NC STATE UNIVERSITY

Use of Calcium Chloride in Bottled Water and Sport Drinks

Sensory and Rat Bioavailability Studies

Jonathan C. Allen[1], J. I. Eledah-Omishakin[1], Mary Ann Drake[1], E. M. Dixon[1], M. D. Lovett[1] and Suren Mishra[2]

[1]Dept. of Food Science, North Carolina State University, Raleigh, NC 27695-7624;

[2]Tetra Technologies, Inc., The Woodlands, Texas 77380

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TETRA

Objectives

- Determine calcium chloride threshold and consumer acceptance in water, flavored water, and sport drinks

- Determine the bio-availability of calcium and vitamin D from water

Hypothesis

- Fortification of bottled water and flavored water with $CaCl_2$ will increase the calcium intake and may improve bone development among the many people who consume less than the RDA for calcium and drink bottled water

What Minerals are in Commercial Bottled Water and Sport Drinks?

Mineral Content of Several Bottled Water Brands and Flavored Drinks (mg/L)

	Calcium	Magnesium	Potassium	Sodium	Zinc
Brand A	0.00	0.00	0.11	0.94	0.00
Brand D	0.03	2.40	3.70	3.10	0.00
Brand E	87.50	21.00	1.00	7.40	0.00
Brand F	3.10	1.10	1.90	3.00	0.00
Brand G	4.80	1.40	4.20	0.00	0.00
Brand L	0.00	0.00	0.00	0.00	0.00
Brand M	20.00	5.80	0.00	8.00	0.02
Brand W	116.00	18.00	1.70	3.20	0.00
Drink G	8.90	6.60	7.10	2.30	2.50
Drink P	16.80	4.40	1.20	103.00	0.10
Drink A	8.40	1.20	1.20	450.00	0.00

Threshold and Acceptance Study

- 3-Ascending Force Choice (3-AFC)
  - Panelists are presented with series of 5 set of samples
  - 3 samples/ series (2 are the same, 1 different)
  - Concentration of samples are in ascending order
  - 30 panelist in duplicate trials
  - Statistical analysis:geometric mean and std error

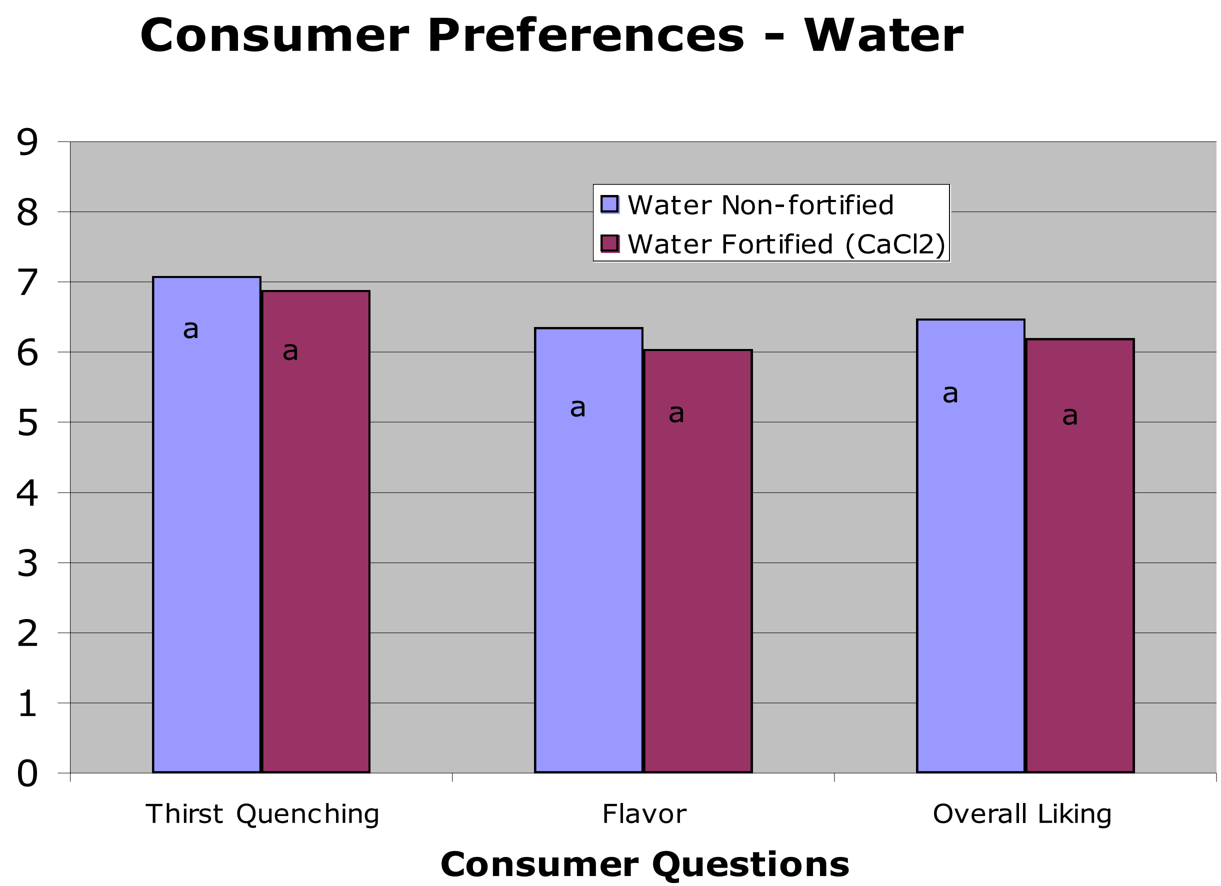
- Acceptance test (n=98) : 9-pt hedonic scale
  - Response are based on likes or dislikes
  - 2 samples are given to panelists one at a time

