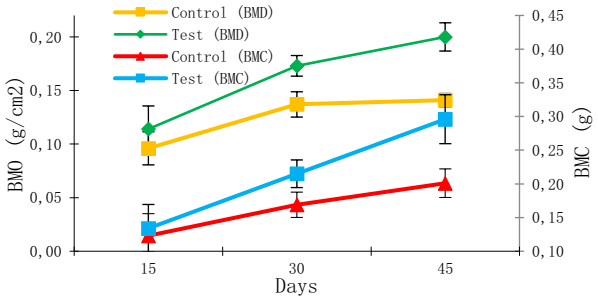
Water Treatment by Magnetic Field Increases Bone Mineral Density of Rats

Abstract: Water treatment using a magnetic field is an attractive but controversial issue with regard to its effects on human health. This study aimed to investigate the effects of water treatment using a magnetic field on the bone mineral density (BMD), bone mineral content (BMC), bone area (BA), bone resistance (BR), blood gas analysis, blood viscosity, and blood biochemical profile of rats. Forty-eight Wistar rats were divided into two groups: control (n = 24) and magnetic water-treated (n = 24). Each of these groups was subdivided into three groups to evaluate three consumption periods (15, 30, and 45 days). The animals were kept in metabolic cages throughout the experiment. A completely randomized design distributed to a 2 × 3 factorial arrangement was used. No significant difference was found in the water intake, dry matter intake, BA, or femoral head resistance between the groups. However, higher anion gap and lower $CHCO_3$ were found in the arterial blood of the magnetic water-treated group. There was significant interaction between the water consumption period and the BR, BMD, and BMC. With 15 days of consumption, there was no difference in the BMC and BR. With 30 days of consumption, the BR (mid shaft), BMD, and BMC showed increases; the increases were greater with 45 days of consumption. In adulthood, every month of the animal is approximately equivalente to 2.5 human years. The consumption of water treated by magnetic field for 45 days provided an effective way to improve BMC in rats (approximately equivalente to 3.75 human years), and can be used to reduce the risk of osteoporosis and fractures.

Keywords: blood biochemical, blood gas, blood viscosity, bone mineral density, drinking water, magnetic field



Bone Mineral Density (BMD, g cm^{-2}) and Bone Mineral Content (BMC, g) of rats drinking magnetically treated water

Introduction: Although magnetism is widely used in the fields of physics, industry, and commerce and its remarkable effects on metals have been known for centuries, there are no conclusive studies on the implications of magnetism on living organisms. However, the Earth is a giant natural magnet that transmits magnetic energy to all living organisms [4]. The development of life is linked to magnetic radiation. Therefore, plants and animals are affected, for better or for worse, by this inevitable phenomenon [4]. One of the applications of magnetism in living organisms is through magnetic water treatment.

Published data on magnetic water treatment are often contradictory, but a physically modified liquid with lower surface tension and higher electrical conductivity, solubility, coagulation and crystallization has been found [1, 21].

From the biological point of view, according to Coey and Cass [9], the influence of the treatment persists for more than 200 h. This persistence of magnetic treatment effects exerts effects on the body after water intake (WI) and is lighter, purer, and smoother compared to water in the normal state. These changes favourably affect the blood flow [20]. Tao and Huang [20] observed that after the blood was exposed to a magnetic field of 1.33 T parallel to the direction of flow for 1 min, the viscosity dropped

from 5.7 cSt to 4.37 cSt (23.3%). They believed that this decrease in the viscosity could be beneficial for blood flow in all kinds of blood vessels. There has also been extensive experimental and theoretical research to determine the magnetic properties of red blood cells. It is generally accepted that red blood cells are paramagnetic with a magnetic susceptibility. Therefore, a strong magnetic field induces dipolar interaction, which causes the aggregation of red blood cells.

Levy et al. [13] observed lower levels of fat in the meat from calves consuming magnetic water. On the other hand, Al-Mufarrej et al. [1] did not observe any differences in the composition of the carcass of broilers that were made to consume magnetic water. Patterson and Chestnutt [16] observed a reduction in dry matter intake (DMI) and poorer feed conversion in lambs, while Sargolzehi et al. [17] did not find any significant differences in the ions and metabolites of blood in lambs.

