original research paper

Acta Agriculturae Serbica, Vol. XV, 29 (2010) 55-76

Acta Agriculturae Serbica
FACULTY OF AGRONOMY • ČAČAK •

Effect of Dietary Supplements of Sodium Bicarbonate on Tissue Calcium (Ca) and Magnesium (Mg) Levels in Beef Cattle

Vladimir S. Kurćubić
Faculty of Agronomy, Čačak, Serbia

Zoran Ilić
Faculty of Agriculture Priština – Zubin Potok, Serbia

Marija Vukašinović
Kraljevo Veterinary Specialist Institute, Kraljevo, Serbia

Radojica Đoković
Faculty of Agronomy, Čačak, Serbia

Abstract: The effect of dietary supplements of Na-bicarbonate administered in the second stage of fattening on Ca and Mg levels in both blood and tissues (bone, muscle, liver and kidney) was evaluated in two groups of animals over a period of 30 days. The control (n=11) and experimental groups (n=14) of beef cattle received identical rations, with the complete compound feed (CCF) fed to experimental group being supplemented with 48.1 g Na-bicarbonate/calf/day.

Mean Ca and Mg levels in beef cattle drinking water (provided by the Morava water supply system) were below those permitted in the Regulation on the Hygienic Safety of Drinking Water (Official Gazette, Republic of Serbia, Nos. 42/98 and 44/99). Mean bicarbonate and alkalinity levels in the drinking water were 309.202 mg/L and 50.688, respectively.

Blood was obtained from experimental beef cattle on days 0, 10, 20 and 30. Bone (the spinous process of the caudal vertebra), muscle, liver and kidneys were sampled at slaughter. Ca and Mg levels in all bovine samples were determined by atomic absorption spectrophotometry (AAS) – Yugoslav standard JUS ISO 6869:2002, using UNICAM 969 apparatus.

Received: 31 October 2009 / *Accepted*: 3 February 2010

No statistically significant differences in serum Ca levels in the test animals were found between the control periods, or between the experimental and control groups. There were no statistically significant differences ($p>0.05$) in serum Mg levels between days 20 and 30 in the experimental group, nor between days 20 and 30 in the experimental group as compared to those in the control group. Statistically significant differences ($p<0.05$) were observed in serum Mg levels in experimental cattle between days 0 and 10 and days 0 and 30, as well as between day 0 in experimental animals and day 30 in the control group. Serum Mg levels showed very significant differences ($p<0.01$) in the experimental cattle (between days 0 and 20, 10 and 20, and 10 and 30), as well as between day 10 in the experimental cattle and day 30 in the control cattle.

No statistically significant differences ($p>0.05$) were found in Ca levels in liver, kidney, muscle and bone samples and in Mg levels in liver, kidney and bone samples between the experimental and control groups. The muscle Mg level in the control group of animals was very significantly above ($p<0.01$) the level in the experimental cattle.

Key words: beef cattle, sodium-bicarbonate, calcium, magnesium, bones, muscle, kidney, liver, AAS.

Introduction

Calcium (Ca) is the most abundant mineral in the body. As much as 98% of the total body stores of Ca functions as a structural component of bones and teeth and the remaining 2% is involved in vital functions (distributed in extracellular fluids and soft tissues) – blood clotting, membrane permeability, muscle contraction, nerve impulse transmission, cardiac regulation, secretion of certain hormones, and activation and stabilisation of certain enzymes.

Calcium requirements are calculated by adding the available calcium required for maintenance, growth, pregnancy and lactation and correcting for the percentage of the dietary calcium absorbed. The Agricultural and Food Research Council (AFRC) has recently used a value of 68% absorption to calculate calcium requirements of beef cattle (TCORN 1991).

Calcium is absorbed primarily from the duodenum and jejunum by both active transport and passive diffusion (McDowell 1992). Vitamin D is required for active absorption of Ca (DeLuca 1979). In natural feedstuffs, Ca occurs in oxalate or phytate form. In alfalfa hay, 20-33% is present as insoluble calcium oxalate which is apparently unavailable to the animal (Ward *et al.*1979).

Calcium deficiency in young animals prevents normal bone development and retards growth and development, resulting in bone softening and fragility leading to fractures. Monti *et al.* (2007) reported the multiple fractures in an 8-year-old child allergic to cow milk to be caused by inadequate dietary amounts of Ca. In adult animals, the deficiency induces osteomalacia. Parathyroid hormone (PTH) is released in response to a reduction in plasma calcium levels. It stimulates the production of 1,25-dihydroxy cholecalciferol (vitamin D_3), which enhances calcium absorption from the intestines and, in conjunction with parathyroid hormone, increases calcium resorption from the bones. Elevated plasma calcium concentrations induce the production of calcitonin that inhibits the production of parathyroid hormone, leading to decreased calcium absorption and bone resorption (National Research Council (U.S.) Subcommittee on Beef

Cattle Nutrition, National Research Council: Nutrient Requirements of Beef Cattle. Minerals 1996).

The calcium content of feedstuffs is affected by plant species, portion of plant consumed, maturity, amount of exchangeable calcium in the soil and climate (Minson 1990). A higher content of calcium is found in legumes than in grasses.

Wacker (1980) reports that more than 300 enzymes are activated by magnesium (Mg). Magnesium is essential, as the complex Mg-ATP, for all biosynthetic processes (glycolysis, energy-dependent membrane transport, formation of cyclic AMP and transmission of the genetic code).

Magnesium is involved in the maintenance of electrical potentials across nerve and muscle membranes and also in nerve impulse transmission. Of the total body magnesium, 65-70% is located in the bone, 15% in the muscle, 15% in other soft tissues and 1% in the extracellular fluid (Mayland 1988).

Dietary requirements for Mg vary depending on age, physiological state and bioavailability from the diet. Recommended Mg requirements are as follows: 3 mg Mg/kg liveweight for replenishment of depleted endogenous stores, 0.45 g Mg/kg gain during cattle growth, 0.12 mg Mg/kg milk during lactation, and 0.12, 021 and 0.33 g Mg/day for early, mid and late gestation, respectively (Grace 1983).

Beef cattle require 7-9 g Mg/day during gestation and 18-21 g Mg/day during lactation to maintain serum Mg levels of 2.0 mg/dl (O' Kelly and Fontenot 1969, 1973).

Magnesium deficiency in calves results in excitability, undernourishment, hyperemia, convulsions, frothing at the mouth, profuse salivation and calcification of soft tissues (Moore *et al*. 1938, Blaxter *et al*. 1954). Grass tetany or hypomagnesemic tetany is characterised by low Mg concentrations in plasma and cerebrospinal fluid and is a problem in lactating beef cattle. Clinical signs include nervousness, reduced feed intake, and muscular twitching around the face and ears. Animals are uncoordinated and walk stiffly. In the advanced stages, cows lie with their head back and go into convulsions. Death occurs quickly unless the animal is treated intravenously or subcutaneously with a magnesium salt solution.

In terms of Mg supply, cows depend primarily on the gastrointestinal tract (GIT) to maintain normal blood Mg concentrations, since homeostatic mechanisms are not sufficient to regulate blood Mg concentrations. Bone Mg levels are high, but mature animals lack the ability to mobilise large amounts of Mg from bone (Rook and Storry 1962). In young calves, at least 30% of the skeletal Mg can be mobilised during Mg deficiency (Blaxter *et al.* 1954).

The rumen is the major site of Mg absorption in ruminants (Grace *et al.* 1974, Greene *et al*. 1983). Magnesium absorption values in mature ruminants fed hay and grass range from 10 to 37 percent (Agricultural Research Council 1980). Magnesium in concentrates is more available than magnesium in forages (Peeler 1972). Magnesium absorption or utilisation is reduced by high dietary levels of potassium (Greene *et al.*, 1983; Wylie *et al*. 1985), as well as by high dietary levels of nitrogen, organic acids, long-chain fatty acids, Ca and P (Fontenot *et al.*1989).

Evidence suggests that Mg absorption from the rumen occurs by an active Na-linked process (Martens and Rayssiguier 1980), and sodium supplementation in a low-sodium diet increases Mg absorption (Martens *et al*. 1987).

Cereal grains generally contain 0.11 to 0.17 % Mg. Plant protein sources contain approximately twice this concentration (Underwood 1981). Legumes are usually higher in Mg than are grasses. Magnesium oxide and magnesium sulphate are good sources of supplemental Mg.