Sensory Threshold of Calcium Chloride

Tap water	***De-ionized water***
93 ± 3 mg/L *34 ± 1.1mg Ca/L* 0.84 ± 0.01 m*M*	101 ± 3 mg/L *36.5 ± 1.1 mg Ca/L* 0.91 ± 0.01 m*M*
Flavored water	***Sport Drink***
857 ± 8.9 mg/L *309 ± 3.2 mg Ca/L* 7.72 ± 0.08 m*M*	844 ± 9.8 mg/L *305 ± 3.5 mg Ca/L* 7.60 ± 0.09 m*M*

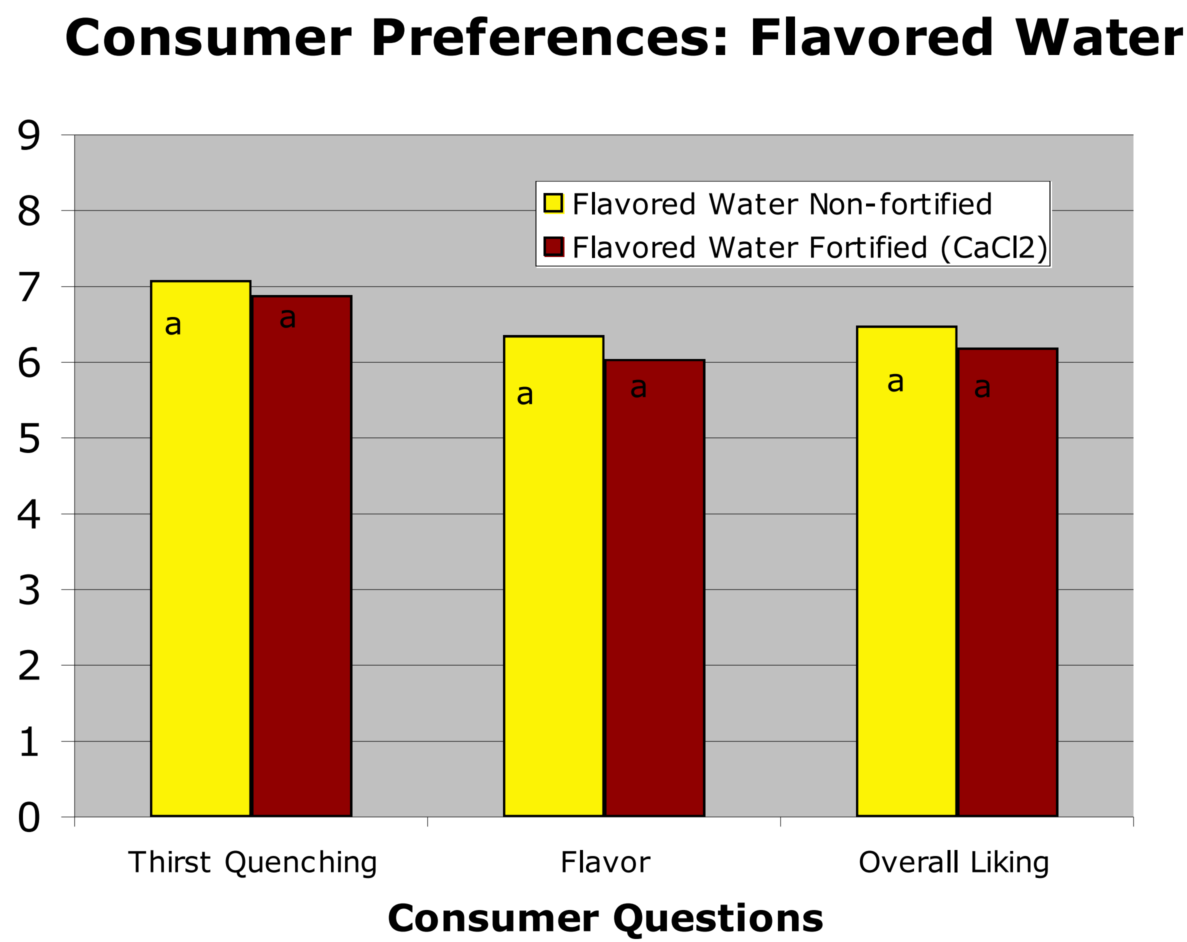
Threshold interpolated from duplicate 3-ascending forced choice tests. N=30

