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Original article

Nickel content in raw cow's, ewe's and goat's milk

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Abstract — Changes in concentrations of nickel present in cow's, ewe's and goat's milk were studied monthly ($n = 360$ samples) by using graphite furnace atomic absorption spectrometry. The mean concentrations of nickel are 15.0 ± 3.81, 18.6 ± 2.50 and 13.6 ± 2.46 $\mu g \cdot kg^{-1}$ of fresh weight for cow's, ewe's and goat's milk respectively. Two-factor (species and time period) variance analyses were made on the three types of milk investigated and Tuckey's mean homogeneity test ($P < 0.05$) was also carried out for the formation of homogeneous groups per species for nickel content. Significant changes ($P < 0.001$) were determined over time in the nickel content of the three species studied and two homogeneous groups were formed, one for cow's and goat's milk and an other for ewe's milk with the higher nickel content. For the three species, the monthly changes of the nickel content correspond to a mathematical model of 3rd grade and these models cannot be utilised to make predictions over time. © Inra/Elsevier, Paris.

raw milk / nickel / graphite furnace absorption atomic spectrometry

Résumé — Teneur en nickel du lait cru de vache, de brebis et de chèvre. Les changements dans les concentrations de nickel présent dans le lait cru de vache, de brebis et de chèvre ont été étudiés chaque mois ($n = 360$ échantillons) utilisant la spectrophotométrie d'absorption atomique par four de graphite. Les concentrations moyennes de nickel ont été de $15,0 \pm 3,81$, $18,6 \pm 2,50$ et $13,6 \pm 2,46$ $\mu g \cdot kg^{-1}$ de poids frais dans le lait de vache, brebis et chèvre, respectivement. Des analyses de variance de 2 facteurs (espèce et période de temps) ont été effectuées sur les trois lait, ainsi que le test de Tuckey sur l'homogénéité moyenne ($P < 0,05$), afin de former des groupes homogènes par espèce selon sa teneur en nickel. Au cours de cette période, des changements significatifs ont été observés dans la teneur en nickel des trois espèces étudiées et deux groupes homogènes sont apparus, l'un comportant du lait de vache et de chèvre et l'autre du lait de brebis, ayant la teneur la plus élevée en nickel. Pour les trois espèces, les changements mensuels dans la teneur de nickel correspondent à un modèle du 3e degré et ces modèles ne peuvent pas être utilisés pour faire des prédictions dans le temps. © Inra/Elsevier, Paris.

lait cru / nickel / spectrométrie d'absorption atomique par four graphite

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1. INTRODUCTION

The present interest in the nutritional significance of minerals and trace elements has markedly increased in function of their participation in many enzymatic reactions and a large number of body functions. With respect to nickel, its functions are not completely understood and to establish this trace element as an essential element in man is a subject under discussion. There is substantial evidence that nickel is essential to animals, and is probably true that it may have a function in the human body [5, 15, 20–24, 30, 33] although the specific biochemical functions of nickel in higher animals, including man, have not yet been defined. For this reason, this element has still not been accepted as essential [25] and the National Research Council [19] has not set up any official recommendations for dietary intake for this trace element since there are no reliable data on which to base estimates of human requirements. However, some suggestions have been made by authors such as Nielsen [20] who proposed a recommended daily nickel intake of 75 μg/d.

On the other hand, data on the actual nickel content of milk and dairy products are limited and the values reported are subject to considerable variation [13]. Significant changes in nickel content of the milk according to different geographical areas were established which indicates that the geographical characteristics of the area and the environmental contamination is closely related to the nickel content in pastures [10]. In general, the mineral content of milk may vary greatly and is influenced by numerous factors involved in its secretion from the mammary gland such as the lactation period [7], climate, season [35], breed of animal, type of feeding, etc. [34]. However, from a food safety and nutritional point of view, it is of special interest to determine the fluctuations in the milk composition of the three main milk-producing species, not taking into consideration individual factors but basing results on milk in bulk throughout a period of time, to see if these fit into a predictable time model.

The objectives of the present study were (1) to determine and compare the nickel content in raw cow's, ewe's and goat's milk and (2) to investigate if there were differences in the concentrations of nickel in the milk of the three main milk-producing species attempting to fit these seasonal changes to a mathematical model in order to make predictions over time.

