

## Iodine concentrations in cow's milk in Central and Northern Bohemia

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**ABSTRACT:** Iodine deficiency and related risks of medical and/or developmental disorders in humans are a world-wide problem. In the last years, Czech endocrinologists and paediatricians have observed a significant increase in the occurrence of goitre in children and adolescents caused by a low dietary intake of iodine. Given the low consumption of seafood, milk and dairy products are the main sources of iodine in the Czech Republic. Iodine contents of milk of dairy cows of different breeds from seven farms located in Central and Northern Bohemia were studied over a period of 17 months (April 2000 through August 2001). Iodine content was determined by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) based on IDF Standard 167 (1994), following alkaline mineralization of the sample. An analysis of the certified reference material CRM 063 was used to examine the accuracy of determination. Two-way analysis of variance (ANOVA) with and without replications, including Scheffe's comparison at a 5% level of significance, was used for statistical evaluation of experimental data. The iodine content of milk ranged from 147 to 605  $\mu\text{g}/\text{kg}$  (mean  $251 \pm 110 \mu\text{g}/\text{kg}$ ) during the winter season, and from 35 to 484  $\mu\text{g}/\text{kg}$  (mean  $212 \pm 104 \mu\text{g}/\text{kg}$ ) during the summer season. The mean iodine concentration in all samples of milk during the studied period was  $225 \pm 109 \mu\text{g}/\text{kg}$ . The wide range of iodine concentrations is connected with differences in iodine saturation of dairy cows and it is a result of multiple factors. Iodine reserves in the soil play an important role and influence the content of iodine in feeds for dairy cows and thereby the iodine levels in milk. The presence of goitrogenic substances in feed for dairy cows is another important factor. The iodine content was found to be statistically significantly lower in the milk from dairy cows fed a diet enriched with rapeseed cakes compared to that of dairy cows on a diet without rapeseed cake. Dairy cows on farm F6, which were fed maize silage without added rapeseed cake during winter, had a statistically higher iodine content in milk during the winter season compared to summer, when they were fed fresh fodder. Fresh fodder is supposed to contain goitrogenic substances which significantly reduce iodine levels in milk. This opinion is supported by experimental findings that the date of milk sample collection has no statistically significant influence on milk iodine levels if the dairy cows are fed a diet containing added rapeseed cake (farms F1, F2, F3, and F4) throughout the year. Compared to the other farms, statistically significantly higher milk iodine content was found on farm F5. The mean iodine content in milk from farm F5 was  $425 \pm 74 \mu\text{g}/\text{kg}$  during the studied period. The main cause is probably that the diet contained no rapeseed cake.

**Keywords:** iodine; dairy cows; milk; alkaline mineralisation; HPLC-electrochemical detection; goitrogenic substances

Iodine deficiency and related risks of medical and/or developmental disorders in humans are a worldwide problem. In the last years, Czech endocrinologists and paediatricians have observed a significant increase in the occurrence of goitre

in children and adolescents caused by a low dietary intake of iodine. In the Czech Republic, an insufficient intake of iodine is evident in particular in the Krkonoše Mts., Orlické Mts., Šumava Mts., Bohemian-Moravian Highlands, Sedlčany

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region, Moravský Úval lowlands, and Beskids Mts. (Zamrazil, 1998). According to the World Health Organization, the optimal daily intake of iodine is 150–300 µg in adults, and less than 100 µg per day is thought to be insufficient; the need for iodine in children is lower, proportional to age, while pregnant and breastfeeding women have higher iodine needs. No medical risks are associated with higher iodine intake (Fiedlerová and Kopecký, 1996). Milk, dairy products, eggs, and cereals are the main sources of iodine in the Czech Republic given the low consumption of seafood (Fiedlerová, 1998). According to Franke et al. (1983), more than 40 percent of iodine intake in inland populations of coastal countries originates from cow's milk.

The iodine content of milk and other foods varies with iodine content in the external environment and with the seasons of the year (Lee et al., 1994; Fiedlerová, 1998). Iodine content in milk as determined by Fiedlerová (1998) ranged from 24 to 748 µg/kg (mean 205 µg/kg) during winter, and from 16 to 345 µg/kg (mean 119 µg/kg) during summer. A large study aimed at determining iodine levels in cow's milk was conducted by Lee et al. (1994). Milk samples were collected in Great Britain in the years 1990–1991 from 13 localities, four times a year. The iodine content in milk ranged from 40 to 310 µg/kg (mean 150 µg/kg). The mean iodine content in milk was 210 µg/kg during winter and 90 µg/kg during summer. The mean iodine level in milk was found to be lower than in the years 1977–1979, when a similar study was conducted and the mean milk iodine content of 230 µg/kg was observed. Based on the repeated study, Lee et al. (1994) concluded that iodine levels in milk were more influenced by the seasons than by the location where dairy cows were kept.