Consumer Acceptance - Water 70 mg/L $CaCl_2$ (25 mg/L Ca)



a : Within a pair, means with the same letter are not significantly different*

Consumer Acceptance -Flavored Water 700 mg/L $CaCl_2$ (250 mg/L Ca)



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Summary

- Flavored water can be more highly fortified with $CaCl_2$ because the threshold is masked
- Consumer acceptance of fortified water is not significantly decreased.

Calcium and Vitamin D Rat Bio-availability Studies

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Phase of Study

- Baseline (21 days old)
  - 5 rats sacrificed
- Depletion Phase (3-4 weeks)
  - Vitamin D-deficient purified diet (AIN 93-G)
    - 5 g Ca/ kg or 2 g Ca/kg
  - Regular water only
  - 10 rats sacrificed
- Repletion Phase: Used fortified water (5-6 weeks)
  - Drinking water fortified
    - 4-5 levels of calcium
    - 4-5 levels of water-soluble vitamin D
  - Group Q and Z (10 each) positive and negative control, respectively

Expt. 1: Vitamin-D complex and Calcium concentrations in water

Vit. D / Ca	0x EDR	.25x EDR	.50x EDR	1.0x EDR	2.0x EDR
0x EDR	Group A		Group B		Group C
.25x EDR		Group D		Group E	
.50x EDR	Group F		Group G		Group H
1.0x EDR		Group I		Group J	
2.0x EDR	Group K		Group L		Group M

Estimated Daily requirement (EDR) for vitamin D= 14.4 µg/L

Estimated Daily requirement (EDR) for Calcium = 2.88 g/L

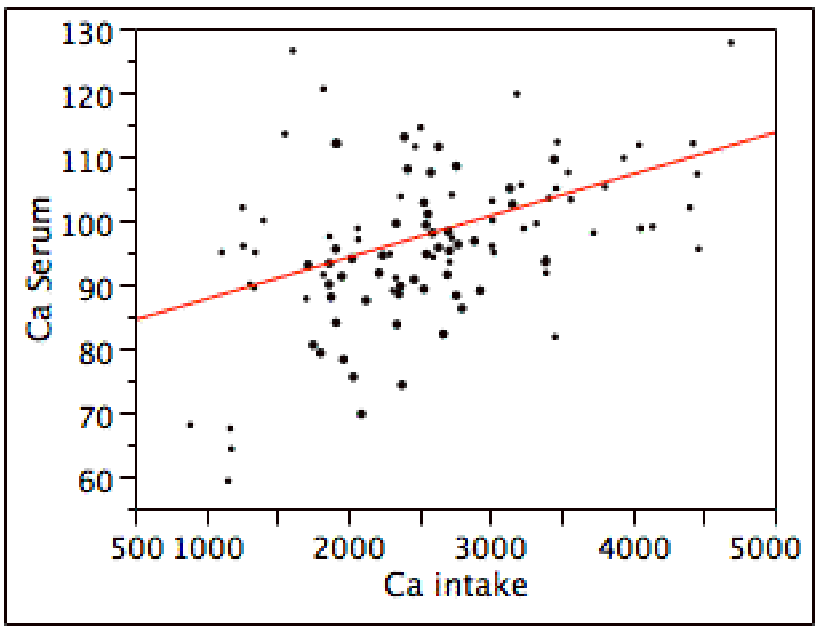
Data collected

- Weight of rats were collected weekly
- Food consumed recorded weekly
- Water consumption recorded weekly
- After each sacrifice
  - Serum and bone were collected in each rat