2. MATERIALS AND METHODS

2.1. Samples

Ten samples of raw cow's, ewe's and goat's milk were taken monthly throughout one year, forming a total of 360 samples. The cow's milk samples (Frisona breed) were collected from a bulked refrigerated collection by Lactaria Andaluza-RAM (Sevilla) before any handling and sampling monthly from 430–450 animals. The ewe's milk samples (Merina breed) were taken from 20 individual milkings from six herds in the Valle de los Pedroches (Córdoba) due to the seasonality of animals giving birth in this species and the lack of cooperatives or centres able to give samples; these milks were mixed and ten samples were taken. The goat's milk samples (Serrana-Andaluza breed) were obtained from refrigerated tanks of 'QUESOL', a goat-keeper's cooperative in the province of Córdoba, and included milk from 74 herds comprising 140–150 animals.

2.2. Sample analysis

The samples were collected in clean hermetically-sealed polypropylene bottles and kept under refrigeration (4 °C) until the time of their processing. In no case was any metal instrument used and the use of glass containers was restricted to the shortest time possible. For the analysis of the samples, the method of [18] was followed. Milk samples (50 g) were weighed in crucibles and, once the sample was dried at 100 °C, this was incinerated in a furnace applying the following mineralization stages: 90–250 °C (ramp time 1 h, hold time 1 h), 460 °C (ramp 2 h, hold time 8 h) and 460 °C–100 °C (ramp time 2 h).

This programme was carried out using a Naberthem N9 furnace equipped with a C16 Program Controller (Lilienthad, Bremen, Germany). The ash was extracted with 2M-HNO_3 (2 mL), dried on a thermostatic hot plate and replaced in the furnace for a further 1 h at 460 °C. The resulting white ash was recovered using 2M-HNO_3 (5 mL) and 0.1M-HNO_3 (20 mL) in a 25 mL volumetric flask and stored in propylene flasks under refrigeration (4 °C).

The determinations were performed with a Perkin-Elmer model 2100 atomic absorption spectrophotometer connected with a Perkin Elmer HGA-700 graphite furnace and M-2 100 Multielement Program Software. Argon as internal and external gas, a hollow cathode lamp for nickel, a deuterium lamp as a background corrector and graphite pyrolytically-coated tubes with L'vov platform were employed. In order to optimize the analytical signal, diverse tests with different lamp intensities (18–25 mA), temperature ranges (800 °C–1 550 °C for pre-atomization and 2 000 °C–2 500 °C for atomization) and different volumes o700f sample injection were applied. A study of standard additions was carried out to prevent nickel losses and to corroborate the linear calibration of the apparatus. With regard to the chemical modifier, 2 g of $NH_4H_2PO_4$ + 0.2 g of Mg $(NO_3)\cdot 6\ H_2O$ in 100 mL of deionized H_2O were tested but no advantageous effect was observed. Instrumental conditions and graphite furnace program settings for nickel assays are shown in *table I*.

To calculate the detection limit (X_{blank} + 3 *SD*), the definition and criteria established by IUPAC were followed [2, 14], as the lowest concentration of a substance that the analytical process can reliably detect using a confidence limit for 1-α of 0.99 (α, significance level or probability of committing a Type I error). The detection limit was 2.65 $\mu g\cdot L^{-1}$ and the concentration limits obtained (minimum detectable concentration) was 1.3 $\mu g\cdot kg^{-1}$. The analytical precision of the method was obtained by calculation of a between-assay variation coefficient from the results of ten different analyses carried out at different times on a sample of dried milk [3, 4]. The resulting coefficient of variation was 28 %. The sensitivity of the assay was 2.2 $\mu g\cdot L^{-1}$.

The accuracy of was monitored by two types of studies: a spiked recovery test and an analysis of *Skim Milk Powder* (BCR 63). For both studies, analyses were done on three replicates of 2 g and these samples were analyzed in parallel following the analytical procedure used in this work. The mean recovery percentages in spiked test was 97 % with a relative standard deviation (RSD) of 10.6 %. The nickel concentration certified in BCR 63 is 11.2 ± 1.7 $\mu g\cdot kg^{-1}$ and the concentration found was 12.1 ± 2.01 (108 %, RSD 16.6 %).