An insufficient amount of iodine in the environment or inadequate iodine intake manifests itself in the health of livestock. For example, endemic goitre was observed in 27% of calves younger than 3 weeks in Southern and Western Bohemia in the years 1988 to 1994 (Kursa et al., 1998). An increased occurrence of abortions and premature deliveries with stillborns was reported in herds of dairy cows with calves suffering from goitre. It is generally accepted that iodine deficiency in dairy cows results in lower iodine content in milk and according to Groppe (1993) in a reduction in milk fat content by 10 to 20 percent as well. Sufficient dietary intake of iodine by dairy cows in the form of iodized salt lick or iodine-enriched feed results in better health

of dairy cows and higher iodine content in milk (Herzig et al., 1999; Kursa et al., 2005). The relation between dietary iodine intake and iodine content in tissues and milk was experimentally confirmed by Anke et al. (1993). The same findings were reported by Kaufmann et al. (1998), when the iodine levels in milk from dairy cows increased from 128 to 472 µg/l after enriching the cows' diet with 150 µg of iodine daily provided as potassium iodide. In addition, in accordance with the report of Wrzol et al. (1999) Kaufmann et al. (1998) found that the increased iodine intake had no influence on milk quality parameters (sensory properties, content of urea, fat, protein and lactose in milk, and number of somatic cells). The effect of iodine-enriched diet on iodine content in milk was confirmed by Brzoska et al. (1999) and Herzig et al. (1999). According to Rambeck et al. (1998), iodine passes into milk from feed much more intensively than into muscles and internal organs of the animal.

Kursa et al. (2005) determined the average iodine concentration of  $310.4 \pm 347.0$  µg/l in a group of 226 randomly selected bulk samples of fresh cow milk from 66 districts of the Czech Republic. Significant variations of iodine content in milk in the variation range from < 10 to > 1 000 µg/l were detected. The variations reflected marked differences in iodine saturation of dairy cows. The average iodine concentration in milk is twice and a half higher than the data acquired before supplementation which was initiated between 1997 and 1999 and is higher than the current European standard. The average concentration of iodine in milk after supplementation determined by Kursa et al. (2005) corresponds to the upper level of daily intake of iodine 0.3 mg that is acceptable on the basis of experimental studies by Bürgi et al. (1982). Kursa et al. (2005) assumed that the occasionally high iodine levels in purchased milk did not pose a threat to human health for the time being. Most of the risk is eliminated by dilution during common purchase of milk and during subsequent processing. The same opinion concerning this problem in Bavaria was shared by Preiss et al. (1997) and Luley (2000). A regular long-time daily intake of 1 litre milk or even more is quite usual in rural families, but no iodine supplementation in the majority of the self-supplier dairy farms is applied and therefore the situation is safe.

Flynn and Power (1985) and later Kaufmann et al. (1998) and Herzig et al. (1999) observed increased iodine content in milk after the cow's mammary

glands had been disinfected with an iodine-containing product (Jodonol). In contrast, Čurda and Rudolfová (2000) proved in their experiments that the milk from dairy cows whose teats were treated with an active iodine-based product Betadine after milking did not have increased iodine levels compared to the control group. However, an increased milk iodine concentration was found after using the same product for uterine lavage.

The presence of goitrogenic substances in the feed for dairy cows has an important effect on iodine content in milk. Kurša et al. (2000) reported that in sheep the intake of glucosinolates in rapeseed meal and nitrates without supplementation induced a low iodine excretion in sheep's milk (15 µg/l), while the addition of iodine into the same diet increased the iodine level to 101 µg per litre of milk. The highest iodine concentration (198 µg/l) in milk was found when the diet contained no rapeseed meal and the feeding dose was enriched with iodine. In addition, Kurša et al. (2000) determined experimentally that the content of iodine was significantly higher in sheep colostrum (334 µg/l) than in sheep's milk (101 µg/l).

According to Březina and Jelínek (1990) 30% of iodine in milk is bound to proteins, 60% to organic compounds of non-protein nature, 4.5% to fat particles and 4% is contained in whey. On the other hand, Rudolfová et al. (2000) determined experimentally that iodine was not bound to milk fat and the highest amount of iodine was contained in milk plasma (approximately 95%), however, the distribution of iodine between casein and other milk components was influenced by different curdling procedures used for the isolation of casein. Inconsistently with the findings of Březina and Jelínek and in accordance with Rudolfová, Flynn and Power (1985) reported that 80 to 90% of iodine was contained in whey and less than 13% was bound to proteins. These findings were confirmed by Sanchez and Szpunar (1999), who found that 78 to 89% of the total milk iodine content was bound to whey, of which 72 to 98% was in the form of iodide. Gushurst et al. (1984) also determined that 84% of the total milk iodine content was in the form of iodide. Dairy products that lose whey during the manufacturing process (cheeses and quarks) and those with high fat content (such as butter) are not as a good source of iodine as milk.

The objective of this paper was to study the effect of the seasons, locality and method of feeding on iodine levels in milk and to provide a long-term

evaluation of iodine content in milk of dairy cows from herds in Central and Northern Bohemia.

## MATERIAL AND METHODS

Iodine content was determined in the milk of three dairy cow breeds from seven farms in Central and Northern Bohemia in a span of 17 months (April 2000 through August 2001).