Serum Calcium vs Total Ca intake (mg/4wk) in rats

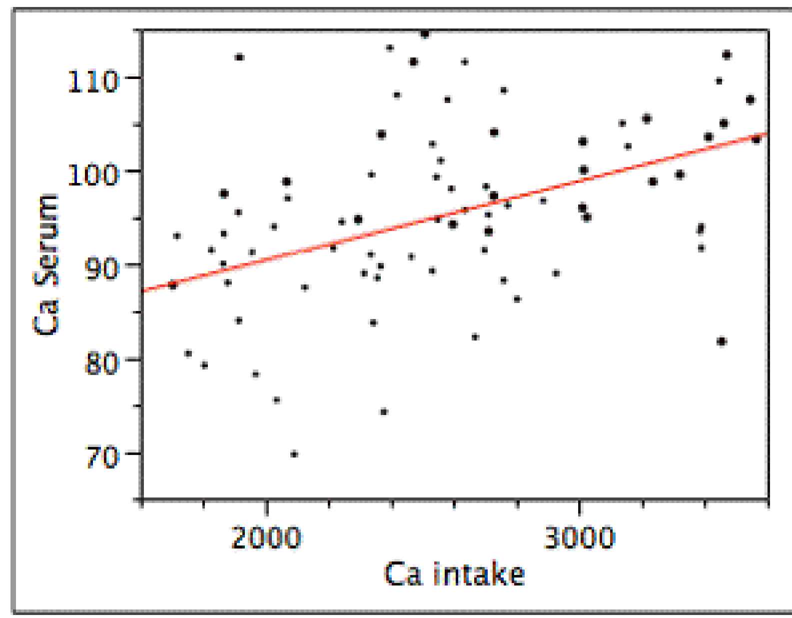
2 g Ca/kg diet

Ca Serum = 81.2+ 0.0065 Ca intake R^2=0.259 *P*= 0.0003*



5 g Ca/kg diet

Ca Serum = 73.6 + 0.0084 Ca intake R^2=0.119 *P*= 0. .015**

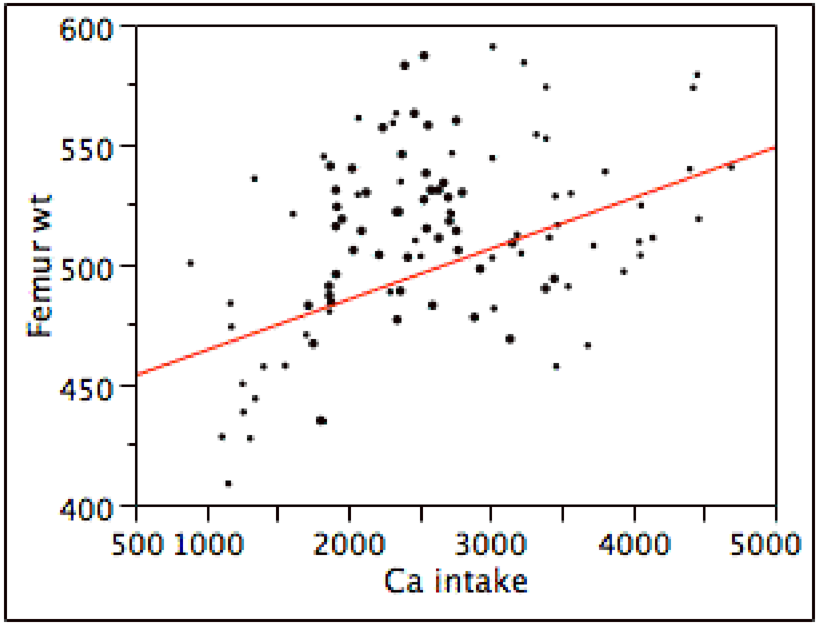


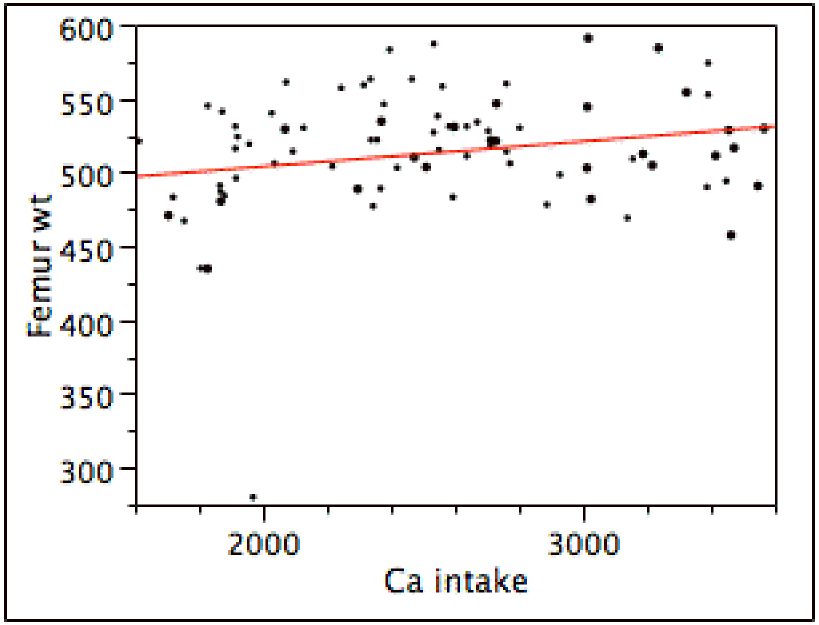
Femur Weight vs Ca intake (mg/4wk) in rats fed 2 g Ca/kg diet (left) or 5 g/kg (right).

