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ORIGINAL ARTICLE

Thiocyanate content in raw milk under the american tropic conditions in relation to the activation of the lactoperoxidase system

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ABSTRACT: The potential risks in the use of thiocyanate salt for the activation of the Lactoperoxidase System (LPs) have been strongly discussed at international level. This study approaches different factors associated to variations of the thiocyanate ion concentrations in milk under the tropic conditions. Mean thiocyanate concentrations in bulk milk are between 0,10-0,15 mmol/liter, with a general mean of 0,140 mmols/L, which indicates that the exogenous activation of the LPs can be done with a lesser salt quantity and that the maximal natural concentration is 2,4 times higher than that established by the guidelines about its use. A criterion regarding of overdose, for the control of use of the LPs is established. Under these conditions, activation is safe and does not involve toxicological risks for consumers.

Keys word: milk, thiocyanate content, Lactoperoxidase System.

Contenido de tiocianato en leche cruda en las condiciones del tropico americano y su relación con la activación del sistema lactoperoxidasa

RESUMEN: Los riesgos potenciales en el uso de una sal de tiocianato para activar el sistema lactoperoxidasa (LP), es un tema de discusión a nivel internacional. El estudio aborda los diferentes factores asociados a la variación de las concentraciones del ion tiocianato en la leche en condiciones del trópico. La concentración media de tiocianato en mezclas se encuentran entre 0,10-0,15 mmoles/litro, con una media general de 0,140 mmoles/L, lo que indica que la activación exógena del sistema LP se puede realizar con una cantidad menor de la sal y que la máxima concentración natural es 2,4 veces superior al establecido por las directrices de uso. Se establece un criterio de sobredosificación para el control de uso del sistema LP. Bajo estas condiciones la activación es segura y no entraña riesgo toxicológico para los consumidores.

Palabras clave: leche, tiocianato, Sistema Lactoperoxidase.

INTRODUCTION

The *Codex Alimentarius* has established a guideline about the use of the LPs. It recommends the addition of 14 mg/L sodium thiocyanate to raw milk (1), besides its natural content, in order to obtain a final concentration between 0,20-0,25 mmol/L, which is the maximal value for the enzyme activity. However, the calculation of the activation dose was established at the end of the 80s. This was based on the existent scientific reports mainly coming from the European countries (2). Some recent reports have pointed out the possible potential risk of this practice since the ingestion of high concentrations of the ion could have toxic effects in humans, due to interference with iodine mechanisms in the thyroid gland (3, 4).

Feeding of ruminants in the tropic (cows, goats, and buffaloes), based on tropical pastures and forages, is a factor to be taken into account since it differs from those used in warm climate countries (5). Other management aspects and lactation characteristics including a lesser dairy production can have an important influence in thiocyanate concentrations. The exogenous activation of the LPs regarding preservation the of raw milk without refrigeration is addressed to bulks of diverse animals, more than to the volume of an individual cow, but individual variations can offer a comparison point for

indicating if activation surpasses or not the maximal natural thresholds. This study integrates results of diverse assays and analyzes different effects in order to establish criteria about the potential toxicity of the LPs activation under the tropic conditions, which is a limited knowledge area but of great interest in the practical use.

MATERIALS AND METHODS

The thiocyanate analysis procedure, recommended by the *Codex Alimentarius* guidelines, was used (1). The study includes three aspects: thiocyanate ion variation in individual samples and in bulk milk from cows under different feeding conditions (extensive and rotational systems) and types of pastures (natural and artificial sugar cane, forages), including star pasture (*Cynodon nlenfuensis*) for being a source of cyanogenic glucosides frequently used in the tropic; the effect of biological factors and herd management such as number, lactation time, breed of animal, colostral phase, mastitis; and a third aspect including the results obtained in large bulk milks in three countries (Cuba, Venezuela and Mexico). A total of 2 091 samples of milk from 444 cows and bulk milk, was analyzed. Bulk milks represented an approximate volume of 4,2 million liters. Fresh samples were preserved in refrigeration (freezer) at a temperature of 4-6°C until their analysis, which was carried out within the first 12 hours of being obtained. The results are expressed in mmol/liter of milk (mmol/) thiocyanate ion (SCN^-), except in the cases referred to the sodium thiocyanate salt which is expressed in mg/L and it is indicated between parentheses.