Reduced urinary Ca excretion in humans as induced by dietary supplements of Na bicarbonate has been reported previously (Lutz and Josephine 1984). In a study on acid balance in humans, Lemann *et al.* (1965) reported reducing faeces and urine Ca levels resulting from dietary supplements of Na bicarbonate. Reidenberg *et al.* (1966) noted that urinary Ca decreases markedly when Na bicarbonate is administered to obese women for correction of metabolic acidosis caused by starvation. Chan (1981) showed that Ca balance becomes more positive when Na bicarbonate is given to young patients with type 1 renal tubular acidosis. Barzel and Jowsey (1969) demonstrated that long-term ingestion of an equimolar solution of Na and K bicarbonate prevents the development of calcium deficiency osteopenia and increases bone formation in rats.

Human metabolic acidosis (a primary decline in bicarbonate concentration) occurs during renal failure (Bushinsky 1995a, Bushinsky 1995b, Madias and Kraut 1989), kidney disease or renal tubular acidosis (Bushinsky 1995, Batlle 1989), being characterised by a decline in systemic pH. There is an abundance of clinical evidence suggesting bone disease in metabolic acidosis and renal failure (Goodman *et al*., 1965, Litzow *et al*. 1967, Mora Palma *et al.* 1983). About 60% of the additional protons are buffered by soft tissues (Levitt *et al.* 1956, Adler *et al.* 1965, Poole-Wilson and Cameron 1975) and bone (Bushinsky 1987, 1988, 1989, 1994, 1995, Bushinsky and Krieger 1992, 1996, Bushinsky and Ori 1993, Bushinsky *et al*. 1983, 1993, Bushinsky and Lechleider 1987. *In-vivo* evidence suggests that acidosis induces a loss of bone Na, K (Bushinsky *et al.* 1986, 1993, 1997, Bergstrom and Ruva 1960, Bettice and Gamble 1975, Burnell 1971) or carbonate (Bushinsky. *et al*. 1993, Bushinsky and Lechleider 1987, Burnell 1971, Bettice 1984) along with Ca (Kraut *et al.* 1984). Bone Na or K loss indicates the proton-cation exchange in the bones, and carbonate loss suggests consumption of this buffer by excess protons.

Urinary Ca excretion is known to be reduced due to an increase in protein intake in women at controlled intakes of Ca and P (Linkswiler 1981). The age-related bone mass decline entails loss of bone calcium. The loss is partly due to the titration of noncarbonic acids with alkaline Ca salts (endogenously produced in response to habitual ingestion of the acid-producing diets typical of Western countries). Persisting diet-induced low-grade metabolic acidoses contribute to the external losses of calcium by direct impairment of renal Ca reabsorptive efficiency characteristic of metabolic acidosis (Lemann 1967).

A study on the effect of K-bicarbonate (KBC) treatment on urinary Ca excretion in postmenopausal women eating a typical American diet reveals that a reduction in urinary excretion of Ca persists throughout the study period of 36 months. More importantly, the women at the greatest risk of body calcium loss had the highest decline in urinary Ca excretion and benefited the most from treatment with KBC (Frasseto *et al.* 2005).

At the beginning of lactation in cows, the homeostatic mechanisms of Ca regulation respond to a profound increase in Ca requirements. Bone calcium

mobilisation and increased absorption from the gastrointestinal tract (GIT) demand reestablishment of homeostasis.

Calcium turnover refers to the rate of Ca movement (loss and replacement) through the exchangeable calcium pool. Cows with a higher Ca turnover are considerably more resistant to hypocalcemia at the onset of lactation than cows with a low turnover, notwithstanding the potentially identical prepartal Ca concentration. Young cows (substantially more resistant to parturient hypocalcemia) have higher Ca turnover than older cows. Parathyreoidectomised cows have a lower calcium turnover than intact cows. Changes in dietary calcium intake do not alter steady state calcium turnover (Ramberg 1976).

The loss of Ca from the exchangeable pool is the product of plasma Ca concentration and the total clearance of Ca from the pool. Total clearance is the sum of the clearances to the bone, gut, foetus and milk.

Plasma Ca concentration is the sum of calcium inflows into the blood from the gut and bone, divided by the total clearance of Ca from the plasma. The inflow of calcium is subject to negative feedback regulation by parathyroid hormone (PTH). Calcium clearances are disturbing signals, and calcium inflow is a controlling signal for plasma calcium homeostasis. Increased urinary Ca clearance tends to lower plasma concentration and invoke endocrine mechanisms to enhance calcium inflow. This metabolic exercise will prepare the calcium homeostatic system to respond more rapidly to the increased Ca clearance at calving.

The onset of lactation requires immediate high Ca rates from the blood of dairy cows. In some cows, the loss of Ca from the mammary gland causes a drastic decline in blood Ca level (from the usual 9-10 mg/dl to <5 mg/dl), leading to the development of the clinical syndrome known as milk fever.

Luick *et al.* (1957) studied the biokinetic aspects of Ca metabolism in dairy cows in an attempt to determine whether cows prefed low Ca:high P diets are better able to maintain normal serum Ca levels than cows prefed high Ca:low P diets. Radioactive calcium (Ca^{45}) was employed as a tracer. It appears that these reservoirs of skeletal Ca are larger and are turning over more slowly in cows prefed low Ca:high P diets. A comparison of bone Ca specific activity to serum Ca specific activity indicates that nearly all the trabecular bone Ca and 7 to 17% of the cortical bone Ca (depending upon dietary Ca/P) may be included in the 'mobilisable Ca pool'.

Goff and Horst (1995) report that high dietary K, but not Ca, induces parturient hypocalcemia in dairy cows.

Successful control of milk fever incidence begins with adequate dietary management of dry cows, preparatal involvement of concentrate feeding during the close-up period to adapt the microflora and forestomach mucosa to the high energy grain diets that will be fed after parturition (Gerloff 1988). Grain feeding may result in subclinical rumen acidosis with compensated metabolic acidosis, growth of the rumen papillae and increased production and absorption of volatile fatty acids (Dirksen *et al.* 1984).

Anion supplementation has been found to play an important role in preventing milk fever incidence through bone Ca mobilisation and increased Ca absorption from the GIT. Liesegang *et al.* (2007) evaluated the effect of different Ca levels in diets supplemented with anionic salts on bone metabolism in dairy cows and suggested that anion supplementation and different dietary Ca levels

have no statistically significant effect on bone Ca resorption. Dietary cation-anion balance is presumably insufficiently low to lead to metabolic acidosis and its positive effects on Ca metabolism.

Acidosis has been shown to regulate Ca homeostasis in cows (Jorgensen 1974, Block 1984). The mechanism involves the effect on the conversion of vitamin D to its active metabolite 1,25 $(OH)_2D_3$.

Rumen acidosis may lead to systemic acidosis if the amount of organic acids absorbed into blood exceeds the removal of these acids from the circulation by the liver and the kidney (Mellau *et al.* 2004).

The metabolite is a key component stimulating intestinal absorption of dietary Ca, bone resorption and renal Ca reabsorption (Lunn and McGuirck 1990).

The resulting metabolic acidosis activates Ca homeostatic mechanisms by enhancing the effect of PTH and $1,25(OH)_2D_3$ on bone, intestinal absorption and renal regulation of calcium (Goff and Horst 1997). These responses follow a negative calcium balance caused by increased urinary excretion of calcium during metabolic acidosis (Wang and Beede 1992). In accordance to this, plasma hydroxyproline and other indicators of bone resorption have also been reported to increase in cows fed acidogenic diets (Block 1984).

Liesegang *et al.* (2000) studied the effect of milk yield on bone Ca mobilisation in two groups of cows – in cows with high (6,500 kg) and low (4,900 kg) milk yields per lactation. Bone Ca mobilisation was considerably more active in high-yielding cows than in low-yielding ones.

Evans *et al.* (1976) evaluated blood serum levels of hydroxyproline, Ca, Mg, P and alkaline phosphatase in Holstein cows over a period of 24 hours and reported lower hydroxyproline and alkaline phosphatase levels in older cows, indicating a decline in bone Ca mobilisation with advancing age. There was a correlation coefficient of only 0.20 between serum Ca and hydroxyprolyne. Serum Ca level remains within a narrow range primarily as a result of homeostatic mechanisms involving bone Ca resorption i.e. the release of Ca and hydroxyproline. Serum hydroxyproline may indicate the degree of homeostasis required to maintain serum Ca.

Anions increase bone Ca resorption and renal Ca production as opposed to cations that lead to changes in both the conformational structure of the PTH receptor and the PTH-receptor interaction as well as to reduced cow's ability to respond to low blood Ca levels (Goff). Monitoring of urine pH indicates the effect of salt on reduced urine pH. It is best to measure urine pH 6 to 9 hours after feeding.

The objective of this study was to use human medical data on osteoporosis treatment with cation supplements and data on the effect of dietary cation/anion balance for dairy cows in an attempt to evaluate the effect of dietary supplements of Na-bicarbonate on calcium (Ca) and magnesium (Mg) deposition in tissues of model beef cattle.

