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Research Article

Latitude and Skimming 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 mineral variation of cow's milk as affected by latitude (north or south Europe; reflecting different feeding practices, i.e. grazing or not), and manufacturing process (different fat content). The mineral content of milk is particularly important to the infant food formula industry, whilst milk products cover a significant proportion of adult requirements in inorganic elements. Milk samples of pasteurized full fat milk were taken according to the origin of milk; southern Europe or north Europe during autumn; reflecting different feeding practices. Additionally, milk samples with different fat content 0, 1.5 and 3.5% were taken during the year, from manufacturing milk supplies. 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. Milk fat removal significantly increased the macro mineral content Ca, P, and Mg. Manufacturing process did not affect micro element content. Latitude did not have significant impact on the content of macro minerals, but significant effect on Cu and Mn content. Higher Cu content in milk of south origin was probably related to higher concentrate to forage ratio fed. Higher Mn content in milk of north origin could be attributed to hay or/and drinking water of high industrialized countries. The elements Zn and Fe were not affected by manufacturing process neither by latitude. It was concluded that only Cu can be substantially manipulated through animal nutrition. Food composition tables should be updated as macro inorganic element 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.

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 manipulate, as so as possible, the end product's composition by natural means. Presently the lacking elements are added artificially into the milk. 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 bound with other organic molecules. In contrast, other researchers report that the content of macro and trace elements in milk depends upon the content of these elements in the soil and feed, which varies considerably between and within countries [3,4]. The reason for these discrepancies might be that too many factors are affecting inorganic element content, such as breed, health [2], feed [5], stage of lactation, milk yield, age of cows [6], season [7], bioavailability of each element in every food and even the type of forage conservation [8]. Thus, it is too hard to perform an *in vivo* feeding trial, excluding all the other factors affecting inorganic milk content. To overcome these obstacles, some researchers have tried to identify the parameters affecting inorganic element composition in milk through sampling by the manufacturing milk supplies [7,9 and 10]. In Greece, due the environmental conditions (high temperatures in the summer; lack of pasture), the vast majority of cows (over 90%; [11] are fed indoors during the year with high concentrates to forage ratio. The main forages used are corn silage and secondly alfalfa hay, with both of these crops having high water demands. The milk is produced by high productive Holstein-Friesian cows on large farms (of over 100 animals), as in the rest of the Europe. This has increased the productivity of animals, but has an impact on the cost of producing milk. Thus, the country is now producing the half of the quota that was allowed to produce in the past and self-sufficiency on cow's milk products is under 30%. In contrast, in northern Europe, a large proportion of cows are grazing during March to October [11], and thus forages are covering a larger proportion of cows' needs in nutrients, especially at this period. These differences in feeding practices, due to latitude, reflect differences in raw milk prices to the farmer which in Greece is about 5-10 cents higher compared to northern European countries. This has led the Greek milk industries to import fresh milk from northern European countries at cheaper milk overlaps transport cost. This situation enables the comparison of milk composition as affected by latitude; which reflect different feeding practices, due to environmental conditions. In the present trial, autumn was selected as the most appropriate sampling period in order to eliminate seasonal variations and extreme differences in environmental conditions. Seasonal differences in the concentration of milk in some elements have been reported [7,12], whilst these seasonal differences were probably caused by different contents of microelements in the feed ration [13]. On the other hand Reykdal et al. [10] note that within country geography doesn't affect the mineral content of milk products. It is the first trial that inter comparison between different countries at the same period of time is reported.

Another question is whether or not manufacturing process is affecting mineral composition in milk, to which extent and which of them [14]. The general perception [15] is that the 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. Thus, the three most critical macro minerals for human nutrition Ca, P and Mg, as though as the four micro minerals where upper limits in the ration have been established Zn, Cu, Fe, Mn according to European legislation, were determined. According to Zambelin et al. [16], 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. An additional aim of the study was to identify the elements where large variation exists and thus which can be easily manipulated through animal nutrition (i.e. Manipulate the end product composition by natural means).

Materials and Methods

Milk samples, from 4 retail markets (3 sub samples of 1 lt for every manufacturer at each market and for every sampling date which was the same with date of production; in order to access different lot numbers; patch), were taken during October (every 6 days; access to differential dates of production and lot numbers), according to raw milk origin (north or south Europe; 60 for every treatment) from 6 different manufacturing milk supplies (20 of each manufacturer; totally 120 samples; 360 sub samples). Also, equal samples (40 for each treatment) were taken in 0, 1.5 and 3.5% fat content from five manufacturers, twice (at the middle of the first and third month) every season (totaling 120 samples; 360 sub samples). The procedure proposed by AOAC [17] (official method 968.12) was followed for sampling and transportation of milk samples. Milk samples for mineral analysis were stored frozen at 3 °C, for 1-2 days, until tested. Prior to analysis milk bottles of 1 lt remained at room temperature for 5 minutes and the temperature was monitored, whilst held on a milk stirrer (AOAC [17]; 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 top of milk bottles). 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 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 [17]; 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 lambs 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 (AOAC [17], official method 965.17). All measurements were made in triplicate and standards were included (QC's from inter laboratory test materials) 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 by one-way ANOVA for non-orthogonal designs, with the use of SAS [18] software. Fixed effects in the first trial included manufacturer and latitude (milk origin), and in the second trial manufacturer, season and manufacturing process (fat removal). The significance of differences between means was estimated by the Tukey's range test at $P < 0.05$.

