh) cows were selected at parturition and then randomly assigned to two groups, control group or the borax group. The study lasted for four weeks. Borax ($Na_2B_40_7 \cdot 5H_2O$, Eti Mine Works Kirka) was orally administered (0.2 mg/kg/day) to all treatment cows shortly after the noon milking for 28 days. The control cows received only water orally administered as a sham treatment. All cows were kept in the same paddock and consumed the same diet. The diet was designed to meet NRC (1989) requirements (Table 1). Diets were split into three equal parts a day and fed as TMR. All feeds in the diet were analysed for crude cellulose, protein, ether extract, ash, and dry matter, according to the Weende Analysis Systems, in addition to ADF and NDF, according to Van Soest. Cows were milked three times daily: at 04:30, 12:30, and 20:30 h. Milk yield was measured and recorded daily for 14 weeks.

Boron and biochemical analyses. Blood samples were collected in serum dry biochemistry tubes via the vena jugularis from all cows on days 0, 7, 15, 21,

Table 1. Feed ingredients and chemical composition of postpartum diets

Feedstuffs	DM
Concentrate mix. (%)	28.2
Corn silage (%)	26.4
Alfalfa (%)	14.8
Brewer's yeast (%)	8
Barley (%)	7.5
Soybean meal (%)	5.5
Cottonseed (%)	5.5
Corn (%)	3.9
Bicarbonate (%)	0.15
Daily feed intake (kg)	23.6
Chemical composition of diet	
DM (%)	55
CP (%)	17.5
Microbial protein (%)	11.8
By pass protein (%)	5.2
NEL (MJ/kg)	1.63
NDF (%)	40.35
ADF (%)	22.42
Ca (%)	0.75
P (%)	0.39
B (%)	0.002

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and 28 of lactation and centrifuged at 5000 × g for 10 min. Then, the plasma was collected, stored at –20 °C, and he boron concentration of the plasma samples and of the total mixed rations (TMR) was determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700X, USA) with a flow injection system.

Measurements of serum non-esterified fatty acids (NEFA; Randox Laboratories Ltd, United Kingdom), beta-hydroxybutyric acid (BHBA; Randox Laboratories Ltd, United Kingdom), total cholesterol (TChol; Biolabo SA, France), total protein (TP; Biolabo SA, France), blood urea nitrogen (BUN; Biolabo SA, France), albumin (ALB; Biolabo SA, France), creatine (CRE; Biolabo SA, France), uric acid (UA; Biolabo SA, France), glucose (GLU; Biolabo SA, France), high-density lipids (HDL; Biolabo SA, France), aspartate aminotransferase (AST; Biolabo SA, France), alanine aminotransferase (ALT; Biolabo SA, France) and gamma-glutamyl transpeptidase (GGT; Biolabo SA, France) were performed in an ELISA reader (Chemwell® Model 2910, Awareness Technology Inc., Palm City, FL, USA) using commercial kits.

Statistical analysis. PASW Statistics (version 18.0; SPSS, Chicago, IL) was used for data analyses. The Mann-Whitney *U*-test was used to compare mean differences between groups. A Wilcoxon signed-rank test was performed after a Friedman test to determine where significance occurred within group variables. A significance level of $P \leq 0.05$ was used. To avoid type 1 alpha errors, Bonferroni correction was used for the Wilcoxon signed-rank test.

RESULTS AND DISCUSSION

TMR boron concentrations were measured and found to be 29.92 mg/kg**.** Serum boron concentration was affected by treatment and treatment × week interaction; see Table 2. Serum B concentration was higher in the borax group than in the control group on Weeks 1, 2, 3, and 4 of the experiment. Serum B concentration did not change in the control group from week to week, but it gradually increased in the borax group week by week. Similarly to these results, it was previously reported that dietary borax supplementation increased plasma boron levels in steers on Days 28, 63, and 77 (Fry et al. 2010), and on Days 2 and 4 (Fry et al. 2011), respectively. In contrast to these findings, plasma boron levels did not change in borax-supplemented steers in either study day by day. Milk yields were not affected by treatment. Peak milk production was observed at Week 10 of lactation for both groups (Control: 28.00 ± 0.90, Boron: 29.67 ± 1.17 litres).

AST, ALT, and glucose levels were similar between groups throughout the study. GGT and creatine levels were found to be similar between groups, whereas the highest level was found at parturition. In accordance with these results, Kabu and Civelek (2012) reported that boron administration did not significantly change serum AST and GGT levels throughout the transition period in dairy cows. They found that AST levels decreased at the second week of lactation, but was not changed during the other weeks of the transition period. In addition, Basoglu et al. (2002) indicated that boron administration did not change serum GGT levels after parturition.