Material and methods

The effect of dietary supplements of sodium-bicarbonate during the second stage of fattening on Ca and Mg levels in blood and tissues (bone, muscle, liver and kidney) was evaluated in beef cattle allocated to the following two treatments:

Control group (11 male Simmental calves aged 6 months) fed meadow hay, maize silage and complete compound feed (CCF) containing a minimum of 14 percent protein (manufactured by the Sto Posto Ltd.) at a daily rate of 7.4 kg without bicarbonate supplements. The beef cattle were provided drinking water in concrete water troughs through the public water supply system;

Experimental group (14 male Simmental calves 6 months of age) receiving meadow hay, maize silage and complete compound feed (CCF) having a minimum of 14 percent protein (manufactured by the Sto Posto Ltd.) and supplemented with 48.1 g Na-bicarbonate/calf/day (673.40 g Na-bicarbonate/day). The watering system used for the experimental group was identical to that for the control group.

Sodium-bicarbonate ($NaHCO_3$) was manufactured by Zorka Pharma Ltd., Šabac, Mr 84.01, quality conforming to Ph.Eur.4th Edition 2002.

The complete feed 1 suitable for cattle 250-300 kg bodyweight manufactured by Sto Posto Ltd. was composed of the following feedstuffs: grains, by-products of the milling, oil, sugar, alcohol and fermentation industries, dried vegetable products, mineral nutrients, salt, and vitamin and mineral premixes.

The shelf-life of the feed was 90 days. The net weight of the complete feed package was 40 kg. The feed quality was controlled by the Laboratory, Faculty of Agriculture, Novi Sad.

The declared physical and chemical composition of the above compound feed used in this study was as follows:

Oats units/kg, minimum	1.00
Crude proteins, minimum %	14.00
NPN protein relative to total proteins, maximum %	35.00
Moisture, maximum %	13.50
Fibre, maximum %	15.00
Ash, maximum %	10.00
Calcium, %	0.80 - 1.00
Phosphorus, %	0.50 - 0.70
Sodium, %	0.20 - 0.30

The following supplements per 1 kg compound feed were used:

Vitamin A	8.000 i.u.
Vitamin D_3	1.200 i.u.
Vitamin E	10 mg
Iron/ANI	50 mg
Manganese/ANI	60 mg
Copper/ANI	10 mg
Zinc/ANI	50 mg
Iodine	0.60 mg
Magnesium	40 mg
B.H.T. (antioxidant)	100 mg
Cobalt/ANI	0.20 mg

Human medical experience was used to calculate sodium-bicarbonate intake (0.1 g/kg BW i.e. 6 g/60 kg BW). According to the average body weight of cattle, the calves received 6.5 g $NaHCO_3$/kg compound feed (48.1 g/calf/day).

The experimental and control groups of beef cattle were kept in loose slatted-floor pens. The experimental cattle were fed CCF at a daily rate of 6 kg supplemented with 4 kg meadow hay. Both groups were fed in two daily feedings (morning and evening).

Water sampling was conducted at the beginning of the trial over nine consecutive days by collecting 1.5 l water from each watering trough into clean plastic (PET) packaging pre-washed with tested water. The water samples were transported to the Chemical Laboratory, Veterinary Specialist Institute, Kraljevo for analysis. Upon sample preparation performed on the day of sample reception, the samples were tested for Ca and Mg levels. The obtained data were subjected to statistical analysis (statistical software SPS ver. 5.0) and the average Ca and Mg levels in the drinking water offered to the beef cattle were evaluated.

Blood samples were secured from the experimental cattle on days 0, 10, 20 and 30 of the experiment and those from the control cattle on day 30. The samples were taken from the caudal vein (v. coccygica) by sterile needles and placed into vacutainer tubes (Terumo® Venoject) (test tubes stored in vertical racks and left at room temperature for 24 hours) where the serum brought in for analysis of Ca and Mg levels was separated). The blood serum samples were wet-digested for analysis conducted following the standard SRB-ISO procedure.

Bone (the spinous process of the caudal vertebra), muscle, liver and kidneys were sampled at slaughter. The muscle tissue was taken from the neck (from the trapezius, without suet).

Sample preparation procedure for Ca analysis – solutions

Standard stock solution of 1000 mg/dm^3 Ca

Standard intermediate solution: 2.5 cm^3 of the standard stock solution was diluted to 50 cm^3 with deionised water; containing 50 μg/ cm^3 Ca.

Preparation of test Ca solutions: aliquots of 1cm^3; 2 cm^3 and 3 cm^3 of the intermediate solution were decanted into 50 cm^3 volumetric flasks. Then, 5 mL of each 1% K and La solution was added and the volume was made up to the mark with 0.6 M HCl solution.

Preparation of the 5% KCl solution: an aliquot of 9.53 g KCl was dissolved in deionised water in a 100 cm^3 volumetric flask.

Preparation of the 5% LaCl solution: an aliquot of 13.37g $LaCl_3$ was dissolved in deionised water in a 100 cm^3 volumetric flask.

The prepared KCl and $LaCl_3$ solutions were diluted to obtain 1% solutions to be subsequently added for Ca determination (20 cm^3 of each solution were decanted into 100 cm^3 volumetric flasks and filled with 0.6 M HCl to the mark).

The digested serum samples were diluted to obtain AAS-readable concentrations, giving a dilution of 1:250 in 25 cm^3 volumetric flasks filled with 2.5 cm^3 of each 1% solution of KCl and $LaCl_3$ prior to making up the volume to the mark with 0.6 M HCl.

Water samples were prepared for analysis by acidifying a 500 ml water blank with nitrate acid (of a concentration of about 2%). The water sample was

evaporated to dryness and the dry residue was dissolved in several ml of aqua regia. The solution was filtered through quantitative filter paper into a 50-ml volumetric flask.

Digestion of serum and tissue (organ) samples for Ca and Mg determination

A serum aliquot of about 3 g was predigested with 20 cm^3 concentrate HNO_3 in a digestion device at a temperature of 430^0C for half an hour. After cooling, 10 cm^3 HNO_3 (1:1) and as much concentrated perchloric acid were added and the digestion continued. Upon digestion and evaporation of the residue to small volume, 0.6 M HCl was added and the sample was transferred into 50 cm^3 volumetric flasks. Calcium and magnesium in all blood serum samples obtained from beef cattle were determined by atomic absorption spectrophotometry (AAS) – Yugoslav Standard JUS ISO 6869: 2002 in a UNICAM 969 spectrophotometre. Ionisation buffers were added for Ca and Mg determination.

Standard solutions for Mg determination were prepared by diluting the stock solutions of the standards for the elements at a concentration of 1.000 mg/L.

The same procedure was used to prepare the bone (the spinous process of the caudal vertebra), muscle, liver and kidney samples collected at slaughter.

Determination of methyl orange alkalinity

A 100 cm^3 aliquot of the test water was decanted into a 500 cm^3 Erlenmeyer flask, two drops of methyl orange were added and the solution was titrated with 0.1 M HCl until the colour changed from yellow to orange. Methyl orange alkalinity is expressed as the number of cm^3 0.1M HCl required to neutralize 1 dm^3 water (the cm^3 spent for the 100 cm^3 x 10 blank). Bicarbonates were calculated by multiplying alkalinity by a factor of 6.1.

Results and discussion

The results of this study are presented in tabular (Tables 1-5) and graphic forms (Figures 1 and 2). Table 1 gives test results on the drinking water offered to beef cattle and sampled over 9 consecutive days.

Mean Ca level in the water offered to beef cattle (provided by the Morava supply system) was 85.683 mg/L, being substantially above the Ca level (Vukašinović *et al*. 2009) of 12.98 mg/L averaged from the range of 6.22-17.56 mg/L in the water supplied to beef cattle in the Municipality of Kraljevo. Values higher than these noted for the Municipality of Kraljevo were reported by Vukašinović *et al.* (1989) for the Ribnica and Studenica waters, as well as by Plavšić (1999) for the waters in the regions of Smederevska Palanka, Rekovac and Srbobran.