RESULTS

The highest thiocyanate concentrations were observed in the milk of cows consuming star pasture fertilized with nitrogen, while in the rest of the groups were very similar, averaging from 0,116 to 0,14 mmol/L, including bulk milk from cows consuming star pasture with the lack or absence of nitrogen fertilization (Table 1). In all cases, means were lower than 0,25 mmol/L, which is the optimal concentration for the activation of the LPs. There were a wide variation between the minimal and maximal value in the case of individual samples, reaching a maximal value of 2,4 times higher than that required for such activation, but being reduced considerably in the bulks. Year season did not have a significant effect on thiocyanate concentrations in milk from cistern cars and jar bulks, having values oscillating between 0.12 and 0.14 mmol/L, which were similar to that previously observed.

There was a high thiocyanate ion concentration in the first 24 hours post birth, until the fourth day of the colostral period; but at the end of the first week, values were normalized between 0,15 and 0,12 mmol/L (Figure 1). Mastitis produced an increment in the thiocyanate concentration and was normalized in milk from healthy cows (Figure 2).

TABLE 1. Thiocyanate concentrations in individual and bulk cow milk in different feeding, and season in Cuba conditions./ *Concentraciones de tiocianato en leche de vacas individuales y mezclas en diferentes sistemas de alimentación, y época del año en las condiciones de Cuba*

Pasture type	N	Mean (Sdx) mmol/L	Concentration	
			Minimal	Maximal
Cows fed with fertilized Star pasture *(Cynodon nlenfluences*)	35	0,21[a] (0,059)	0,06	0,60
Cows fed with guinea pasture (*Panicum maximun*) and Glycinia (*Neotonia Wighitii*)	41	0,14[b] (0,042	0,04	0,62
Bulks: Milk of cows fed with non-fertilized Star Pasture	35	0,140[b] (0,0038)	0.08	0.156
Bulks: : Milk of cows fed with different tropical pastures	74	0,121[b] (0,0031)	0.084	0.156
Bulks: Samples collected in jars and cistern cars	379	0,116[b] (0,0037)	0,019	0,181
Bulks: Dry season (Nov.-April). Samples collected of tanks in farms and cisterns	178	0,135[b] (0,0023)	0,05	0,20
Bulks: Wet season (May-October). Samples collected of tanks in farms and cisterns.	201	0,13[b] (0,0049)	0,09	0,21

Sdx, Standard deviation of the mean. Different letters $p<0,05$

FIGURE 1. Effect of the colostrum period on the thiocyanate content in milk./ *Efecto del periodo calostral sobre el contenido de tiocianato en leche.*

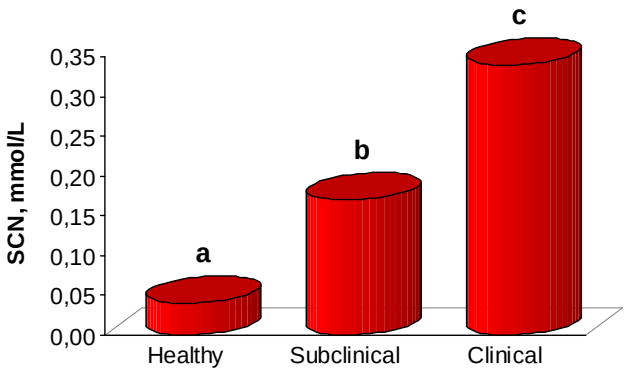

FIGURE 2. Effect of the mastitis affectation on thiocyanate content in milk./ *Efecto de la incidencia de mastitis sobre el contenido de tiocianato en leche.*

Thiocyanate concentrations in bulk milk in cows consuming pastures and forages as basic diet (rustic breeds) and concentrated foods (specialized breeds), coming from tropical areas of Cuba, Venezuela and Mexico, were as it is shown in Table 2. In all the cases, the absolute thiocyanate values were higher in milk from more rustic breeds (zebu-like breeds or its crossbreeds with European breeds), though difference was only significant in the case of Mexico. Means were also within the values previously observed, also the minor concentration in specialized breed would be related with a higher production of milk volume.

