Water and Manufacturing Process Effect on Cow's Milk Content in Essential Inorganic Elements

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Abstract

The objective of this study was to determine the effects of mineral content of drinking water on mineral content of cow's milk, and to evaluate the effect of skimming process on mineral content of milk. The mineral content of milk is particularly important to the infant food formula industry, whilst milk products cover significant proportion of adult requirements in inorganic elements. Presently water is not considered feed and official controls in EU are restricted to complete feed for ruminants (complementary feed and forages), excluding water quality and content. Nevertheless, water is the main constituent (87%) of milk. Thirty Holstein Friesian cows, with the same milk production; parity and stage of lactation where randomly allocated in to two equal groups and fed the same ration but had access to different water supply, in a changeover design. Additionally, milk samples with different fat content 0, 1.5 and 3.5% were taken during the year, from manufacturing milk supplies, representing the 90% of Greek milk pool. The elements determined were Ca, Mg, Zn, Mn, Cu, Fe by the use of atomic absorption spectroscopy, and P was determined through UV-VIS spectrophotometer. Water did not have significant effect on the content of Ca and P but significant effect on Mg, Cu and Mn content of milk. Milk fat removal significantly increased the content of Ca, P, and Mg. Manufacturing process did not affect micro element content. The elements Zn and Fe were not affected by manufacturing process neither by drinking water. Food composition tables should be updated as macro mineral content of milk is reduced gradually corresponding to higher yielding cows; whilst trace element content tend to increase as a result of higher proportion of concentrates fed.

Keywords: drinking water; cow's milk; inorganic elements; skim milk.

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1. Introduction

Minerals are constituents of the bones, teeth, soft tissue, muscle, blood, and nerve cells. They act as catalyst for many biological reactions within the body, and the utilization of nutrient in food [1]. Thus, it is extremely crucial, for the consumer, to ensure that all factors affecting end product quality and composition are appropriate accessed and controlled. There is a debate whether or not milk content in inorganic elements can be altered through nutrition. According to NRC [2] diet doesn't affect macro mineral content since a very small proportion exists as free ions in milk, but are bind with other organic molecules and especially caseins, thus the content of those elements is correlated to the protein content of milk [3]. In contrast other researchers report that the content of macro and trace elements in milk depends upon the content of these elements in soil and feed, which varies considerably among and within countries [4,5]. The reason for these discrepancies

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might be that too many factors are affecting inorganic element content in milk, such as breed [6], health [7], herd [3], feed [8], altitude [9], stage of lactation [10], milk yield, age of cows [11], season [12], bioavailability of each element in every food and even the type of forage conservation [13]. Presently water is not considered feed and official controls in EU are restricted to complete feed for ruminants (complementary feed and forages), excluding water quality and content. Nevertheless, water is the main constituent (87%) of milk. Drinking water effect on milk composition is neglected in animal studies although its importance has been recognized in human studies [14,15]. Recently Khan et al. [16] have reported the marginal effect of drinking water on production status of buffaloes, whilst Zhou et al. [17], in a cohort study, have reported a correlation between the inorganic element composition of water and that of milk. It is the first trial that direct effect of drinking water on milk synthesis is reported.

Additionally, it has not been reported yet the mineral content of Greek milk pool and the effects of skimming process on end product composition. The general perception [18] is that the de fattening process doesn't negatively affect inorganic elements' content but it hasn't been reported yet whether different manufactures and season plays any role on this issue [19]. Thus, the three most critical macro minerals for human nutrition Ca, P and Mg, as though as the four micro minerals Zn, Cu, Fe, Mn where upper limits in complete feed (without taking into account water) have been established, according to European legislation [20], were determined. According to Zambelin et al. [21], there is insufficient research work on the content of minerals in milk and dairy products, which is paradox as the milk may provide 10-20% of daily dietary intake of minerals in Europe.

2. Materials and methods

Thirty Holstein Friesian cows, with the same milk production (of the previous lactating period); parity (2nd and 3rd) and stage of lactation (middle lactation 3-4 months) where randomly allocated in to two equal groups and fed the same ration (Table 1; total mixed ration, TRM) but had access to different water supply; immediately after milking (outdoor), in a changeover design, with a period of 15 days, and a 10 days washout period. The animals were milked twice daily; the production was recorded at the 5 last days of each period, and milk samples (1% of every milking) where taken at 10th and 15th day of each period, and composed for every day.