Tab. 1. Levels of Ca, Mg, alkalinity and bicarbonates in the samples of water offered to experimental and control beef cattle

No	Sample design.	Ca mg/L	Mg mg/L	Alkalinity	Bicarbonates mg/L
1	4377/1	84.15	29.87	50.30	306.83
2	4429	92.32	32.89	51.30	312.93
3	4431	83.82	29.81	59.40	362.34
4	4443	86.02	30.93	49.60	302.56
5	4458	86.22	30.68	48.40	295.24
6	4523/1	84.45	32.18	48.60	296.46
7	4523/2	85.72	30.56	49.60	302.56
8	4523/3	84.65	33.50	49.20	300.12
9	4523/4	83.80	30.31	49.80	303.78
	$\bar{x}$	**85.683**	**31.192**	**50.688**	**309.202**

The Regulation on the Hygienic Safety of Drinking Water (FRY Official Gazette Nos. 42/98 and 44/99) prescribes limits for Ca at 200 mg/L water, being considerably above the levels in this study. The maximum tolerable level for Mg, as prescribed by the said Regulation, is 50.00 mg/L water, being above the present values (mean Mg level in the cattle drinking water was 31.192 mg/L). Mean bicarbonate and alkalinity levels were 309.202 mg/L and 50.688, respectively.

Tab. 2. Serum Ca levels in the experimental (day 0, 10, 20 and 30) and control (day 30) groups of beef cattle

No.	**Sample designation Experimental group**	**Ca** mg/kg **day 0**	**Ca** mg/kg **day 10**	**Ca** mg/kg **day 20**	**Ca** mg/kg **day 30**	**Sample design. Control group**	**Ca** mg/kg **day 30**
1	7181758513	125.29	155.13	103.37	107.52	5146	inadequate
2	7102001429	92.26	102.41	40.87	142.10	8486	112.74
3	7171906123	87.93	114.59	102.96	96.04	6293	97.55
4	7121758498	106.16	96.54	98.63	92.52	8479	149.35
5	7161907726	178.22	96.17	110.89	108.96	1693	inadequate
6	7112001443	84.56	150.66	100.27	80.37	6236	93.80
7	7111907658	84.74	141.91	102.73	164.21	1651	83.22
8	7181905157	155.50	88.38	97.20	99.90	8479	149.35
9	7141907567	77.27	127.54	96.99	78.93	8463	108.21
10	7101906249	82.69	134.55	inadequate	inadequate	4248	105.27
11	7191758522	227.71	97.54	95.82	97.51	7501	103.86
12	7121758587	83.63	90.67	135.89	146.54		
13	7121758506	114.39	102.06	178.40	inadequate		
14	1818	99.66	130.96	100.97	91.05		
Total		**1600.01**	**1629.11**	**1464.99**	**1305.65**		**1003.35**
Mean value		**114.2864**	**116.365**	**112.6915**	**108.8042**		**111.4833**

The analysis of variance did not show statistically significant differences in the serum calcium (Ca) in beef cattle between the control periods, nor between the experimental and control groups of cattle (Table 2, Figure 1).

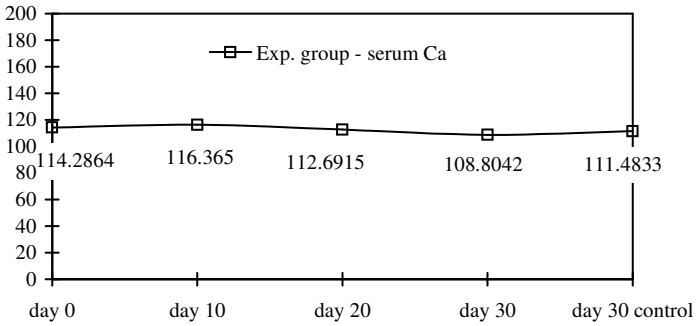

Fig.1. Serum Ca levels (mg/kg) in the experimental (days 0, 10, 20 and 30) and control (day 30) groups of beef cattle

As illustrated by Figure 1, the curve shows no significant oscillation, and a mild increase in serum Ca level is observed in the experimental beef cattle on day 10, followed by a slight decrease on days 20 and 30 down to a level below serum Ca level in the control cattle fed standard non-supplemented rations.

Blood Ca level is not a good indication of Ca status, as plasma Ca level is maintained between 9 and 11 mg/dL by homeostatic mechanisms (National Research Council (U.S.) Subcommittee on Beef Cattle Nutrition, National Research Council: Nutrient requirements of beef cattle. Minerals, 1996, 54-74).

Tab. 3. Serum Mg levels in the experimental cattle (days 0, 10, 20 and 30) and the control group (day 30)

No	**Sample designation Experimental group**	**Mg** mg/kg **day 0**	**Mg** mg/kg **day 10**	**Mg** mg/kg **day 20**	**Mg** mg/kg **day 30**	**Sample designation Control group**	**Mg** mg/kg **day 30**
1	7181758513	28.12	47.32	20.89	20.53	5146	inadequate
2	7102001429	33.37	34.29	7.61	32.12	8486	27.57
3	7171906123	27.18	32.76	19.99	24.25	6293	23.40
4	7121758498	25.62	34.84	22.87	24.88	8479	29.98
5	7161907726	32.56	32.38	28.11	39.52	1693	30.16
6	7112001443	25.11	45.02	22.69	21.79	6236	20.80
7	7111907658	25.40	51.94	23.59	28.89	1651	17.23
8	7181905157	42.43	56.66	23.02	25.77	8479	29.98
9	7141907567	28.86	36.46	25.49	22.56	8463	22.01
10	7101906249	26.94	33.40	21.93	inadequate	4248	27.76
11	7191758522	54.49	31.96	21.47	20.12	7501	28.01
12	7121758587	37.02	31.84	inadequate	inadequate		
13	7121758506	29.69	33.31	32.82	inadequate		
14	1818	34.41	30.65	22.76	25.89		
Total		**451.20**	**532.83**	**293.24**	**286.32**		**256.90**
Mean value		**32.22857**	**38.05929**	**22.55692**	**26.02909**		**25.69**

The analysis of variance revealed statistically significant differences in the serum levels of magnesium (Mg) between the control periods and between the experimental and control groups. The LSD test (least significance difference test – the statistical software SPS ver. 5.0) used showed significant (*– $p<0.05$) and very significant (**– $p<0.01$) differences between individual control periods and between the experimental (E) and control (C) groups of beef cattle (Table 4).

Tab. 4. LSD test results for serum magnesium (Mg) levels in beef cattle between the control periods and between the control and experimental groups

	E group day 0	**E group day 10**	**E group day 20**	**E group day 30**	**C group day 30**
E group day 0	/	5.83*	9.817**	5.832*	6.776*
E group day 10	5.83*	/	15.647**	11.662**	12.606**
E group day 20	9.817**	15.647**	/	3.985^{ns}	3.041^{ns}
E group day 30	5.832*	11.662**	3.985^{ns}	/	0.944^{ns}
C group day 30	6.776*	12.606**	3.041^{ns}	0.944^{ns}	/

^{ns} – $p>0.05$; * - significant difference – $p<0.05$; ** - very significant difference – $p<0.01$

The LSD test used showed no statistically significant differences ($p>0.05$) in serum Mg level between days 20 and 30 in the experimental cattle, between days 20 and 30 in the experimental group as compared to the values measured on day 30 in the control cattle.

There were statistically significant differences ($p<0.05$) in serum Mg levels between days 0 and 10 in the experimental cattle, between days 0 and 30 in the experimental group, and between day 0 in the experimental group and day 30 in the control group of cattle.

Statistically very significant differences ($p<0.01$) were found in serum Mg levels between days 0 and 20, 10 and 20, and 10 and 30 in the experimental cattle, as well as between day 10 in the experimental cattle and day 30 in the control group.

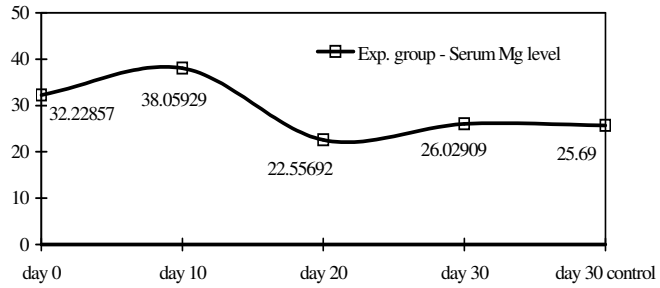

Fig. 2. Serum Mg levels in the experimental cattle (days 0, 10, 20 and 30) and the control cattle (day 30)

As presented in Figure 2, the curve shows oscillations in serum Mg levels in the experimental group of beef cattle between all days of the study (an increase in Mg level on day 10 as compared to day 0, a decrease on day 20 as compared to both day 0 and day 10, a mild increase on day 30 relative to the level on day 20, the level, however, being below the value measured on day 0). Serum Mg levels in the experimental cattle on days 0, 10 and 30 were above the level in the control cattle. Those measured on day 20 were below the average value observed for the control group.