A summary or guide table was elaborated from these results, in order to establish criteria for the LPs management under the tropic conditions. These should be based on assuring the maximal activity of the system and a lesser toxicological risk associated to thiocyanate ion (Table 3).

The general mean concentration obtained was of 0,14 mmol/L. From such value, the amount of thiocyanate necessary for the activation of the LPs is calculated in 0,11 mmol/L to reach the optimal concentration required by the lactoperoxidase enzyme which is 0,25 mmol/L. An expected range regarding overdose was established in 0,251-0,35mmoles/L, when concentration surpasses the value 0,35 mmol/L. This criterion is obtained when considering the sum of the maximal natural concentration in a bulk milk (0,20 mmol/L in cows consuming star pasture), plus a standard deviation of the mean value (0,03 mmol/L) and the exogenous concentration which should be added (0,11mmol/L) to reach a total sum of 0,25 mmol/L of the SCN ion concentration.

DISCUSSION

The highest thiocyanate concentrations in the milk of cows consuming star pasture fertilized with nitrogen as a basic diet, coincide with the highest cyanogenic potential reported for this tropical grass (6), similar to that observed in the milk of cows consuming mandioc (7). The tendency to concentrations slightly superior in rustic breed cows or their crossbreeds with specialized breeds seems to be associated to: first, the use of feeding based on tropical pastures and forages and second, the concentration effect of solids in milk and in the components of the LPs in the animals due to low milk productions (5,8). The increment of thiocyanate in cows having subclinical and/or clinical mastitis, and in their colostral period, is adjusted to the defensive role attributed to the LPs at mammary gland level, and also to the physiological stage which increases mammary tissue permeability (8). In both cases, such

TABLE 2. Ion thiocyanate concentration of bulk milks in different type of cattle from tropical areas of three countries./ *Concentración del ion tiocianato en mezclas de leche en diferentes tipos de Ganado en áreas de tres países tropicales*

Country	Rustic Cattle Mean, (Sdx), n	Specialized Cattle Mean, (Sdx), n
Cuba	0,122[a] (0,031), 106	0,11[a] (0,043), 273
Venezuela	0,13[a] (0,026), 82	0,11[a] (0,083), 94
Mexico	0,18[a] (0,031), 315	0,12[b] (0,004), 390

Different letters between columns p<0,05. Letras diferentes entre columnas p<0,05

TABLE 3. Extreme concentrations, mean values, activation concentration and overdose of the thiocyanate ion (mmol/L) in the activation of the Lactoperoxidase System in the tropic./ *Concentraciones extremas, valores medios, concentración de activación y sobredosis del ion tiocianato (mmol/L) en la activación del sistema Lactoperoxidasa en el trópico*

Source	Minimal	Maximal
Individual cows	0.04	0.63
Value in raw milk bulks	0.08	0.21
Tropical Mean Concentration	0.140	
Enzyme Optimal Concentration	0.25	
Exogenous Addition according to CAG 13, 1991	0,173 (14 mg/L of the salt NaSCN)	
Proposal of activation for the tropic	0,11 (9 mg/L of the salt NaSCN)	
Overdose Suspicion	0.251 - 0.35	
Overdosing	+0.35	

Between brackets the equivalence of the SCN ion in weight from the thiocyanate sodium salt
Valores entre paréntesis corresponde al peso de la sal tiocianato de sodio equivalente al ion tiocianato

concentration is reduced when reaching a normal udder health condition. The slight ion increase starting from the 5th or more lactation could also be associated to conditions in the oldest cows´ udder.