The serum albumin concentration differed between the two groups at parturition, but it was similar in the last week of the study. In the control group, albumin levels gradually decreased in the first three weeks of the study but began to increase during the last week. Albumin levels were similar in the borax group week by week. The TP level for the borax group was higher than for the control group only for the last week. TP levels gradually increased week by week in the borax group, whereas

Table 2. Serum boron concentrations (mg/l) in the boron and control groups at weekly intervals

Group	Calving	Week				*P*-value
		1	2	3	4	
Control	0.226 ± 0.023	0.238 ± 0.032^{a}	0.317 ± 0.084^{a}	0.252 ± 0.04^{a}	0.272 ± 0.025	0.615
Boron	0.221 ± 30^{A}	9.913 ± 0.247^{Bb}	11.321 ± 0.818^{Bb}	15.649 ± 0.706^{Bb}	17.549 ± 0.635	0.042
P-value	0.922	0.025	0.034	0.014	0.017	

Different superscripts in the same row indicate significant differences a, b ($P < 0.05$) over time during the postpartum period for a given group. Different superscripts in the same column indicate significant differences A, B ($P < 0.05$)

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Table 3. Serum biochemical indicators (AST, ALT, GGT, total protein, ALB, BUN, UA and CRE) in dairy cows during postpartum (4 weeks) after oral treatments: Boron group (0.2 mg/kg/day) and control group (n = 10 per group). Results are expressed as means ± standard deviations

Indicators	Group	Calving	Week				Significance
			1	2	3	4	
AST (μkat/l)	control	1.28 ± 0.07	1.28 ± 0.09	1.27 ± 0.05	1.29 ± 0.05	1.33 ± 0.06	> 0.05
	boron	1.37 ± 0.06	1.37 ± 0.06	1.41 ± 0.05	1.45 ± 0.06	1.42 ± 0.05	> 0.05
Significance	*P*	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
ALT (μkat/l)	control	0.4 ± 0.01	0.36 ± 0.02	0.35 ± 0.02	0.39 ± 0.02	0.40 ± 0.01	> 0.05
	boron	0.42 ± 0.01	0.39 ± 0.02	0.34 ± 0.03	0.35 ± 0.02	0.37 ± 0.05	> 0.05
Significance	*P*	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
GGT (μkat/l)	control	0.32 ± 0.01^{a}	0.24 ± 0.01^{b}	0.24 ± 0.01^{b}	0.31 ± 0.02^{ac}	0.32 ± 0.01^{ac}	< 0.001
	boron	0.33 ± 0.01^{a}	0.23 ± 0.01^{b}	0.24 ± 0.01^{b}	0.31 ± 0.02^{ac}	0.32 ± 0.01^{ad}	< 0.01
Significance	*P*	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
Total protein (g/l)	control	62.3 ± 1.15^{ab}	63.5 ± 2.24^{ab}	57.3 ± 2.19^{b}	65.9 ± 2.35^{a}	64.9 ± 1.21^{Aa}	< 0.05
	boron	61.0 ± 1.16^{a}	64.4 ± 1.27^{a}	64.0 ± 2.57^{a}	66.1 ± 1.42^{a}	71.8 ± 0.67^{Bb}	< 0.05
Significance	*P*	> 0.05	> 0.05	> 0.05	> 0.05	< 0.01	
ALB (g/l)	control	35 ± 0.62^{Aa}	30 ± 0.54^{b}	27.1 ± 1.54^{b}	29.6 ± 1.53^{b}	29.8 ± 1.17^{b}	< 0.01
	boron	29.2 ± 2.21^{B}	31.2 ± 1.51	29.7 ± 1.02	27.2 ± 0.84	30.7 ± 1.14	> 0.05
Significance	*P*	< 0.01	> 0.05	> 0.05	> 0.05	> 0.05	
BUN (mmol/l)	control	3.89 ± 0.26^{a}	4.35 ± 0.22^{a}	4.60 ± 0.26^{a}	4.73 ± 0.31^{a}	5.54 ± 0.12^{b}	< 0.01
	boron	4.01 ± 0.21^{a}	4.29 ± 0.18^{a}	5.15 ± 0.21^{b}	4.67 ± 0.36^{abc}	6.64 ± 0.11^{c}	< 0.05
Significance	*P*	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
UA (mmol/l)	control	0.061 ± 0.01	0.064 ± 0.02	0.06 ± 0.02	0.067 ± 0.01	0.069 ± 0.02^{A}	> 0.05
	boron	0.062 ± 0.02	0.063 ± 0.01	0.06 ± 0.01	0.062 ± 0.01	0.058 ± 0.01^{B}	> 0.05
Significance	*P*	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
CRE (μmol/l)	control	124.78 ± 2.65	102.54 ± 3.51	101.28 ± 3.52	102.24 ± 3.68	100.43 ± 4.21	> 0.05
	boron	124.06 ± 3.53^{a}	02.5 ± 3.52^{b}	98.9 ± 1.68^{b}	101.28 ± 3.54^{b}	99.1 ± 3.68^{b}	< 0.05
Significance	*P*	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

Different superscripts in the same row indicate significant differences a,b ($P < 0.05$), c,d ($P < 0.01$), e,f ($P < 0.001$) over time during the postpartum period for a given group. Different superscripts in the same column indicate significant differences A,B ($P < 0.05$), C,D,F ($P < 0.01$)

values remained relatively constant in the control group except for Week 2, when they reached their lowest level. These results indicate that the control cows may have expended more protein for energy metabolism than the borax cows. In the early lactation period, BUN levels were found to be similar between the two groups whereas the highest level was found in the last week. Uric acid levels for the borax group were lower than for the control only in the last week (Table 3). In contrast to these results, we previously found that boron administration increased serum BUN levels at Week 1 of lactation, but that the levels were similar between groups in the other weeks of the transition period, before and after parturition (Kabu and Civelek 2012).