There are many relationships between blood gas, biochemical profile, viscosity, and other parameters, which help elucidate the mechanisms underlying the effects of magnetic water on bone densitometry. The purpose of the present study was to investigate the effects of magnetic water treatment on the bone mineral density (BMD, g cm^{-2}), bone mineral content (BMC, g), bone area (BA, cm^2), bone resistance (BR, kN m^{-1}), blood gas analysis (bicarbonate, mmol L^{-1}; anion gap, mOsm kg^{-1}), blood viscosity, and blood biochemical profile of rats.

Materials and Methods: This study was carried out at the São Paulo State Agency Agribusiness Technology, Secretary of Agriculture and Food Supply, (APTA-SAA-SP), Department of Basic Science and Department of Veterinary Pathology, São Paulo State University (UNESP), SP - Brazil.

Forty-eight Wistar males rats were divided into two groups: control (n = 24) and magnetic water-treated group (n = 24). Each of these groups was further subdivided into 3 subgroups (n = 8) and paired by body weight to evaluate 3 consumption periods (15, 30, and 45 days) in eight replicates. All the animal experiments were performed under approved conditions in accordance with protocols approved by the Institutional Animal Care and User Committee from APTA-SAA (CEEA/IZ 0178/2013). A completely randomized design with two treatments and three consumption periods, distributed to a 2 × 3 factorial arrangement, was used.

The animals were fed during the experimental period with the same ration (Table 1). The water treatment was performed using a commercial magnetic conditioner (Sylocimol) designed to generate a strong magnetic monopole field of 32,000 Gauss. These devices were inserted into the water troughs of the metabolic cages. The water chemical composition was analysed according APHA [2] and dissolved oxygen was analysed according to the Winkler method [15] (Table 2).

The rats were kept in individual steel metabolic cages throughout the experiment. The initial average weight of the animals was 286 g; the weights of the animals increased to 357–390 g by the end of the experimental period. The rats were randomly assigned to individual steel metabolism cages equipped with stainless steel feeders and individual troughs. The average room temperature during the trial ranged between 22.8 ± 1.0°C (minimum) and 29.2 ± 2.4°C (maximum). The body weight gain (BWG) and metabolic weight ($kg^{0.75}$), WI and DMI, nitrogen balance, and digestibility were observed to determine the effect of magnetic water treatment. The rats were allowed to acclimatize to the crates, and total collection of the faeces and urine was performed in the last 7 days.

At the end of each consumption period, blood samples were collected from the left femoral artery using a cannula for blood gas analysis, blood cell counts, and biochemical profile analysis. A blood sampling kit was used for blood gas analysis (3-mL ventilated syringes with 23G l needle containing freeze-dried lithium heparin) [10]. These blood samples were immediately analysed in a calibrated blood gas analyser set at the body temperature of rats. The samples in the tubes with or without EDTA were centrifuged at 1000 rpm for 5 and 10 min to obtain the plasma and serum, respectively, which were then poured into clean tubes using pipettes. The serum samples were stored at -20°C until analysis. The chemical tests were performed using commercial test kits (LabtestDiagnóstica S.A. Brazil) and a semiautomatic spectrophotometer was used (LabquestDiagnóstica) at wavelengths specific for each blood component for analysis. The blood collected in 10-mL tubes with the anticoagulant K_2EDTA was processed to obtain haematological parameters.

Arterial blood viscosity was measured within 1 h from blood withdrawal after mixing the blood sample with heparin (35 UI mL^{-1}). Blood viscosity was evaluated at shear rates of 450 (η450) and 225 (η225) s-1. A 0.5-mL aliquot was used to determine the shear force (shear stress SS, N m^{-2}) under the shear rate (shear rate, SR, s-1) corresponding to 30 and 60 rpm. Viscosity measurement was performed at 37°C using a cone-plate viscometer (Wells-Brookfield DV-III, Stoughton, USA) equipped with a CP-40 spindle. The determinations were carried out in buffer (for each run) and in the samples at intervals of 1 min between speed increments with an additional 30 s for stabilization.

The BMD (g cm^{-2}), BMC (g), and BA (cm^2) of the right femur were measured using the DPX-Alpha Lunar® densitometer, and BR (kN m^{-1}) of the mid shaft and head of the femur was measured using Universal Test Machine EMIC®, DL3000.

The tests were conducted from late September until early November, 2013. A paired t-test was used to determine statistical significance of the differences between two treatments. The null hypothesis was that the difference in the mean values was zero (H0: mA – mB = 0). The trapping data were analysed with two-way ANOVA (factors: periods (3 levels) and kind of water (2 levels)) using Proc GLM [18], with Tukey's mean separation test (P = 0.10) used for significant factors.