2 g Ca/kg diet

5 g Ca/kg diet

- Femur Wt = 443 + .021 Ca Intake, R^2=.315, *P= 0.0001**
- Femur Wt = 470 +.017 Ca Intake, R^2=.027, *P= 0.26*

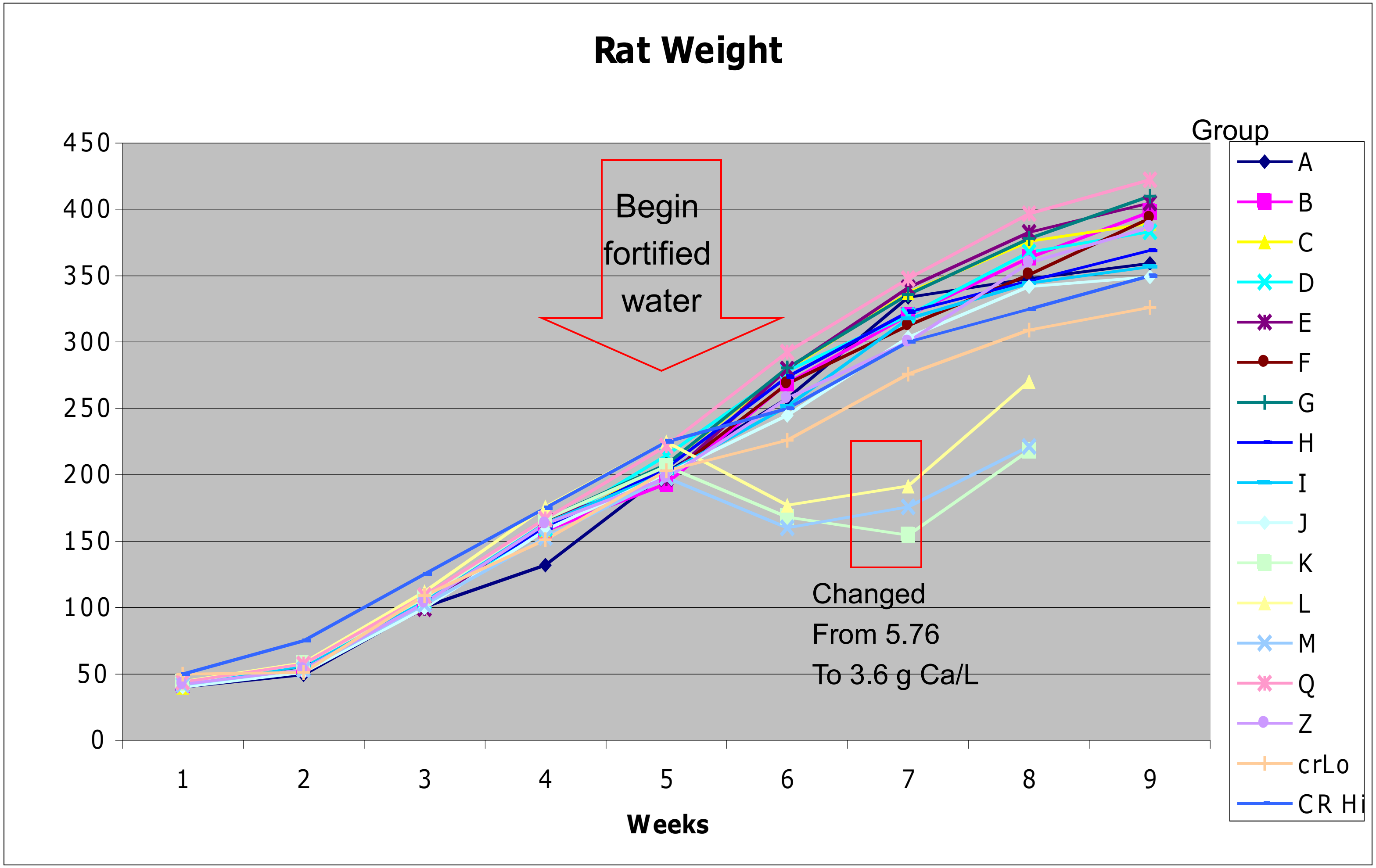




Bioavailability: Calcium Metabolism Measures vs Total Ca intake (mg/4wk) in rats

	2 g Ca/kg diet		5 g Ca/kg diet	
variable	**R^2**	**P (regression)**	**R^2**	**P (regression)**
Final Body Weight (g)	0.118	0.0168*	0.00015	0.9332
Femur Ash Wt (mg)	0.397	.0001*	0.023	0.29
Calcium in Ash (%)	0.165	.0041*	0.001	0.787
Femur Diameter (mm)	0.11	.021*	0.011	0.465
Femur Length (mm)	0.141	.008*	0.008	0.5373
Bone Strength	0.11	.021*	0.006	0.579