The general mean concentration obtained in all studies was higher than that established in the international preceding reports (1,9), which indicates that the activation of the LPs under the American tropic conditions could be carried out with lesser amounts of thiocyanate salt than that recommended by the guidelines. Similitude in concentrations obtained in bulk milk in the three countries under study, indicates that such values could be taken as reference Stability of such concentrations during a year is also a favorable element for assuming a unique concentration for the activation of the LPs, which is established in 0,11 mmol/ L thiocyanate ion, equivalent to 9 mg/100 liters of salt. It should be considered that the activation of the LPs is addressed to the preservation of bulk milk without refrigeration and not to its use in individual animals (1,9). Thus, keeping a thiocyanate mean value in bulk milk within very narrow limits is also a safety aspect. In this sense, the maximal natural concentration found in raw milk from individual cows (0,62 mmol/L) was 2,4 times higher than the concentration threshold recommended for the enzyme activity, including the exogenous activation with sodium thiocyanate salt (0,25mmol/L). Regarding sodium thiocyanate salt weight, bulk milk concentration varies between 8-12 mg/100 ml of milk, while in individual cows is of 4-49 mg/100 ml.

A suspicion of overdose range (0,251-0,35 mmol]/ L) and a value above which could be considering that the is there is a thyocianate ion overdose (+ of 0,35 mmol) in the exogenous activation of the LP system, has not been established before and constitutes an important element related to the security of the use and the practical control of the method.

Some reports, which have pointed out the potential thiocyanate risk due to the possible interference in the thyroid metabolism (3,4,10), have used much higher concentrations than those recommended by the *Codex Alimentarius* guidelines, or the conclusions referred to milk are speculative. The inocuity of the activation method has been demonstrated since the 80s up to now (2,5,9,11,12). The criterion about the toxicological damage referred to the guidelines, is a misinterpretation due to the following reasons: A simple calculation of the annual thiocyanate ingestion indicates that those countries which do not apply the LPs but consume an equivalent of 200 kg/year or more (developed countries), drink a greater quantity of thiocyanate from natural origin than those countries with an equivalent of 120 kg/year or less (developing countries), though activating the LPs in all milk. Another misinterpretation is the fact that the natural ion content in milk from individual cows can be 2,4 times higher than the total concentration obtained in bulk milk, once activating the LPs. A third aspect is that people drink more thiocyanate in the consumption of some vegetables and mandioc (9,13) than during the consumption of milk previously activated with the LPs. Regarding the term risk-benefit, it could be more dangerous to limit the consumption of milk than the potential risk which implies the activation of the system using small quantities of thiocyanate; and also iodine deficiencies in the areas affected by endemic goitern (14) could be covered by the supply of iodized salt.

The characterization carried out in this study indicates the need of adjusting (reducing to a half) the amount of thiocyanate salt established for the activation of the LPs under the tropic conditions and also the

need of using suspicion and overdose indicators as a way of incrementing the control of use of such method. The previous results assure a greater safety in the activation of the system than that established in the *Codex Alimentarius* guidelines; and also do contribute to its improvement.

CONCLUSIONS

The mean concentration of thiocyanate in raw bulk milk under tropic conditions is between 0,10-0,15 mmoles/L, with a high variability on individual animals between 0,05-0,6 mmoles/L. The exogenous activation of the LPs only needs between 8-9 mg of sodium thiocyanate per liter, half less than that indicated in the *Codex Alimentarius* guideline.

The activation of the LPs in bulk milk with the thiocyanate quantity indicated is 2,4 times lesser than the maximum values found in milk of individual cows. The criterion to identify the over dosage was established from 24 mg/L or higher than 0,40 mmoles /L, and between values for the suspicion of over dosage (0,26-0,40 mmoles/L). The previous results assure a higher security margin in the system activation than that established by the *Codex Alimentarius* guideline and contribute to its improvement.

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