Results: There was no significance interaction between water treatment or the consumption period and DMI and WI, digestibility, and N balance (P > 0.05). However, the weights of the rats treated with magnetic water showed significantly reduced weights compared to the control rats at the end of the experimental period (Table 3).

DMI, WI, nitrogen intake, urine volume, and nitrogen excretion in the faeces were similar between the groups. Nitrogen balance was positive in both groups, but nitrogen retention by the body weight was increased in the rats treated with magnetic water, which probably was the reason for the decreased nitrogen excretion in the urine (Table 3). The ammonium excreted in the urine is produced in the kidneys, where glutamine is metabolized into ammonium ions and bicarbonate [11]. These data not only point to the normal digestion of food proteins, but also point to changes in renal the excretion of ammonium in systemic pH regulation.

Further, no significance interaction was observed between water treatment or the consumption period and the viscosity, biochemical profile, and blood gas level (P > 0.05). The levels of $CHCO_3$ and CO_2 showed unusual reductions with the same pH and anion gap in the arterial blood of the rats in the treatment group (Table 4). There was no difference in blood pH because of the systemic acid-base balance. The skeleton is

actively involved in maintaining stable systemic acid-base balance [7]. Thus, although there was no difference in the blood pH, the additional hydrogen ions on control water (Table 2), could was buffering by bone mineral, including phases as a $CaHPO_4$ and CO_3, associated with calcium, potassium and/or sodium, which has an important roles in regulating bone formation. The additional hydrogen ions led metabolic acidosis and withdrawal of mineral from the bone. In the same way, four hours after the intraperitoneal injection of ammonium chloride into rats, bone sodium level falls by approximately 28% [5]. Rat bones lose approximately 7 mEq/kg of exchangeable bone sodium after 5 h of metabolic acidosis [6]. In the presence of high acid concentrations, bone minerals and calcium can be solubilized and released [7], and these minerals and calcium can bind with hydrogen. In the rats in the treatment group, the increased iCa was not accompanied by addition of hydrogen ions (Table 5). Approximately 60% of the administered hydrogen ions are buffered outside of the extracellular fluid by soft tissue and bone [19]. In addition, the reduction in the number of hydrogen ions in magnetic water may favour bone densitometry by reducing the competition for calcium binding sites.

Water treatment and the consumption period showed a significant interaction with BR, BMD, and BMC ($P < 0.05$). In the first analysis, with fifteen days of consumption, there was no difference ($P < 0.05$) in the BMC and BR between the two groups. In the second evaluation, with thirty days of comsumption, the BR (mid shaft), BMD, and BMC were significantly increased ($P < 0.05$). With forty five days of consumption, the BR, BMD, and BMC showed further increases, all of which were significant ($P < 0.05$), which also in turn increased the differences between the groups (Table 5).

In summary, the BMD, BMC, and BR of the treatment group were higher than those of the control group. Furthermore, consumption of water treated by magnetic field for the longest periods resulted in the highest BMD, BMC, and BR in the rats.

Lin and Yotvat [9] observed a reduction in the fat in sheep carcasses and Levy et al. [8] observed lower fat content in the meat of calves upon consumption of magnetic water. Patterson and Chestnutt [11] observed that consumption of magnetically treated water tended to reduce food intake and the rate of carcass gain and resulted in less efficient conversion of food to carcass gain in lambs. Balieiro Neto et al. [3] observed significantly lower subcutaneous fat thickness in dairy cows consuming magnetic water. Consistent with these reports, our findings showed that the weight gain of rats consuming magnetically treated water is significantly reduced. The lowest weight gain and fat reduction may be mainly due to the increased solubility of calcium and increased lipid metabolism enzymes. It is known that calcium is mainly absorbed by active transport in the upper small intestine; therefore, it moves against a concentration gradient of energy, a process that requires an additional energy source [14].

The results of tests are not always straightforward, which often makes them challenging to interpret and explain. The bicarbonate formed in the kidneys is transported across membranes to the extracellular fluid, where it restores blood bicarbonate levels that were neutralized by systemic metabolic acid production. This bicarbonate production occurs in association with ammonium excretion due to glutamine metabolism. The rate of bicarbonate and ammonium production in the kidneys is regulated in response to changes in the systemic acid-base status and decreases during metabolic alkalosis [11, 17]. It is important to consider that hydrogen ions are not accounted for on the cation side in the anion gap (cations minus anions),

but the decrease in bicarbonate buffer compensation would appear as a bicarbonate deficit, thus increasing the anion gap.