2.3. Statistical analysis

Data obtained from the chemical analysis of the samples were evaluated statistically by descriptive parameters; analysis of variance (ANOVA) and Tuckey's mean homogeneity test 'Honest Significant Differences', which allowed

Table I. Instrumental conditions and graphite furnace programme for determination of nickel. **Tableau I.** Conditions instrumentales et programme du four graphite pour déterminer le nickel.

Wavelength	232.0 nm
Slit width	0.2 nm
Intensity	25 mA
Injection volume	10 µL
No. of injections per sample	2
Standards	5, 10, 15 and 20 $\mu g\cdot L^{-1}$

	Furnace steps				
	Drying		Pre-atomization	Atomization	Cleaning
Temp (°C)	110	300	1 000	2 200	2 650
Ramp (s)	10	15	10	0	1
Holp (s)	30	30	10	5	2
Ar $mL\cdot min^{-1}$	300	300	300	0	300

the formation of homogeneous groups by an association of classes of statistically similar concentrations [17, 31].

3. RESULTS AND DISCUSSION

Table II shows the mean nickel concentrations in raw milk from the three main milk-producing species (cow, ewe and goat), determined monthly throughout one year, and the results obtained broadly agreed with those of the literature for cow's milk, but for ewe's and goat's milk are lower than the quoted values, although there was a wide variability in the latter (*table III*). By means of two-factor (species and time) variance analyses, statistically significant differences ($P < 0.001$) were determined for the nickel content of the three milks investigated and throughout the time period studied. The interaction between species and time was also determined and showed a minor significant grade ($P < 0.01$).

In view of these results, Tuckey 'Honest Significant Differences' (HSD) ($P < 0,05$) test were performed for the formation of homogeneous groups from annual concentrations of nickel among species and, for ewe's milk, a single group was formed, which displayed the highest levels of nickel *(table II)*. Tuckey tests ($P < 0.05$) were also performed between the monthly concentrations in the different species (*table II*) and their trends did not correspond, in any of the three species, to any defined seasonality for the nickel content. This phenomenon may be due to the action of some factor of a

Table II. Nickel content (μg·kg^{-1} fresh weight) of raw milk samples from different† species (mean ± s.d.).

Tableau II. Concentration en nickel (μg·kg^{-1} de poids frais) dans les échantillons de lait cru † provenant des différentes espèces (moyenne ± d.t.).

	COW	EWE	GOAT
September	$15.5^{a, b, c} \pm 2.1$	$20.0^{a, b} \pm 1.4$	$14.5^{a} \pm 4.9$
October	$19.0^{a} \pm 0.2$	$18.0^{a, b} \pm 1.6$	$15.5^{a} \pm 2.1$
November	$18.0^{a, b} \pm 4.2$	$17.0^{a, b} \pm 0.3$	$11.5^{a} \pm 2.2$
December	$14.0^{a, b, c} \pm 1.1$	$18.5^{a, b} \pm 0.5$	$15.0^{a} \pm 1.4$
January	$10.5^{b, c} \pm 2.1$	$13.5^{b} \pm 2.2$	$12.0^{a} \pm 1.1$
February	$11.0^{b, c} \pm 1.4$	$22.0^{a} \pm 2.3$	$12.0^{a} \pm 0.6$
March	$13.0^{a, b, c} \pm 2.8$	$16.5^{a, b} \pm 0.7$	$11.0^{a} \pm 1.4$
April	$20.0^{a} \pm 0.7$	$20.5^{a, b} \pm 0.3$	$18.5^{a} \pm 0.7$
May	$10.0^{b, c} \pm 0.5$	$17.5^{a, b} \pm 0,1$	$13.5^{a} \pm 0.1$
June	$15.0^{a, b, c} \pm 1.4$	$20.0^{a, b} \pm 1.4$	$12.5^{a} \pm 0.8$
July	$15.0^{a, b, c} \pm 1.3$	$21.0^{a, b} \pm 1.5$	$13.5^{a} \pm 0.6$
August	$20.0^{a} \pm 2.7$	$19.0^{a, b} \pm 1.2$	$14.0^{a} \pm 1.3$
Total	$15.0^{x} \pm 3.8$	$18.6^{y} \pm 2.5$	$13.6^{x} \pm 2.5$

† 120 milk samples obtained during 1 year for each species from 430–450 cows, 120 ewes and 140–150 goats. $^{a, b, c}$ Tuckey homogeneous ($P < 0.05$) groups between time. $^{x, y}$ Tuckey homogeneous ($P < 0.05$) groups between milk.