Tab. 5. Levels of Ca and Mg in the liver, kidney, muscle and bone of the experimental and control group of beef cattle (mg/kg)

Designation	liver Ca	liver Mg	kidney Ca	kidney Mg	muscle Ca	muscle Mg	bone Ca	bone Mg
EXPERIMENTAL GROUP								
7181758513	147.08	226.40	95.48	225.67	223.20	223.00	63.262	1491
7102001429	63.96	214.45	77.30	220.43	634.24	197.36	44.272	1779.28
7171906123	64.31	206.19	80.17	239.37	217.70	210.20	70.996	1500
7121758498	98.95	195.23	97.32	234.81	320.33	206.60	73.096	1910
7172001443	83.78	226.40	98.87	236.96	240.00	200.39	84.943	1326
7181905157	90.66	214.37	112.79	295.93	64.70	220.90	62.844	1678
7141907567	79.23	221.59	98.40	231.50	2.707	216.99	66.428	1578
7191758522	79.08	203.74	93.60	232.91	42.20	201.58	77.722	2357.30
7121758587	85.17	220.90	112.42	262.05	213.83	194.64	73.551	1890
1818	78.31	215.62	117.77	254.78	137.00	204.56	86.199	1781
$\bar{x}$	**87.05**	**214.489**	**98.412**	**243.441**	**480.03**	**207.622**	**70,331.30**	**1729.058**
CONTROL GROUP								
5146	56.45	212.80	116.10	235.31	63.20	241.68	55.893	1323
8486	74.29	210.00	70.61	233.10	141.63	220.97	55.459	1540
8479	71.16	202.25	74.91	223.00	155.00	253.72	95.300	2000
1693	56.21	201.26	87.11	253.91	84.36	196.11	80.445	2136
6236	94.25	210.38	99.27	232.43	78.74	232.18	79.113	1756
1651	102.30	280.06	90.95	231.29	112.63	236.91	54.660	1917.90
8479	77.97	214.12	91.05	225.82	102.13	218.27	86.890	1960
8463	122.49	218.73	76.85	275.00	95.88	236.14	99.548	2192
4248	90.16	219.49	101.60	264.36	236.60	210.21	64.922	1637
7501	159.50	247.70	117.42	251.52	157.57	249.70	67.039	2049
$\bar{x}$	**90.478**	**221.679**	**92.587**	**242.574**	**122.77**	**229.589**	**73,926.90**	**1851.09**

Blincoe *et al.* (1973) evaluated the Mg, Ca and Sr content of bovine bone ash. The caudal vertebra, eighth rib and the femur contained identical levels of the three elements, but statistically significant differences were observed between the other bones and teeth. The caudal vertebra served as a representative sample of the skeleton and the fact that it is easily secured *in vivo* makes it attractive for studies of skeleton composition. Magnesium and calcium levels increased between 3 and 12 months of age, following which they remained relatively constant.

Complying with the above results, the caudal vertebra of the beef cattle tested in this study was sampled for analysis of the bone content of Ca and Mg.

The analysis of variance used for Table 5 data indicates no statistically significant differences ($p>0.05$) between the experimental and control cattle in

terms of the Ca content of the liver, kidney, muscle and bone samples and Mg content of the sampled liver, kidney and bone.

Based on the analysis of variance results, an LSD test (least significance difference test) was used, showing the muscle Mg level to be significantly higher ($p<0.01$) in the control group than in the experimental group of cattle.

The liver content of Ca in the experimental and control groups averaged 87.05 mg/kg and 90.478 mg/kg, respectively, being substantially below the average of 534.04 mg/kg (the content ranging between 125.57 and 1943.63) reported by Vukašinović *et al*. (2009).

The average kidney content of Ca in the experimental and control cattle was 98.412 mg/kg and 92.587 mg/kg, respectively, as significantly opposed to the results of Vukašinović *et al*. (2009), who measured the average content in beef cattle of 252.44 mg/kg (ranging from 189.44 to 296.22).

The measured muscle Ca averaged 480.03 mg/kg in the experimental cattle, and 122.77 mg/kg in the control cattle. Vukašinović *et al*. (2009) reported the average Ca level of 729.07 mg/kg (falling within the range of 133.07 – 2616.24). The Ca content in the experimental cattle substantially exceeded the beef level (10 mg/100g) determined by Rogovski (1981), but the average muscle content of Mg in the control group was comparable to the value reported by Rogovski (1981).

The average bone content of Ca in the experimental and control cattle was 70,331.30 mg/kg and 73,926.90 mg/kg, respectively.

Remzi Gonul *et al.* (2009) compared bone and blood serum levels of minerals in cattle raised in northwestern Turkey and established higher bone levels of Ca and P (333.23±163.02 and 132.76±17.84 mg g^{-1}, respectively) and lower Mg levels (2.38±0.32 mg g^{-1}). The comparison showed statistically significant difference in Ca and Mg levels, particularly between the Kirklareli and Edime provinces ($p<0.001$).

As for blood mineral levels, the above authors reported the Ca level in the test regions to correspond to the normal reference values. The measured values were statistically significantly higher ($p<0.001$) in the provinces of Istanbul and Edime than in Tekirdag Province. Magnesium levels were found to be higher in Istanbul, Kirklareli and Edime Provinces than in Tekirdag Province, the values for Istanbul and Edime being higher than the reference values, and those for Kirklareli and Tekiridag being within the reference range. Statistically significant differences were observed between the regions ($p<0.001$). Phosphorus levels were not statistically different between the provinces reporting values above the reference level.

The blood serum levels of Ca (mg dL^{-1}) secured from the above provinces had the following average values and standard deviation: 11.75±0.5 in Istanbul, 9.17±0.5 in Tekirdag, 10.25±1.32 in Kirklareli, 12.05±0.52 in Edime, giving an average of 10.80±0.71.

The average values and standard deviation for Mg level (mg dL^{-1}) in the blood serum samples from different provinces were as follows: 2.54±0.71 in Istanbul, 1.97±0.8 in Tekirdag, 2.2±0.4 in Kirklareli, and 2.79±0.83 in Edime, averaging 2.37±0.68. The objective of such studies is to determine criteria used in identifying metabolic diseases at an early stage and enable continued control of animal productivity within a herd.

Beighle *et al.* (1994) reported significant differences in bone ash values of P, Ca and Mg in animals of different age, sex and breed. Accordingly, the above studies conducted in Turkey involved dry bone samples collected from female cattle aged 2 years in order to eliminate the above variations and ensure maximum reliability of results.

The comparison of the findings in Turkey with those of Beighle *et al.* (1994) suggests that the bone mineral levels were higher than those available in the related literature (203.68±1.0 and 108.25±0.41 mg g^{-1}), whereas the Mg level was below the values previously reported (5.51±0.05 mg g^{-1}) by Bjorkman (1994).

The study conducted in Thailand by Tumwasorn Sornthep *et al.* (1983) confirmed differences in blood plasma Ca levels between different groups of cattle. Calcium levels were lowest in pregnant cows (8.2 mg/100 ml) and highest in young calves (10.4 mg/100 ml).

Kaya *et al.* (2008) observed that Ca levels (8.76 ± 0.3 mg/dl) in hypophosphatemic animals were higher before treatment ($P<0.05$) than in healthy control cattle (7.71 ± 0.1 mg/dl).

Tab. 6. Criteria for classifying adult cattle according to serum levels of Ca, inorganic P and Mg (Puls 1988)

Status	Ca, mg/dL	Inorganic P, mg/dL	Mg, mg/dL
Deficiency	1-6	<0.5 – 4.5	<1.1
Marginal	7-9	4.0 – 4.6	1.2 – 1.8
Adequate	8-11	4.5-6	1.8 – 3.5
High	>12	8.0-12	>4.0

Respective mean serum Ca levels in the test beef cattle were 114.2864 mg/kg, 116.365 mg/kg, 112.6915 mg/kg, 108.8042 mg/kg and 111.4833 mg/kg. Based on the reference values given in Table 6, the measured serum Ca levels indicated an adequate to high status of beef cattle in this study, depending on the day of testing.

Respective mean serum Mg levels in the test beef cattle were 32.22857 mg/kg, 38.05929 mg/kg, 22.55692 mg/kg, 26.02909 mg/kg and 25.69 mg/kg, suggesting an adequate serum Mg status.

Evaluation of blood mineral levels showed that Ca levels in all regions were consistent with the normal reference range of 9.7-12.4 mg dL^{-1}. The reference range for Mg level is 1.8-2.3 mg dL^{-1} (Carlson, 1990). These values are above those obtained in this study.