Table 1. Composition of lactating cows diet (%, as-fed basis)

Item	Diet
Alfalfa hay	5.0
Corn silage	44.0
Soybean meal, 44% crude protein	19.5
Whole cotton seed	5.0
Barley grain	10.0
Wheat grain	10.0
Wheat bran	4.0
Dicalcium phosphate	0.65
Limestone, ground	0.85
Salt	0.4
Vitamin premix [1]	0.1
Trace minerals[2]	0.5
Net energy for lactation (Mcal/kg)	1.57
Crude protein (%)	15.6
NDF (%)	37.1
ADF (%)	22.2
Calcium (%)	0.91
Phosphorus (%)	0.64
Magnesium (%)	0.3
Copper (ppm)	22.2
Iron (ppm)	93.3
Manganese (ppm)	62.8
Zinc (ppm)	83.4

1) Supplied per kg of concentrates mixtute: vitamin A (acetate), 6,600 IU; vitamin D_3 (cholecalciferol), 880 IU; vitamin E (DL-α tocopheryl acetate), 44 IU; vitamin K (as menadione sodium bisulfate complex), 15 IU.

2) The inorganic trace minerals were added at a concentration to provide 100% of the NRC (2001) requirement estimates for Cu, Fe, Mn, and Zn. Cu (sulfate); Fe (sulfate); Mn (oxide); Zn (oxide).

Proximate chemical analysis

Dry matter (DM) was determined by drying in an oven [22; method 934.01]. Ash was determined by ignition in a muffle furnace [22; method 942.05]. The CP was measured as Kjeldahl N x 6.25 [22; method 984.13] and accordingly for milk. Ether extract (EE) was determined according to AOAC [22; method 920.39]. Acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined using AOAC [22; method 973.18] and NDF using AOAC [22; official 2002.04]. The NDF was assayed without a heat stable amylase and expressed inclusive of residual ash. Additionally, ADF was expressed inclusive of residual ash.

Mineral analysis of water

Atomic absorption spectrophotometer (PerkinElmer 700 USA) was used to quantify the inorganic minerals in the water samples by using the method reported by [23]. The following elements were determined in water samples in (mg/l): Calcium (Ca); Magnesium (Mg); Iron (Fe); Zinc (Zn); Manganese (Mn); Copper (Cu). Water sampling and analysis was performed every 5 days (Table 2).

Table 2. Mean content of different water sources in essential minerals

Item (ppm) (n3)	Water 1 (8 samples)	Water 2 (8 samples)	SEM	P
Ca	104a	169b	8	*
Mg	21a	84b	4	*
Zn	0.1a	0.03b	0.001	*
Fe	0.02a	0.04b	0.0007	*
Cu	0.05a	0.02b	0.0008	*
Mn	0.003a	0.08b	0.0002	*

a, b: Mean values with different letters in the same row significantly differ ($P<0.05$)
n= number of independent samples analyzed for each sample

Sampling and Analysis of milk samples

Sampling trial 1

Milk samples from each cow were taken at 10th and 15th day in every period (60 for every treatment). Milk samples for mineral analysis were refrigerated at 4 °C, for 1-2 days, until tested. Prior to analysis milk samples remained at room temperature for 5 minutes and the temperature was monitoring, whilst held on a milk stirrer [22; official method 925.21 about preparation of milk test samples]. A reduced sample of 2 ml was taken of each bottle by the use of a sterile syringe (equally; from the bottom, the middle and the top of milk bottles).

Sampling trial 2

Additionally, equal samples (40 for each treatment) were taken for 0, 1.5 and 3.5% in fat content from five manufacturers (covering the 90% of Greek milk pool), twice (at the middle of the first and third month) every season (totally 120 samples; 360 sub samples). The procedure proposed by AOAC [22; official method 968.12] was followed for sampling and transportation of milk samples.

Inorganic elements' chemical analysis

Wet mineralization in a microwave Xmars oven (CEM Corporation Matthews, North Carolina USA) in the presence of a mixture of 6 ml nitric oxide 65% and 1 ml H_2O_2 30% (Merck; HPLC grade), in accordance with the relevant standards of the manufacturer, was applied to diet and milk samples prior to analysis. The elements Ca, Mg, Zn, Cu, Fe, Mn were determined by the use of flame atomic absorption spectroscopy by the use of a Perkin Elmer AA analyst 700 (Perkin Elmer co., Waltham, Massachusetts USA) according to AOAC [22; official method 965.09 revised; for the determination of the micro elements Cu and Mn, two extra working solutions were used for building the standard curves; 0.1 ppm and 0.5 ppm] single element lamps were used for higher accuracy. P was determined by the use of a Perkin Elmer Lamda 25 UV-VIS reflectance spectrophotometer (Perkin Elmer Inc., Waltham, Massachusetts USA) according to [22; official method 965.17]. All measurements were made in triplicate and standards were included (CRM) in each run (according to ISO 17025). Certified standard solutions were used for standard curves (sigma-aldrich co.**;** certified analysis standard solution material 1000 μg ±1/ml for all the elements; for P reflects orthophosphate content). Ultraviolet extra pure water MilliQ instrument (Merck Inc., Darmstadt, Germany) was used for 0 curve point and for rinsing the instrument parts (plastics) that came in contact with a milk sample before each run.