TChol, NEFA, and BHBA levels were found to be similar between groups; the highest levels of these parameters were found in the last week, first week, and third week of the study, respectively. NEFA decreased week by week and was found to be at the lowest level in the last week for both groups. The HDL level for the borax group was lower than for the control group for the third week only (Table 4) and was similar between groups in other weeks of lactation. Positive effects of boron on lipid metabolism have been reported in some studies (Naghii and Samman 1997a; Devirian and Volpe 2003; Basoglu et. al. 2010). In these experiments we did not detect any significant effects of boron administration on lipid metabolism, which is in agreement with our earlier study in which we

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Table 4. Serum biochemical parameters (Glucose, Total Cholesterol, HDL, NEFA and BHBA) in dairy cows during postpartum (4 weeks) after oral treatments: Boron group (0.2mg/kg/day) and control group (n = 10 in each group). Results are expressed as means ± standard deviations

Parameters	Group	Calving	Week				Significance
			1	2	3	4	
Glucose (mmol/l)	control	3.11 ± 0.23	2.58 ± 0.08	2.75 ± 0.04	2.70 ± 0.07	2.51 ± 0.08	> 0.05
	boron	3.04 ± 0.19	2.80 ± 0.09	2.79 ± 0,06	2.82 ± 0.07	2.62 ± 0.11	> 0.05
Significance	*P*	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
T.Chol (mmol/l)	control	2.25 ± 0.05^{a}	2.37 ± 0.08^{a}	2..43 ± 0.1^{a}	2.80 ± 0.06^{b}	3.06 ± 0.07^{c}	< 0.001
	boron	2.27 ± 0.07^{a}	2.43 ± 0.08^{ab}	2.50 ± 0.06^{bc}	2.70 ± 0,05^{c}	3.12 ± 0.05^{d}	< 0.01
Significance	*P*	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
HDL (mmol/l)	control	2.53 ± 0.1^{a}	2.35 ± 0.11^{a}	2.33 ± 0.15^{a}	3.17 ± 0.17^{Ab}	3.01 ± 0.05^{b}	< 0.01
	boron	2.61 ± 0.9^{a}	2.62 ± 0.1^{a}	2.47 ± 0.12^{a}	2.45 ± 0.09^{Ba}	2.94 ± 0.06^{b}	< 0.05
Significance	*P*	> 0.05	> 0.05	> 0.05	< 0.01	> 0.05	
NEFA (mmol/l)	control	0.63 ± 0.04^{a}	0.73 ± 0.05^{a}	0.69 ± 0.04^{a}	0.58 ± 0.02^{a}	0.49 ± 0.03^{b}	< 0.05
	boron	0.66 ± 0.04^{acd}	0.77 ± 0.06^{acd}	0.72 ± 0.02^{ac}	0.61 ± 0.03^{ad}	0.50 ± 0.02^{b}	< 0.05
Significance	*P*	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
BHBA (mmol/l)	control	0.80 ± 0.03^{ac}	0.80 ± 0.02^{a}	0.98 ± 0.04^{ac}	1.00 ± 0.06^{b}	1.00 ± 0.04^{c}	< 0.01
	boron	0.83 ± 0.03^{a}	0.81 ± 0.02^{a}	1.00 ± 0.04^{b}	1.05 ± 0.05^{b}	0.96 ± 0.05^{b}	< 0.05
Significance	*P*	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

Different superscripts in the same row indicate significant differences a,b ($P < 0.05$), c,d ($P < 0.01$), e,f ($P < 0.001$) over time during the postpartum period for a given group. Different superscripts in the same column indicate significant differences A,B ($P < 0.05$), C,D ($P < 0.01$)

did not observe any effects of boron administration on serum TChol, HDL, NEFA, or BHBA levels after parturition (Kabu and Civelek 2012).

The present results demonstrate that oral administration of borax increased serum boron concentrations in Weeks 1, 2, 3 and 4 and did not exert any negative effects on bovine health. Likewise, no notable effects on metabolic indicators in Austrian Simmental (Fleckvieh) cows during early lactation were detected. However, further studies are needed in order to determine the effectiveness of borax administration on boron dependent-proteins and the effects of B on calcium and magnesium metabolism in dairy cows, especially during early lactation.

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