† 120 échantillons de lait obtenu sur 1 année pour chaque espèce, provenant de 430–450 vaches, 120 brebis et 140–150 chèvres. $^{a, b, c}$ Groupes homogènes de Tuckey ($P < 0,05$) entre temps. $^{x, y}$ Groupes homogènes de Tuckey ($P < 0,05$) entre types de lait.

Table III. Nickel content ($\mu g \cdot kg^{-1}$) in raw milk from other authors.
Tableau III. Concentrations en nickel ($\mu g \cdot kg^{-1}$) du lait cru indiqué par d´autres auteurs.

Milk type	Mean ± S.D.	Range	Reference
Cow milk			
		10–250	Heikonen (1973)
	94 ± 42	28–180	Franco et al. (1981)
	20.8 ± 6.8		Pertoldi et al. (1984)
	18.9 ± 1.7		Gabrielli and Pertoldi (1984)
		16–81	Fischbach and Potter (1986)
	60 ± 9		Alegría et al. (1988)
		65–129	García et al. (1990)
	25	4–60	Souci et al. (1994)
Ewe milk			
	225 ± 32	181–297	García et al. (1981)
	101 ± 22	49–220	Franco et al. (1981)
	230		Souci et al. (1994)
Goat milk			
	186 ± 21	142–216	García et al. (1981)
	86 ± 23	56–118	Franco et al. (1981)
	190		Souci et al. (1994)

greater weight, such as for instance the strong influence of the type of feeding, the agroclimate conditions, the environmental contamination and the lactation period [7, 10, 34] in the variability of the nickel content of the milk.

In *figure 1*, the mean concentrations of nickel are shown for each month, with 95 % intervals of confidence around them, together with the annual intervals of confidence of the three species (A) and the monthly changes of each species for the trace element studied (B). It can be observed that the annual intervals for cow's and goat's milk overlap each other while the interval corresponding to ewe's milk is independent from the rest of the species (*figure 1A*). *Figure 1B* shows the curves calculated as optimal fits to the monthly evolution of each species which in all cases corresponds to 3rd grade polynomial algorithms. These models supply acceptable fits but cannot be used as prediction models and are therefore of a merely illustrative character. From an observation of the seasonal changes, it can be deduced that the three milk types showed wider specific margins of confidence (*figure 1A*) and monthly fluctuations (*figure 1B*) in their nickel content, possibly due to the effect of the reduced number of individuals contributing milk to each sampling. In any case, the fluctuations of the goat and ewe milk may be due to what was found by Ford et al. [7], who indicated that the seasonal variations were mainly due to alterations in nutrition and that a mineral supplement corrected these variations.

3.1. Nutritional estimation of the nickel content in the three milk types

To calculate the nickel dietary intakes, only data on the consumption of cow's milk in Spain are available and not for the other species. In the case of the cow's milk, the mean intake in Spain is 301 mL/d [16] and,