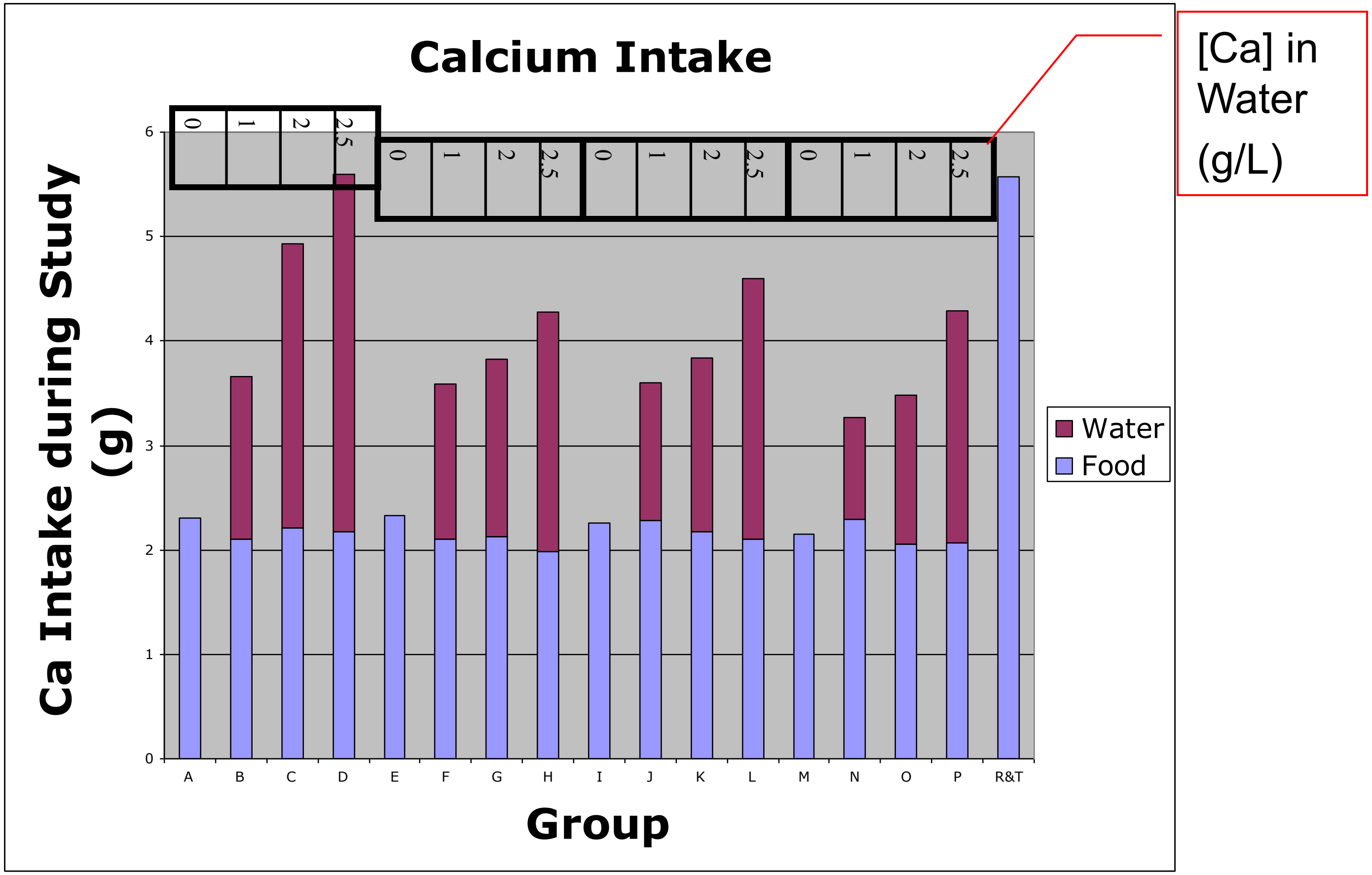
Rat Growth - Trial 1



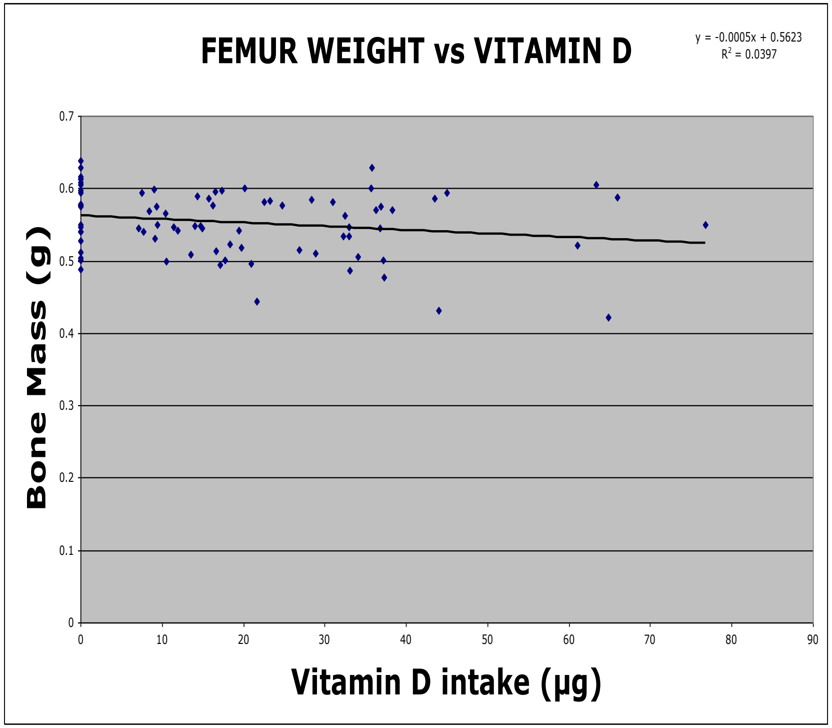
Experiment 2: Calcium and Vitamin-D-Fortified Flavored Water

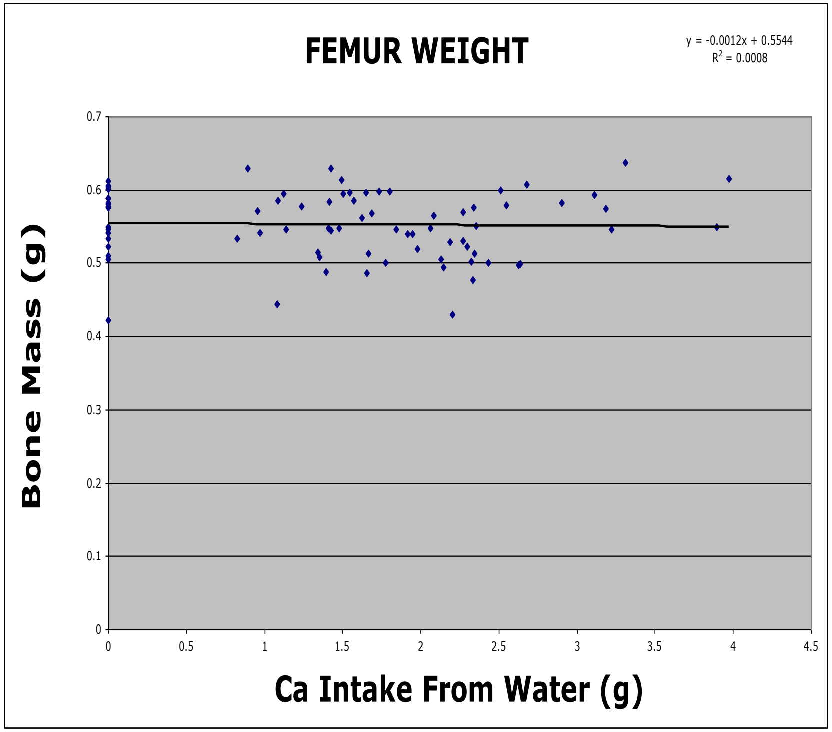
[Ca] (g/L)/ [Vitamin] D µg/L	**0**	**1.0**	**2.0**	**2.5**
0	A = 5 rats	B = 5 rats	C = 5 rats	D = 5 rats
10	E =5 rats	F = 5 rats	G = 5 rats	H = 5 rats
20	I = 5 rats	J = 5 rats	K = 5 rats	L = 5 rats
40	M = 5 rats	N = 5 rats	O = 5 rats	P = 5 rats