Results

Latitude significantly affected Cu and Mn content (P<0.05). In opposite, the levels of Ca, Mg, P, Zn and Fe were not affected significantly by the latitude (Table 1). The 0% fat milk had a 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 2).

Table 1: Effect of latitude on mineral content of full fat (3.5%) milk.

Item (ppm)	South	North	SEM	P
Ca	926	905	65	NS
P	962	977	67	NS
Mg	113	120	8	NS
Zn	4.4	4.3	0.24	NS
Fe	0.42	0.44	0.05	NS
Cu	0.31a	0.22b	0.03	*
Mn	0.064a	0.087b	0.0007	*

a, b: Mean values with different letters in the same row significantly differ (P<0.05).

Table 2: Effect of skimming process (fat removal) on mineral content of milk.

Item (ppm)	Full fat milk (3.5%)	Semi skimmed milk (1.5%)	Skimmed milk (0%)	SEM	P
Ca	879a	931b	978c	59	*
P	893a	1003b	1039b	62	*
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	0.075	0.0006	NS

a, b, c: Mean values with different letters in the same row significantly differ (P<0.05).

Discussion

Minerals and trace elements contribute to the buffering capacity of milk, the maintenance of milk pH, the ionic strength of milk and the milk's osmotic pressure. Mean Ca content in the present study was 916 mgL^{-1} which is somewhat below the normal range [19]. 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 tends to reduce [6]. Sikiric et al. [5] 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 the major minerals found in milk, required by young, and are mostly associated with the casein micelle structure NRC [2], thus large variations are not expected for these elements. On the other hand, the manufacturing process is affecting Ca content with skim milk having higher Ca content (Table 2). It has been reported that skimming increases the concentration of mineral nutrients [9]. This is probably happening because Ca is mostly distributed in the colloidal phase of the milk [20]. Mean P content in the present study was 969 mgL^{-1} which in accordance with the values reported by Coulon et al. [8]. According to the present study, milk's P content cannot be easily manipulated through nutrition. 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. The present study indicates that there is no meaning of increasing P levels above standard levels as its content is relatively stable in milk. Manufacturing process affects P content in the same pattern as Ca. 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 hormone functions. The average Mg content was 117 mgL^{-1} which is a very common value [12,16]. Mg content was not affected by latitude. The manufacturing process is affecting Mg content linearly where the lower fat content results in higher Mg content.

Average milk content in Zn was 4.4 mgL^{-1} which is higher compared with values reported by other researchers [1,4], 3.5 mgL^{-1}), but within the range of normal values [21]. Zinc is required for the structure and the activity of more than 300 enzymes, whilst adequate Zn intake is necessary for many physiologic systems as immunity, reproduction, taste etc. [22]. Anyhow, according to present findings, Zn was not affected substantially by latitude neither manufacturing process. The vast majority of values for Zn content in cow's milk is between 3000–5000 μgL^{-1} [13] and in very rare cases values out of that range have been reported [23,24]. Zn in milk is mostly bound to casein, but some are bound to lactoferrin [25]. These results confirm that Zn cannot be largely manipulated through nutrition, which is in line with Flynn and Power [26]. Mean Fe content in the present study was 0.44 mgL^{-1} which is lower compared to other reports [4]; 0.78 mgL^{-1}) but within the normal values [27]. 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 latitude neither manufacturing process. Mean Cu content in present trial 270 μgL^{-1} was higher compared to previous reports [13], but at the same range with Sikiric et al. [5]. According to present study, it seems that Cu content can be manipulated through nutrition, and the Cu level can be easily doubled. In contrast 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 [16]. Nevertheless, milk is not a sufficient source of Cu as it contains negligible amounts.

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 the regulation of gut absorption and changing metabolic demands [28]. Probably, this is not the case for Cu as values ranged between 1.98-160 μg/l in cow's milk has been reported [13]. Additionally, higher Cu content in cow's milk during the indoor period than during the outdoor grazing period has been reported by O' Brien et al. [7]. These differences can be attributed to the higher content of Cu in concentrates (premixtures) and the higher concentrate to forage ratio of the ration during the indoor period.

Average Mn levels were 75 μgL^{-1} which is higher in comparison with previous reports [1]; 26-55 μgL^{-1}, [13]; 20 μgL^{-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 [29] stated that Mn concentrations in milk could be altered by dietary means. Extreme values ranged from 1.09 μgL^{-1} [24] till 65 μgL^{-1} Rojas et al. [14] have been reported. The present data suggest that latitude has a significant effect on Mn content in milk. Anyhow, the higher Mn content in milk of northern countries origin could be attributed to drinking water, due to environmental pollution of the highly industrialized countries in the north. Johnson et al. [30] 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%). Drinking water effect has never been taken into account in animal studies, although its importance has been recognized in human studies [31]. Manufacturing process did not affect significantly milk content in Mn, even though this element is found to milk fat membranes [7]. Mn was the mineral with the lowest content in cow's milk in the present study. The contents of three trace elements out of four tested, were higher compared to previous reports revealing that higher concentrate to forage ratio fed to high producing ruminants (sorter grazing period compared to previous decade) increase micro mineral content of milk.

Conclusion

Environmental pollution of northern EU countries is obvious in milk composition, and specifically Mn content. Latitude, reflecting different feeding practices, did not affect significantly milk content in Ca, P, and Mg. The inorganic elements that showed large variation and thus can be easily manipulated by natural means are first Cu and secondly Mn. 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 an impact on inorganic element content of milk.

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