Human medical experience was used to calculate sodium-bicarbonate intake (0.1 g/kg BW i.e. 6 g/60 kg BW). According to the average body weight of cattle, the calves received 6.5 g $NaHCO_3$/kg compound feed (48.1 g/calf/day). A possible explanation for the absence of increase in Ca and Mg deposition in the blood and the test organs (liver, kidney, muscle, bone) lies in the lower rate of the Na-bicarbonate supplement used. As reported by Sinovec (2006), the cation sources most commonly used include carbonates (sodium, potassium), bicarbonates (sodium, potassium) and oxides (magnesium) used at a rate of about 200 g/day/calf. The use of high rates of cations is justified in maintaining

dietary cation-anion balance as cations combat the incidence of ruminal acidosis. Supplementation of cationic salts to the feed of lactating cows has positive effects on performance results, primarily during the first 100 days of lactation.

Conclusion

Human medical data on osteoporosis treatment with cation supplements and data on the effect of dietary cation/anion balance for dairy cows were used in an attempt to evaluate the effect of dietary supplements of Na-bicarbonate on Ca and Mg levels in both blood serum and tissues (bone, muscle, liver and kidney) of model beef cattle.

The current study, the results reported, the statistical analysis presented and the comparison with the findings of other authors suggest the following:

- The Regulation on the Hygienic Safety of Drinking Water (FRY Official Gazette Nos. 42/98 and 44/99) prescribes limits for Ca at 200 mg/L water, being substantially above the levels in this study (85.683 mg/L). The maximum tolerable level for Mg, as prescribed by the said Regulation, is 50 mg/L water, (above the average value measured in this study - 31.192 mg/L);
- Differences in the Ca level observed in the bovine serum were not statistically significant between the controls, nor between the experimental and control groups. The mean levels of Ca measured in bovine sera were 114.2864 mg/kg, 116.365 mg/kg, 112.6915 mg/kg, 108.8042 mg/kg and 111.4833 mg/kg on days 0, 10, 20 and 30 in the experimental cattle and day 30 in the control group, respectively;
- Blood Ca level is not a good indication of Ca status, as plasma Ca level is maintained between 9 and 11 mg/dL by homeostatic mechanisms;
- The LSD test used showed no statistically significant differences ($p>0.05$) in serum Mg level between days 20 and 30 in the experimental cattle, between days 20 and 30 in the experimental group as compared to the values measured on day 30 in the control cattle;
- There were statistically significant differences ($p<0.05$) in serum Mg levels between days 0 and 10 in the experimental cattle, between days 0 and 30 in the experimental group, and between day 0 in the experimental group and day 30 in the control group of cattle;
- Statistically very significant differences ($p<0.01$) were found in serum Mg levels between days 0 and 20, 10 and 20, and 10 and 30 in the experimental cattle, as well as between day 10 in the experimental cattle and day 30 in the control group;
- The mean serum levels of Mg in beef cattle were 32.22857 mg/kg, 38.05929 mg/kg, 22.55692 mg/kg, 26.02909 mg/kg and 25.69 mg/kg, as measured on days 0, 10, 20 and 30 in experimental cattle and on day 30 in the control ones, respectively;
- No statistically significant differences ($p>0.05$) were observed between the experimental and control cattle in terms of Ca content of the liver, kidney, muscle and bone samples and Mg content of the sampled liver, kidney and bone (analysis of variance);
- LSD test showed the muscle Mg level to be significantly higher ($p<0.01$) in the control group than in the experimental group of cattle;

- A possible explanation for the absence of increase in Ca and Mg deposition in the blood and the test organs lies in the lower rate of the Na-bicarbonate supplement used, which is in line with the related literature reporting the use of about 200 g cation source/day/calf, relative to the 48.1 g Na-bicarbonate/calf/day used in this study.

Acknowledgements: This study is part of the Technological Development project Ref. No. 20015 "Contemporary Tendencies in Production, Reproduction, Disease Prevention and Health Control in Ruminants Aimed at Their Welfare and Biosafety" supported by the Ministry of Science and Technological Development of the Republic of Serbia

References

Adler S., Roy A., Relma A.S. (1965): Intracellular acid-base regulation. I. The response of muscle cells to changes in CO2 tension or extra-cellular bicarbonate concentration. *J. Clin. Invest.,* **44**: 8-20.

Agricultural Research Council (1980): The Nutrient requirements of Ruminant Livestock. Slough, U.K.: Commonwealth Agricultural Bureaux.

Barzel U.S., Iowsey I. (1969): The effects of chronic acid and alkali administration on bone turnover in adult rats. *Clin Sci.*, **36**: 5l7-24.

Batlle D.C. (1989): Renal tubular acidosis. In: Seldin DW, Giebisch G, editors. The regulation of acid-base balance. New York: Raven Press, 353-390 pp.

Beighle D.E., Boyazoglu P.A., Hemken R.W., Serumaga-Zake P.A. (1994): Determination of calcium, phosphorus and magnesium values in rib bones from clinically normal cattle. *Am. J. Vet. Res*., **55**: 85-89- PMID: 81 41 502.

Bergstrom W.H., Ruva F.D. (1960): Changes in bone sodium during acute acidosis in the rat. *Am. J. Physiol.*, **198**: 1126-1128.

Bettice J.A., Gamble J.L.Jr. (1975): Skeletal buffering of acute metabolic acidosis. *Am. J. Physiol.*, **229**: 1618-1624.

Bettice J.A. (1984): Skeletal carbon dioxide stores during metabolic acidosis. *Am. J. Physiol.* (*Renal Fluid Electrolyte Physiol*. 16), **247**: F326-F330.

Bjorkman C. (1994): Concentrations of sodium, potassium, calcium, magnesium and chlorine in the muscle cells of downer cows and cows with parturient paresis. *Res. Vet. Sci*., **57**: 53-57. PMID: 7973093.

Blaxter K.L., Rook J.A.F., MacDonald A.M. (1954) Experimental magnesium deficiency anaemia in calves. *Br. J. Nutr.,* **11**: 234-246.

Blincoe C., Lesperance A.L., Bohman V.R. (1973): Bone Magnesium, Calcium and Strontium Concentrations in Range Cattle. *J. Anim. Sci.*, **36**: 971-975.

Block E. (1984): Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. *J. Dairy Sci*., **67**: 2939-2948.

Burnell J.M. (1971) Changes in bone sodium and carbonate in metabolic acidosis and alkalosis in the dog. *J. Clin. Invest.*, **50**: 327-331.

Bushinsky D.A., Krieger N.S., Geisser D.I. (1983): Effects of pH on bone calcium and proton fluxes in vitro. *Am. J. Physiol.* (*Renal Fluid Electrolyte Physiol.* 14), **245**: F204-F209.

Bushinsky D.A., Levi-Setti R., Coe F.L. (1986): Ion microprobe determination of bone surface elements: effects of reduced medium pH. *Am. J. Physiol*. (*Renal Fluid Electrolyte Physiol.* 19), **250**: F1090-F1097.

Bushinsky D.A. (1987) Effects of parathyroid hormone on net proton flux from neonatal mouse calvariae. *Am. J. Physiol.* (*Renal Fluid Electrolyte Physiol.* 21), **252**: F585-F589.

Bushinsky D.A., Lechleider R.J. (1987) Mechanism of proton-induced bone calcium release: calcium carbonate-dissolution. *Am. J. Physiol.* (*Renal Fluid Electrolyte Physiol.* 22), **253**: F998-F1005.

Bushinsky D.A. (1988): Net proton influx into bone during metabolic, but not respiratory, acidosis. *Am. J. Physiol.* (*Renal Fluid Electrolyte Physiol.* 23), **254**: F306-F310.

Bushinsky D.A. (1989): Internal exchanges of hydrogen ions: bone. In: Seldin DW, Giebisch G, editors. The regulation of acid-base balance. New York: Raven Press, 69-88 pp.

Bushinsky D.A., Krieger N.S. (1992): Role of the skeleton in calcium homeostasis. In: Seldin DW, Giebisch G, editors. The kidney: physiology and pathophysiology. New York: Raven Press, 2395-2430 pp.

Bushinsky D.A., Ori Y. (1993): Effects of metabolic and respiratory acidosis on bone. *Curr. Opin. Nephrol. Hypertens.*, **2**: 588-596.

Bushinsky D.A., Lam B.C., Nespeca R. (1993): Decreased bone carbonate content in response to metabolic, but not respiratory, acidosis. *Am. J. Physiol.* (*Renal Fluid Electrolyte Physiol.* 34), **265**: F530-F536.

Bushinsky D.A., Wolbach W., Sessler N.E. (1993): Physicochemical effects of acidosis on bone calcium flux and surface ion composition. *J. Bone Miner Res.*, **8**: 93-102.

Bushinsky D.A. (1994): Acidosis and bone. *Miner. Electrolyte Metab.*, **20**: 40-52.