4-Week Calcium Intake

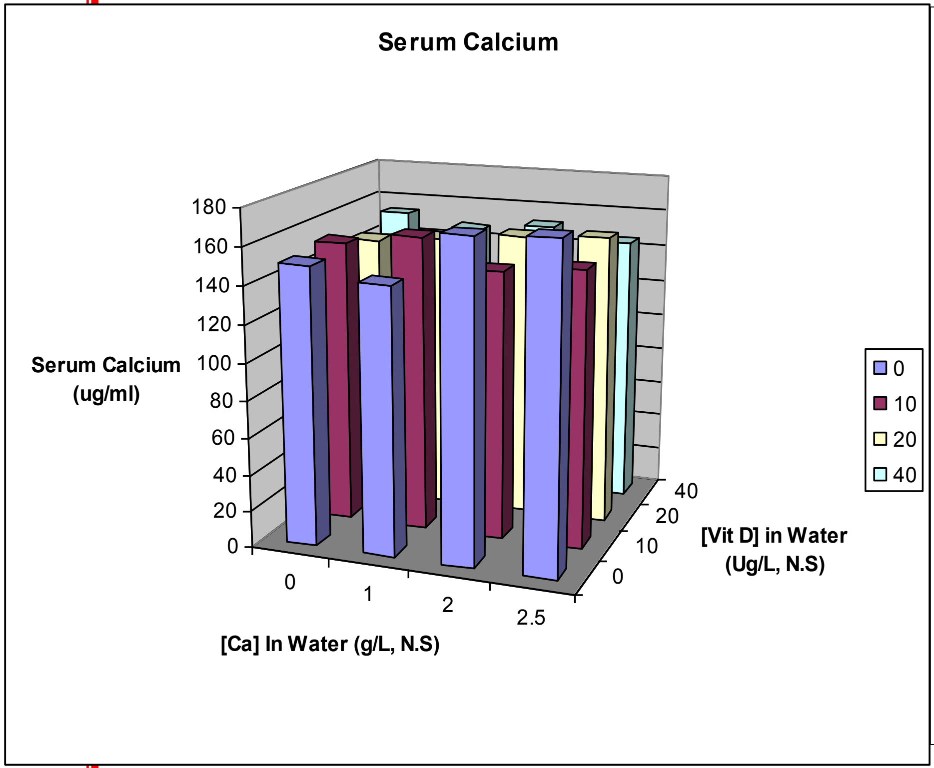


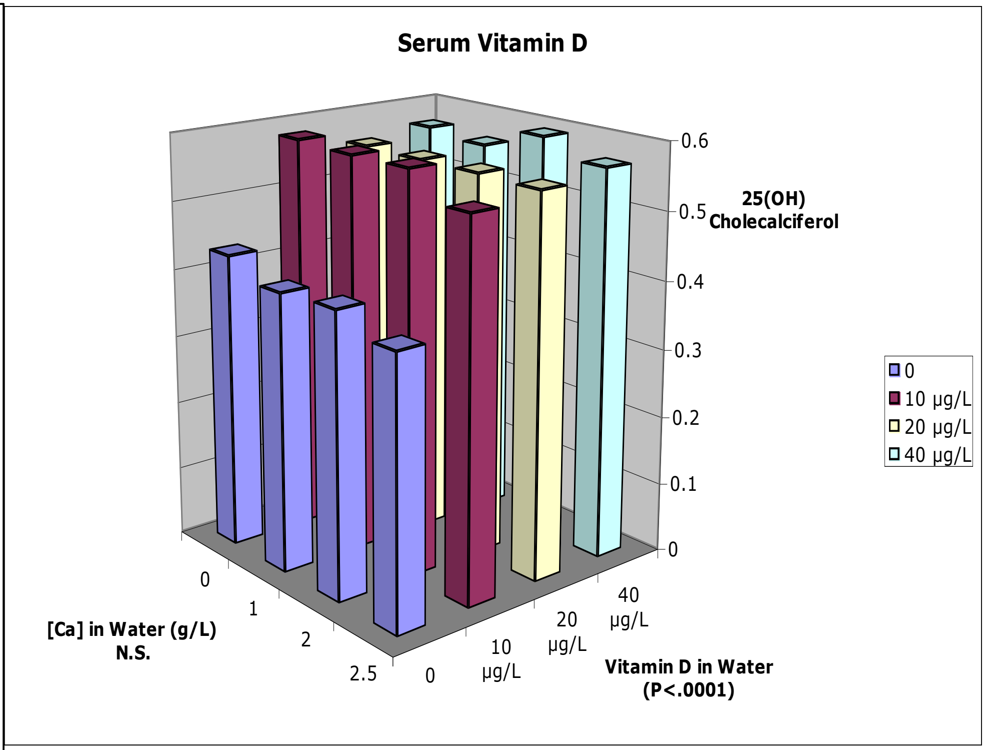
Calcium and Vitamin D Intake from Water did not Affect Bone Mass





Water Fortification Effects on Serum [Calcim] and [Vitamin D]





Conclusion

- Bottled water and similar thirst-quenching beverages could be fortified with $CaCl_2$ at higher levels than currently used in beverages to increase calcium intake
- Water fortification at the threshold for taste would be labeled “not a significant source”; sport drink fortification at the threshold could provide 15% of DV per serving
- Outcomes for bone development would be similar to other dietary calcium sources