Statistical analysis

The data were analyzed statistically with the use of SAS [24] software. The analysis of the results was carried out using the mean data of the last week (2 measurements) of each experimental period (changeover design). Fixed effects in the first trial included cow, water source and period (interactions proved not significant and excluded from the model). In the second trial (repeated measures design) manufacturer, season and manufacturing process (fat removal) were set as fixed effects. The significance of differences between means was estimated by the Tukey's range test at P < 0.05.

3. Results and discussion

Drinking water significantly affected Mg, Cu and Mn content (P<0.05). In opposite, the levels of Ca, P, Zn and Fe were not affected significantly by the source of water (Table 3).

Table 3. Effect of water on mineral content of cows' milk

Item (ppm) (n3)	Water 1 (60 samples)	Water 2 (60 samples)	SEM	P
Milk yield	26.4	26.7	1.6	NS
Fat content	3.7	3.8	0.2	NS
Protein content	3.5	3.4	0.1	NS
Ca	967	955	55	NS
P	973	969	57	NS
Mg	114a	122b	7	*
Zn	4.3	4.1	0.23	NS
Fe	0.41	0.42	0.05	NS
Cu	0.32a	0.21b	0.03	*
Mn	0.062a	0.089b	0.0007	*

a, b: Mean values with different letters in the same row significantly differ (P<0.05)
n= number of independent samples analyzed for each sample

The 0% fat milk had higher content in Ca, P and Mg compared to 3.5%. Additionally, the 1.5% milk had higher Ca and P content compared to 3.5%, indicating that fat removal increases Ca, P and Mg content, linearly (P<0.05). Manufacturing process did not affect Zn, Cu, Fe, and Mn content (Table 4).

Table 4. Effect of manufacturing process (fat removal) on mineral content of milk

Item (ppm) (n 3)	Full fat milk (3.5%) (40 samples)	Low fat milk (1.5%) (40 samples)	Skim milk (0%) (40 samples)	SEM	P
Ca	916a	941b	978c	49	*
P	969a	1003b	1039b	52	*
Mg	109a	114ab	121b	5	*
Zn	4.5	4.4	4.3	0.19	NS
Fe	0.46	0.44	0.47	0.04	NS
Cu	0.27	0.27	0.25	0.02	NS
Mn	0.075	0.076	0.08	0.0006	NS

a, b, c: Mean values with different letters in the same row significantly differ (P<0.05)
n= number of independent samples analyzed for each sample

Minerals and trace elements contribute to the buffering capacity of milk, the maintenance of milk pH, the ionic strength of milk and milk's osmotic pressure. Mean Ca content in present study was 916 mg L^{-1} which is somewhat below the normal range [25]. This could be attributed to the fact that milk yield of cows is increasing due to higher genetic potential and thus the content of macro elements tend to reduce [11]. Sikiric et al. [8] have reported that farms fed the same ration but different hay had large variations in Ca content. It seems that milk Ca content could be affected by the type of nutrition but in a very narrow range. Ca and P are the major minerals found in milk, required by young, and are mostly associated with the casein micelle structure [2], thus large variations are not expected for these elements. These could explain that drinking water did not affect Ca content of milk (Table 3). On the other hand, manufacturing process affected Ca content with skim milk having higher Ca content (Table 4). It has been reported that skimming increases the concentration of mineral nutrients [26]. This is probably happens because Ca is mostly distributed in the colloidal phase of the milk [27].

Mean P content in present study was 969 mg L^{-1} which in accordance with the values reported by Coulon et al. [13]. P is important for energy production, muscle contraction, bone health and many other biochemical reactions. Lately, there is increased awareness about environmental pollution caused by P excretion. Present study indicates that there is no meaning of increasing P levels above standard levels as its content is relatively stable in milk; whilst moderate changes in ingested P does not affect milk yield [28]. Manufacturing process affects P content at the same pattern as Ca (Table 4).

Mg content in water is positively correlated with Mg concentration in milk. Mg is a key player in energy metabolism, as it acts as an activator of many enzymatic reactions such as glycolysis, fat and protein metabolism, it is necessary for membrane stability and neuromuscular, cardiovascular, immune and hormonal functions. The average Mg content of Greek milk pool was 109 mg L^{-1} which is a very common value [29, 21]. Manufacturing process is affecting Mg content linearly where the lower fat content results in higher Mg content.