Bushinsky D.A. (1995a): Metabolic acidosis. In: Jacobson HR, Striker GE, Klahr S, editors. The principles and practice of nephrology. St. Louis, MO: Mosby, pp. 924-932.

Bushinsky D.A. (1995b): The contribution of acidosis to renal osteodystrophy. *Kidney Int*, **47**: 1816-1832.

Bushinsky D.A., Krieger N.S. (1996): Regulation of bone formation and dissolution. In: Coe F, Favus M, Pak C, Parks J, Preminger G, editors. Kidney stones: medical and surgical management. New York: Raven Press, pp. 239-258.

Bushinsky D.A., Gavrilov K., Stathopoulos V.M. (1996): Effects of osteoclastic resorption on bone surface ion composition. *Am. J. Physiol.* (*Cell Physiol.* 40), **271**: C1025-C1031.

Bushinsky D.A, Gavrilov K., Chabala J.M. (1997): Effect of metabolic acidosis on the potassium content of bone. *J. Bone Miner Res.*, **12**: 1664–1671.

Carlson G.P. (1990): Clinical Chemistry Tests. 1st Edn. Large Animal Internal Medicine. In: Smith, B.P. (Ed.). Part IV, Chapter, Mosby Company, USA, **22**: 390, ISBN: 0-8016-5062-3.

Chan JCM. Nutrition and acid base-base metabolism. (1981) *Fed. Proc.*, **40**: 2423-8.

DeLuca H.F. (1979): The vitamin D system in the regulation of calcium and phosphorus metabolism. *Nutr. Rev.,* **37**: 161-193.

Dirksen G., Liebich H.G., Brosi G., Hagemeister H., Mayer E. (1984): Morphologie der Pansenschleitmhaut und Fettsaureresorption beim Rind-Bedeutende Faktoren fur Gesundheit und Leistung. *Zbl. Vet. Med. A.*, **31**: 414-430.

Evans J.L., Fish R.E., Lelkes Z.B., Trout J.R. (1976): Hydroxyproline in Serum as a Homeostatic Index for Calcium in Cattle. *Journal of Dairy Science,* **(59) 10:** 1838-1841.

Fontenot J.F., Allen V.C., Bunce G.E., Goff J.P. (1989): Factors influencing magnesium absorption and metabolism in ruminants. *J. Anim. Sci*., **67**: 3445-3455.

Frassetto L., M., Curtis R., Anthony S. (2005): Long-Term Persistence of the Urine Calcium-Lowering Effect of Potassium Bicarbonate in Postmenopausal Women. *The Journal of Clinical Endocrinology & Metabolism*, **90 (2)**: 831-834.

Gerloff J.B. (1988): Metabolic diseases of ruminant livestock: Feeding the dry cow to avoid metabolic disease. *Vet. Clin. Nort. Am. Food Anim. Pract.*, **4**, 379-390.

Goff J.P., Horst R.L.(1995): Dietary potassium, but not calcium (Ca), induces milk fever in dairy cows. *J. Dairy Sci.,* **78**: 185 (abst.).

Goff J.P., Horst R.L. (1997): Physiological changes in parturition and their relationship to metabolic disorders. *J. Dairy Sci*., **80**, 1260-1268.

Goff J.O.: Pathophysiology of Calcium and Phosphorus Disorders. USDA Service, Ames, IA 50010.

Goodman A.D., Lemann J. Jr, Lennon E.J., Relman A.S. (1965): Production, excretion, and net balance of fixed acid in patients with renal acidosis. *J. Clin. Invest.*, **44**: 495-506.

Grace N.D. (1983): Manganese. in The Mineral Requirements of Grazing Ruminants, N.D. Grace, ed. Occasional Publ. No. 9. New Zealand: New Zealand Society of Animal Producers, 80-83 pp.

Grace N.D., Wyatt M.J., Macrae J.C. (1974): Quantitative digestion of fresh herbage by sheep. III. The movement of Mg, Ca, P, K and Na in the digestive tract. *J. Agric. Sci.,* **82**: 321-330.

Greene, L.W., K.E. Webbe, Jr., and J.P. Fontenot (1983) Effects of potassium level on site of absorption of magnesium and other macroelements in sheep. *J. Anim. Sci.,* **56**: 1214-1221.

Jorgensen N.A. (1974): Combating milk fever. *J. Dairy Sci*., **57**, 933-944.

Kaya A., Yakup Akgül Y., Yüksek N. (2008): Studies on the aetiology and treatment of hypophosphataemia developed naturally in cattle from Van region of Turkey). *Medycyna Wet*., **64** (2), 171-174.

Kraut J.A., Mishler D.R., Kurokawa K. (1984): Effect of colchicine and calcitonin on calcemic response to metabolic acidosis. *Kidney Int*., **25**:608–612.

Lemann J. Jr., Lennon E.J., Goodman A.D., Litzow J.R., Relman A.S. (1965): The net balance of acid in subjects given large loads of acid or alkali. *I Clin Invest*; **44**: 507-l7.

Lemann, Jr J, Litzow, J.R., Lennon, E.J. (1967) Studies of the mechanism by which chronic metabolic acidosis augments urinary calcium excretion in man. *J. Clin. Invest*., **46**: 1318-1328.

Levitt M.F., Turner L.B., Sweet A.Y., Pandiri D. (1956): The response of bone, connective tissue, and muscle to acute acidosis. *J. Clin. Invest*., **35**: 98–105.

Liesegang A., Eicher R., Sassi M.L., Risteli J., Kraenzlin M., Riond J.L., Wanner M. (2000): Biochemical markers of bone formation and resorption around parturition and during lactation in dairy cows with high and low standard milk yields. *J.Dairy.Sci.*, **83 (8)**: 1773-81.

Liesegang A., Chiappi C., Risteli J., Kessler J., Hess H.D. (2007): Influence of different calcium contents in diets supplemented with anionic salts on bone metabolism in periparturient dairy cows. *Journal of Animal Physiology and Animal Nutrition*, **91** (**3-4)**: 120-119.

Linkswiler H.M., Zemel M.B., Hegsted M., Schuette S. (1981): Protein-induced hypercalciuria. *Fed. Proc.*, **(40) 24**: 29-33.

Litzow J.R., Lemann J. Jr, Lennon E.J. (1967): The effect of treatment of acidosis on calcium balance in patients with chronic azotemic renal disease. *J. Clin. Invest.*, **46**: 280-286.

Luick J.R., Boda J.M., Kleiber M. (1957): Some biokinetic Aspects of Calcium Metabolism in Dairy Cows. *Am. J. Physiol.,* **189**: 483-488.

Lunn D.P., Mc Guirck S.M. (1990): Renal regulation of electrolyte and acid base balance in ruminants. *Vet. Clin. North. Am. Food Animal Pract.*, **6**:1-28.

Lutz Josephine (1984): Calcium balance and acid-base status of women as affected by increased protein intake and by sodium bicarbonate ingestion. *The American Journal of Clinical Nutrition* **39**: 281-283.

Madias N.E., Kraut J.A. (1989): Uremic acidosis. In: Seldin DW, Giebisch G, editors. The regulation of acid-base balance. New York: Raven Press, 285-317 pp.

Martens H., Rayssiguier Y. (1980): Magnesium metabolism and hypo-magnesaemia. In Digestive Physiology amd Metabolism in Ruminants. Y. Ruckebusch and P. Thivend, eds. Lancastor, U.K.: MTP Press Limited, 447-466 pp.

Martens H., Kubel O.W., Gabel G., Honig H. (1987): Effects of low sodium intake on magnesium metabolism of sheep. *J. Agric. Sci. Camb.,* **108**: 237-243.

Mayland H.F. (1988): Grass tetany. Pp. 511-522 in Ruminant Animal Digestive Physiology and Nutrition, D.C. Churh, ed. Englewood Cliffs, N.J.: Prentice Hall.

McDowell L.B. (1992): Minerals in Animal and Human Nutrition. New York: Academic Press.

Mellau L.S.B., Jørgensen R.J., Bartlett P.C., Enemark J.M.D., Hansen A.K. (2004): Effect of Anionic Salt and Highly Fermentable Carbohydrate Supplementations on Urine pH and on Experimentally Induced Hypocalcaemia in Cows. *Acta vet. scand*., **45**: 139-147.

Minson, D. J. (1990): Forages in Ruminant Nutrition, 1990, New York: Academic Press.

Monti G., Libanore V., Marinaro L., Lala R., Miniero R., Savino F. (2007): Multiple Bone Fractures in an 8-Year-Old Child with Cow's Milk Allergy and Inappropriate Calcium Supplementation. *Ann. Nutr. Metab.*, **51**: 228-231.