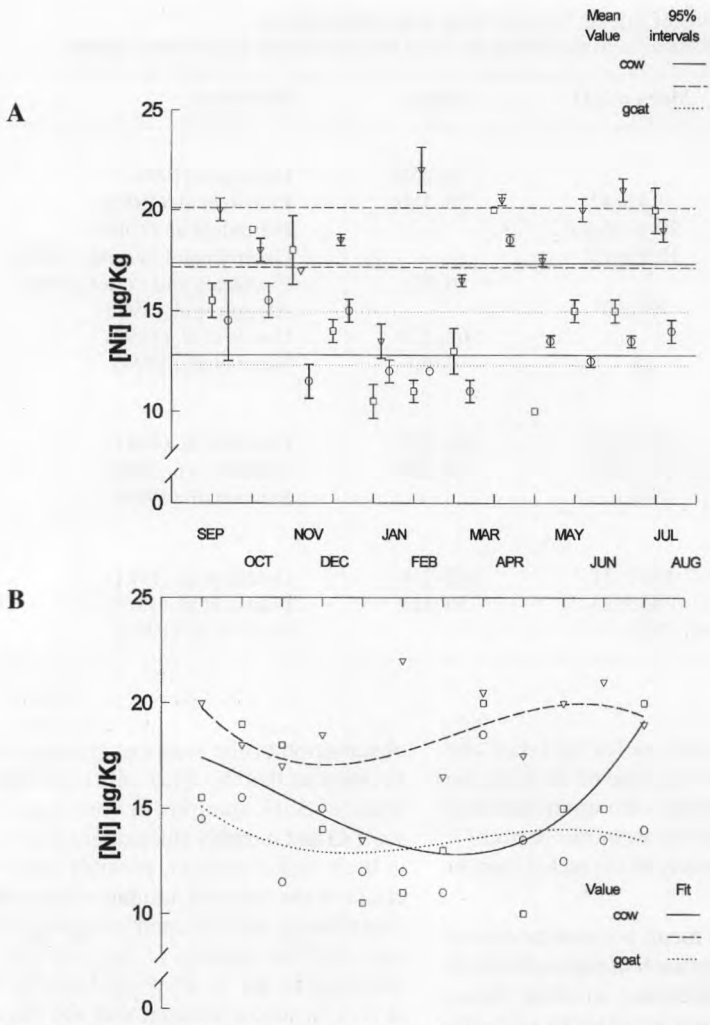

Figure 1. Changes in monthly contents of nickel in cow's, ewe's and goat's milk. **A**. Means ± margins of confidence for monthly and annual concentrations. **B**. Adjustments calculated.

Figure 1. Changements dans les concentrations mensuelles en nickel dans le lait de vache, de brebis et de chèvre. **A**. Moyenne ± intervalle de confiance pour les concentrations mensuelles et annuelles. **B**. Ajustements calculés.

on the basis of the mean nickel concentrations determined, this type of milk provides 4.51 µg/d. We shall indicate as a consumption guide the mineral contribution afforded by a cup of milk (250 mL or 8 fl oz) of the different species [26], obtaining nickel intakes of 3.7, 4.6 and 3.4 (µg/d) for cow's, ewe's and goat's raw milk respectively.

To assess if the statistically significant changes in nickel content between cow's, ewe's and goat's milk have any nutritional significance, nutrient density (ND) values and percentages of recommended daily nickel intakes were calculated. Nutrient density (ND) supply information about the contribution of a nutrient by the consumption of

a foodstuff according to its total energy value. Renner et al. [29] indicated that a useful evaluation of the nutritional significance of minerals and trace elements in milk can be obtained by computing the nutrient density for each of the elements in the following way:

$$ND\ (\%) = [(N_p/E_p) / (N_r / E_r)] \cdot 100$$

where Np = nutrient concentration (mineral element) in the food, Ep = energy supplied by food, N_r = recommended daily intakes of nutrient (mineral element) and E_r = recommended energy intake. This nutritional concept has the advantage of being independent of the amount of the food consumed and a nutrient density of 100 % or more indicates that the food, if consumed in sufficient quantities, contributes substantially to the intake of that particular nutrient. Datum of E_p was considered for cow's (whole) milk, ewe's milk and goat's milk reported by Souci et al. [32] and E_r value for men with a reference body size and 19–50 years of age [19]. The percentages of nutrient density for nickel content are less than 100 % in the three types of milk, 87.7, 74.9 and 78.2 % for cow's, ewe's and goat's raw milk respectively, and do not therefore contribute substantially to the intake of nickel and are not a good food source of this mineral element.

The National Research Council [19] has not set up any official recommendations for dietary intake nickel, although suggestions have been made by several authors, such as Nielsen [20] who have proposed a recommended daily nickel intakes of 75 µg/d. The reported percentages of recommended daily nickel intake assumed a 100 % availability of the essential element although there are many factors that may condition the availability of the nutrient and, therefore, the values of daily intakes and percentages of recommended daily nickel intakes supplied. The nickel content of cow's, ewe's and goat's milk supplies percentages of recommended daily nickel intake of less than 2 % (0.50, 0.62 and 0.45 % respectively) and therefore, according to the criteria of nutrition labelling indicated by Porter et al. [28], the three types of milk are judged being an inadequate source of this mineral element.

As a conclusion, it can be confirmed that, according to the lower percentages of nutrient density and recommended nickel daily intake, cow's, ewe's and goat's raw milk are poor sources of nickel in the diet and, although ewe's milk has a higher concentration of nickel and its percentage of recommended nickel intake is greater, its nutrient density is lower than the other two species in function of its energy content. These results confirm that the significant changes in the nickel concentration of cow's, ewe's and goat's milk have no nutritional significance.

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