Average milk content in Zn was 4.5 mg L^{-1} which is higher compared with values reported by other researchers [1, 5] 3.5 mg L^{-1}, but within the range of normal values [30]. Zn is required for the structure and activity of more than 300 enzymes, whilst adequate Zn intake is necessary for many physiologic systems as immunity, reproduction, taste etc [31]. Anyhow, according to present findings, Zn is not affected substantially by water source neither manufacturing process. The vast majority of values for Zn content in cow’s milk is between 3 – 5 mg L^{-1} [32] and in very rare cases values out of that range have been reported [33, 34]. Zn in milk is mostly bound to casein, but some is bound to lactoferrin [35]. These results confirm that Zn cannot be largely manipulated through nutrition which is in line with Flynn and Power [36].

Mean Fe content in present study was 0.46 mg L^{-1} which is lower compared to other reports [5] 0.78 mg L^{-1} but within the normal values [37]. It is well known that milk is lacking Fe which is an essential element for oxygenation of the body tissues. Fe in milk is bound to lactoferrin, xanthine oxidase (an enzyme associated with the cell membrane) and some to caseins. Fe content was relatively constant and was not affected by the water source neither manufacturing process.

According to present study, it seems that drinking water affects Cu content of milk ($P<0.05$). It has been reported that the transition element cations (Cu, Mn, Zn) have concentrations in blood, tissues and milk that are largely independent of the intake, as they relate to regulation of gut absorption and changing metabolic demands [38]. Probably, this is not the case for Cu as values ranged between 1.98-160 μg/l in cow’s milk have been reported [32].

Mean Cu content in present trial 270 μg L^{-1} was higher compared to previous reports [32], but at the same range with Sikiric et al. [8]. These differences can be attributed to the higher content of Cu in concentrates (premixtures) and the higher concentrate to forage ratio of the ration; fed to high producing ruminants. Accordingly, higher Cu content in cow’s milk during indoor period than during the outdoor grazing period has been reported by O’ Brien et al. [12]. Moreover, in countries where grazing is a common practice throughout the year such differences could not be detected [39].

Manufacturing process does not affect Cu content significantly. Cu is bound to the caseins, to b-lactoglobulin, lactoferrin and a small proportion to the milk fat membranes [21]. Nevertheless, milk is not a sufficient source of Cu as it contains negligible amounts, thus the increment of Cu content of milk through diet and water is desirable.

Present data suggests that drinking water has significant effect on Mn content of milk and this is in line with Zhou et al. [17] who found a positive correlation between water and milk concentration in Mn. One reason could be that the absorption of Mn in feed is much lower than other minerals. Accordingly, the 2001 Nutrient Requirements of Dairy Cattle [40] put Mn availability from feedstuffs at 1%. Johnson et al. [41] in a human study reported that the absorption of Mn from the water (7.8-10.2%) is higher compared to food (1.4-5.5%). Manganese oxide is the most common form of Mn found in well waters. Although Mn is commonly included as one of the minerals to test for, in the assessment of water quality, there are no reports of adverse effects in livestock from drinking water high in Mn [42]. In opposite, it has been reported that Mn in infant formulas during reconstitution could exceed maximum levels, with detrimental effects on infant health [43]. The EPA [44] water quality guidelines for human drinking water have a secondary standard maximum guideline of 0.05 ppm for Mn. Present findings supports the opinion that microelements absorption from water is higher in comparison with feed; and, additionally, the inorganic element content of drinking water is directly affecting milk synthesis. Average Mn levels were 75 μg L^{-1} which is higher in comparison with previous reports [1] 26-55 μg L^{-1} and [32] 20 μg L^{-1}. Mn is a cofactor for a number of important enzymes. Mn has been rarely studied in cow's milk and the factors affecting its content are not well known. Iyengar [45] stated that Mn concentrations in milk could be altered by dietary means. Extreme values ranged from 1.09 μg L^{-1} [34] till 65 μg L^{-1} [46] have been reported. Manufacturing process did not alter significantly milk content in Mn; even though this element is found to milk fat membranes [12]. Mn was the mineral with the lowest concentration in cow's milk in present study.

The contents of three trace elements out of four tested, were higher compared to previous reports. This is probably revealing that higher concentrate to forage ratio fed to high producing ruminants, accompanied with sorter or without grazing period, compared to previous decade [47] increase micro mineral content of milk.

4. Conclusions

Drinking water is clearly affecting end product composition; thus upper limits in the ration should take into account drinking water source. Inorganic element content in the drinking water has never been taken into account in animal studies; whilst this is an extremely important factor especially at micro level. People consuming skim milk do not miss anything regarding inorganic element intake. Food tables reporting milk synthesis should be updated, as higher milk yields, over the time, have impact on inorganic element content of milk.

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