After 45 days, (approximately equivalente to 3.75 human years, [12]), the BR, BMD, and BMC in the treatment group were significantly increased ($P < 0.05$) and the differences between the groups also increased. These effects were attributed to reduced competition with hydrogen ions for calcium binding sites. Given that calcium is a major structural element in bones and is a key player in bone health, calcium absorption and higher iCa favours better densitometry.

There are too many positive and negative ions in body fluids. Most ion channels simply allow ions to flow in or out of the cell. The normal tendency is for the contents inside and outside a cell to balance out in this way. The flow of body fluids means ion flow and results in an electric chain. If there is a magnetic flow, the flow of ions changes and become organized and faster, thus stimulating the ions channel that carry body fluids, increasing the ion flow or increasing to inside and outside of the ions from cell membrane, until there is a balance [9].

Conclusions:

Our findings indicate that consumption of magnetically treated water for more than 45 days provides an effective way to improve BMC in rats and can reduce the risk of osteoporosis and fractures.

Conflict of interest:

There are no conflicts of interest to declare.

Acknowledgements:

We are grateful to Agribusiness Technology State Agency (APTA) and Universidade Estadual Paulista (UNESP) for their contributions and for providing us access to their laboratories for our study.

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Tables

Table 1. Chemical composition of feed on a dry matter basis

Nutrient	Unit	Level	Nutrient	Unit	Level
Dry Matter	g kg^{-1}	870	Vitamin A	UI kg^{-1}	25,500
Crude Protein	g kg^{-1}	230	Vitamin D3	UI kg^{-1}	4,000
Ether Extract	g kg^{-1}	40	Vitamin E	UI kg^{-1}	82
Crude Fibre	g kg^{-1}	50	Vitamin K3	mg/kg	6.4
Ash	g kg^{-1}	100	Vitamin B1	mg/kg	11
Calcium	g kg^{-1}	12	Vitamin B2	mg/kg	12
Phosphorus	mg kg^{-1}	8500	Niacin	mg/kg	219
Sodium	mg kg^{-1}	2,700	Pantothenic acid	mg/kg	90
Magnesium	mg kg^{-1}	500	Vitamin B6	mg/kg	11
Iron	mg kg^{-1}	180	Folic acid	mg/kg	12
Copper	mg kg^{-1}	30	Biotin	mg/kg	0.16
Manganese	mg kg^{-1}	110	Vitamin B12	mcg/kg	40
Zinc	mg kg^{-1}	110	Choline	mg/kg	1,800
Iodine	mg kg^{-1}	1	Lysine	g/kg	12.50
Cobalt	mg kg^{-1}	2	Methionine	mg/kg	3,500
Selenium	mg kg^{-1}	0.20			

Table 2. Chemical composition and characteristics of the magnetically treated water

		Treatments	
	Unit	**Control**	**Magnetic**
Sodium	mg L^{-1}	3.0	3.3

Calcium	mg L^{-1}	6.8	7.0
Magnesium	mg/L	2.6	2.6
Total hardness	mg/L	30	20
Turbidity	NTU*	0.88	0.14
pH "in situ"		6.90	7.31
Total alkalinity	mg L^{-1}	46	45
Carbonate alkalinity	mg L^{-1}	0	0
Total residual chlorine	mg L^{-1}	0.01	0.01
Total chlorine	mg L^{-1}	0.02	0.01
Dissolved oxygen	mg L^{-1}	3.45	4.60
Iron	mg L^{-1}	<0.01	<0.01
Soluble iron	mg L^{-1}	<0.01	<0.01
Fluoride	mg L^{-1}	0.27	0.23
Chloride	mg L^{-1}	1.5	1.0
Sulphate	mg L^{-1}	1.0	1.0
Nitrate as NO_3	mg L^{-1}	<0.15	<0.15
Nitrate as N	mg L^{-1}	0.2	0.2
Nitrite as NO_2	mg L^{-1}	<0.001	<0.001

*NTU = nephelometric turbidity units.