Moore L.A.,. Hallman E.T, Sholl L.B. (1938): Cardiovascular and other lesions in calves fed diets low in magnesium. *Arch. Pathol*., **26**: 820-838.

Mora Palma F.J., Ellis H.A., Cook D.B. (1983): Osteomalacia in patients with chronic renal failure before dialysis or transplantation. *Q. J. Med.,* **52**: 332-348.

National Research Council (U.S.) Subcommittee on Beef Cattle Nutrition, National Research Council (1996): Nutrient requirements of beef cattle. Minerals, 54-74.

O' Kelley R.E., Fontenot J.P. (1969): Effects of feeding different magnesium levels to drylot-fed lactating beef cows. *J. Anim. Sci.,* **29**: 959-966.

O' Kelley R.E., Fontenot J.P. (1973): Effects of feeding different magnesium levels to drylot-fed gestating beef cows. *J. Anim. Sci.,* **36**: 994-1000.

Peeler H.T. (1972): Biological availability of nutrients in feeds: Availability of major mineral ions. *J. Anim. Sci.,* **35**: 695-712.

Plavšić K. (1999): Primena atomske apsorpcione spektrofotometrije za određivanje mikroelemenata u zemljištu i vodama. Magistarski rad, Univerzitet u Kragujevcu, Prirodno matematički fakultet.

Poole-Wilson P.A., Cameron I.R. (1975): Intracellular pH and K+ of cardiac and skeletal muscle in acidosis and alkalosis. *Am. J. Physiol.*, **229**: 1305-1310.

Pravilnik o higijenskoj ispravnosti vode za piće ("Sl. list SRJ" 42/98).

Pravilnik o higijenskoj ispravnosti vode za piće ("Sl. list SRJ" 44/99).

Ramberg C.F., Mayer G.P., Kronfeld D.S., Potts J.T. (1976): Dietary calcium, concrentrations and parathyroid hormone concentration in cows. *J. Nutr.* **106**: 671-679.

Reidenberg M.M., Haag B.L., Channick B.I., Shuman C.R., Wilson T.O.G. (1966): The response of bone to metabolic acidosis in man. *Metabolism,* **15**: 236-41.

Remzi Gonul, Abdulah Kayar, Tanay Bilal, Erman O.R.M., Cagla Parkan D.V.M., Tamer Dodurka H., Tevfik Gulyasar, Bora Barutcu (2009): Comparison of Mineral Levels in Bone and Blood Serum of Cattle in Northwestern Turkey. *Journal of Animal and Veterinary Advances* **8** (7): 1263-1267.

Rogovski B. (1981): Die Ernahrungsphysiologische bedeutung von Fleisch und Fett. Bd. 2 der Kulmbacher Reihe, Kulmbach.

Rook J.A.F., Storry J.E. (1962): Magnesium in nutrition of farm animals. *Nutr. Abstr. Rev.* **32**: 1055-1077.

Sinovec Z. (2006) Ishrana i metabolički poremećaji krava u peripartalnom periodu Clinica Veterinaria 2006, 26.06-29.06.2006., Neum, Bosna i Hercegovina.

Sornthep T., Chinatat N., Chairerk K., McDowell L.R., Conrad J.H., Ellis G.L. (1983): Mineral supplementation for beef cattle in the central Thai Villages. *Tropical Animal Production*, **8**: 196-205.

TCORN (1991): A reappraisal of the calcium and phosphorus requirements of sheep and cattle. *Nutr. Abstr. Rev. Ser. B.* **61**: 573-612.

Underwood E.J. (1981): The Mineral Nutrition of Livestock, 2nd Ed. Slough, U.K.: Commonwealth Agricultural Bureaux.

Vukašinović Marija, Mihajlović R., Kostović D. (1989): Kvalitet vode reke Studenice u toku 1987. i 1988.godine. *Zaštita voda '89*, 177-185.

Vukašinović Marija, Kurćubić V., Jovanka Lević (2009): Uticaj koncentracije mikro i makroelemenata u hrani i vodi na njihovu zastupljenost u tkivima junadi u tovu. *XIII međunarodni simpozijum Tehnologija hrane za životinje. 1. Workshop Feed-to-Food, FP7 REGPOT-3. "Tehnologija hrane za životinje, kvalitet i bezbednost*", 29.09.- 01.10.2009. godine, Novi Sad. Zbornik radova, 307-318.

Wacker W.E.C. (1980): Magnesium and Man. Cambridge, Mass.: Harvard University Press.

Wang C., Beede D.K. (1992): Effects of magnesium on acid base status and calcium metabolism of dairy cows fed acidogenic salts. *J. Dairy Sci.*, **75**: 829-836.

Ward G., Harbors L. H., Blaha J. J. (1979): Calcium-containing crystals in alfalfa: Their fate in cattle, *J. Dairy Sci*., **62**: 715-722.

Wylie M.J., Fontenot J.P., Greene L.W. (1985): Absorption of magnesium and other macrominerals in sheep infused with potassium in different parts of the digestive tract. *J. Anim. Sci.,* **61**: 1219-1229.

UTICAJ DODAVANJA NATRIJUM-BIKARBONATA OBROCIMA NA ZASTUPLJENOST KALCIJUMA (Ca) I MAGNEZIJUMA (Mg) U TKIVIMA JUNADI U TOVU

- originalni naučni rad -

Vladimir S. Kurćubić
Agronomski fakultet, Čačak

Zoran Ilić
Poljoprivredni fakultet Priština – Zubin Potok

Marija Vukašinović
Veterinarski specijalistički institut, Kraljevo

Radojica Djoković
Agronomski fakultet, Čačak

Rezime

Ispitivanje uticaja dodavanja Natrijum-bikarbonata u obrok tovne junadi u drugoj fazi tova na koncentraciju Ca i Mg u krvi i tkivima (kosti, mišićno tkivo, jetra i bubreg) izvedeno je na dve grupe životinja, tokom 30 dana. Kontrolna (n=11) i ogledna grupa (n=14) tovne junadi su dobijale identičan obrok, pri čemu je u potpunu krmnu smešu (PKS) za ishranu ogledne grupe umešavano 48.1 g Na-bikarbonata po junetu dnevno. Sistem napajanja tovne junadi obe grupe je istovetan..

Srednje vrednosti koncentracije Ca i Mg u vodi za napajanje tovne junadi (sa moravskog vodovoda) su ispod vrednosti dozvoljenih Pravilnikom o higijenskoj ispravnosti vode za piće ("Sl.list SRJ" 42/98 i 44/99). Srednja vrednost za koncentraciju bikarbonata u vodi bila je 309.202 mg/L, a za alkalitet iste bila je 50.688.

Krv je uzorkovana od tovne junadi ogledne grupe nultog (0.), 10., 20. i 30. dana ispitivanja. Kost (trnasti nastavak repnog pršljena), mišićno tkivo, jetra i bubrezi su uzieti na liniji klanja. Sadržaj Ca i Mg u svim ispitivanim uzorcima poreklom od tovne junadi su određeni metodom atomske apsorpcione spektrofotometrije (AAS) - JUS ISO 6869: 2002, na aparatu UNICAM 969.

Nisu utvrđene statistički značajne razlike u sadržaju Ca u krvnim serumima tovne junadi između kontrolnih perioda, kao i između ogledne i kontrolne grupe. Ne postoje statistički značajne razlike ($p>0.05$) u koncentraciji Mg u serumima tovne junadi između 20. i 30. dana ispitivanja ogledne grupe, između 20. i 30. dana ogledne grupe u poređenju sa vrednostima dobijenim za kontrolnu grupu. Statistički značajne razlike ($p <0.05$) su utvrđene između vrednosti koncentracije Mg u serumima ogledne grupe 0. i 10. i 0. i 30. dana ispitivanja, kao i između 0. dana ispitivanja ogledne grupe i 30. dana ispitivanja kontrolne grupe tovne junadi. Statistički veoma značajne razlike ($p<0.01$) dokazane su između vrednosti koncentracije Mg u serumima ogledne grupe (0. i 20., 10. i 20., 10. i 30. dana ispitivanja), kao i u poređenju vrednosti 10. dana ispitivanja ogledne grupe sa 30. danom ispitivanja kontrolne grupe tovne junadi.

Nema statistički značajnih razlika ($p>0.05$) između sadržaja Ca u uzorcima jetre, bubrega, mišića i kosti i sadržaja Mg u uzorcima jetre, bubrega i kosti junadi ogledne i kontrolne grupe. Sadržaj Mg u mišićima tovne junadi kontrolne grupe bio veoma značajno viši ($p<0.01$) u odnosu na sadržaj Mg u mišićima junadi ogledne grupe.