Table 3. Effect of the consumption of magnetically treated water in rats

	Control	Test	CV	SEM	*P*-value
Initial BW (g)	287.69	286.64	7.26	20.86	0.927
Final BW (g)	390.80[a]	357.53[b]	8.41	31.49	0.071
BWG (g day^{-1})	2.291[a]	1.575[b]	27.50	0.531	0.026
DMI (g day^{-1})	25.66	24.35	17.38	4.34	0.646
WI (mL)	35.14	32.51	16.90	5.72	0.487

Urine(mL)	19.12	18.02	15.02	2.79	0.551
DMD (%)	60.35	61.31	7.46	4.54	0.746
NI (g day^{-1})	0.944	0.896	17.34	0.15	0.647
NF (g day^{-1})	0.262	0.234	43.98	0.10	0.695
NA (g day^{-1})	0.682	0.661	9.28	0.06	0.608
NANI (%)	72.24	73.77	9.49	7.01	0.812
NU (g day^{-1})	0.054	0.056	18.63	0.01	0.765
NR	0.626	0.606	8.73	0.05	0.561
NR/BW (g kg^{-1})	1.534 [b]	1.692 [a]	4.84	0.07	0.012
$NR/BW^{0.75}$(g kg^{-1})	6.888 [b]	7.350 [a]	5.13	0.36	0.080
UN (g kg^{-1})	40.12 [a]	37.08 [b]	14.87	5.732	0.092
UF (g kg^{-1})	38.20	38.20	17.17	6.56	1.000
UREA (mg dL^{-1})	54.85	53.83	9.53	5.18	0.729

dry matter intake (DMI), water intake (WI), body weight gain (BWG), urine volume, dry matter digestibility (DMD), N intake (NI), N excreted in the faeces (NF), N absorption (NA), NA in % of NI (NANI), N excreted in the urine (NU), N retention (NR), N retention per BWG, and N retention per $BWG^{0.75}$ (NR BWG^{-1}, NR $BWG^{-0.75}$), urinary N concentration (UN), faeces N (UF) concentration, and plasma urea concentration (UREA).

Table 4. Effect of the consumption of magnetically treated water on viscosity, biochemical profile, and blood gas level.

	Control	Test	CV	SEM	*P*-value
Viscosity 30 (cP)	3.79	3.67	22.36	0.833	0.819
Viscosity 60 (cP)	2.63	2.18	35.58	0.848	0.405
Na (mmol L^{-1})	139.96	139.37	2.08	2.913	0.720
K	4.007	4.005	18.07	0.724	0.996

iCa	1.288 [b]	1.339 [a]	3.433	0.045	0.066
pH	7.32	7.31	0.545	0.039	0.697
pO_2 (mm Hg)	69.38 [b]	86.02 [a]	16.77	13.14	0.043
pCO_2 (mm Hg)	53.85 [a]	46.40 [b]	15.284	7.624	0.081
pHt	7.29	7.28	0.546	0.039	0.572
pO_{2t} (mm Hg)	77.33	96.87	16.42	14.43	0.033
pCO_{2t} (mm Hg)	60.11	51.73	15.32	8.52	0.080
SO_2	91.75 [b]	94.60 [a]	2.593	2.419	0.057
tHb	17.66	15.91	12.17	2.036	0.150
Hct	53.05	47.68	12.16	6.103	0.142
$CHCO_3$	28.66 [a]	25.04 [b]	11.08	2.962	0.050
$ctCO_2$	24.63	22.01	11.78	2.735	0.113
Osmolality (mOsm kg^{-1})	278.8	277.7	1.941	5.402	0.734
Cl	101.10	102.07	1.876	1.907	0.404
Ânion gap	14.70 [b]	16.95 [a]	10.65	1.705	0.047

Means not bearing the same superscript letters within rows are significantly different (P > 0.05)

Table 5. Effects of the consumption of magnetically treated water in rats.

Days of consumption	Control	Test	CV	SEM	*P*-value
	Bone Mineral Content				
15	0.123	0.134	26.48	0.034	0.511
30	0.169 [b]	0.215 [a]	10.19	0.019	0.0003
45	0.201 [b]	0.296 [a]	12.01	0.030	<0.0001

		Bone Mineral Density			
15	0.096 [b]	0.114 [a]	17.82	0.018	0.075
30	0.137 [b]	0.173 [a]	6.92	0.010	<0.0001
45	0.141 [b]	0.200 [a]	6.09	0.010	<0.0001
		Mid shaft Femoral Resistance			
15	317.94	349.89	19.51	65.17	0.377
30	438.33 [b]	531.22 [a]	15.66	75.47	0.033
45	436.06 [b]	567.61 [a]	14.37	72.15	0.005

Within rows, means with different letters are significantly different ($P < 0.05$). Bone mineral density (BMD, g cm^{-2}), bone mineral content (BMC, g), bone area (BA, cm^{-2}), and bone resistance (BR, kN m^{-1})