The characteristics of the respective farms were as follows:

**Farm F1:** Soil conditions/type: illuviated soils formed on loess deposits, pH: moderately acid, breed: Holstein, number of dairy cows: 450, housing: free box stalls, feeding: the cows were fed maize silage and haylage all the year round, rapeseed cake was added to the feed mixture.

**Farm F2:** Soil conditions/type: brown soils, pH: moderately acid, breed: Czech Pied Cattle, number of dairy cows: 480, housing: free box stalls, feeding: the cows were fed a mixture of maize silage and rapeseed cake all the year round, the cows were released to summer pasture during the dry period.

**Farm F3:** Soil conditions/type: brown earth on loess, pH: moderately acid, breed: Holstein (70%) and Czech Pied cattle (30%), number of dairy cows: 250, housing: free box stalls, feeding: the cows were fed maize silage and haylage all the year round, rapeseed cake was added to the feed mixture, no summer grazing.

**Farm F4:** Soil conditions/type: brown soils, pH: moderately acid, breed: Holstein, number of dairy cows: 500, housing: tie stalls, feeding: the cows were fed maize silage and haylage all the year round, rapeseed cake was added to the feed mixture, no summer grazing.

**Farm F5:** Soil conditions/type: chernozem on loess, pH: neutral, breed: Holstein, number of dairy cows: 230, housing: free box stalls, feeding: the cows were fed maize silage and haylage all the year round, no rapeseed cake was added to the feed mixture, no summer grazing.

**Farm F6:** Soil conditions/type: brown soils, pH: moderately acid, breed: Holstein and Czech Pied Cattle, number of dairy cows: 450, housing: free box stalls, feeding: maize silage during winter, green feed without rapeseed cake was fed during the vegetation period.

**Farm F7:** Soil conditions/type: brown soils, pH: moderately acid to acid, breed: Jersey, number of dairy cows: 90, housing: free, feeding: the cows

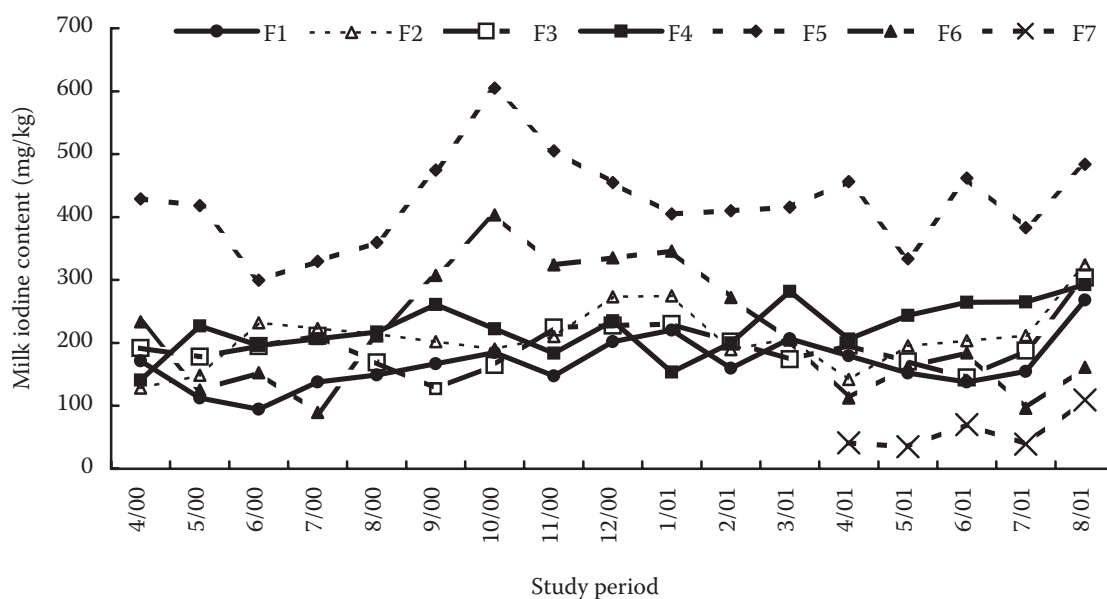


Figure 1. Iodine content in milk on individual farms during the studied period

were fed maize silage and haylage all the year round, rapeseed cake was added to the feed mixture, the cows were released to summer pasture during the dry period.

The milk from all 7 farms was carted to the same dairy.

Neither iodine supplementation of feeds nor the treatment of mammary glands with disinfectants on basis of active iodine was applied on any farm.

Aliquots of fresh milk samples (1 g) collected directly on farms were dried for iodine determination and degraded using alkaline mineralisation in the presence of KOH, ZnSO<sub>4</sub> and KClO<sub>3</sub> according to the procedure described by Fiedlerová (1998). The iodine was determined by high-performance liquid chromatography with electrochemical detection system on the NOVA-PAK C-18 reverse phase column (Hejtmánková et al., 2005) using the modified procedure according to provisional tech-

nical standard of the International Dairy Federation IDF–Standard 167 : 1994, describing direct determination of iodine content in fresh milk or dried milk after recovery. All fresh milk samples were analyzed in triplicate with the mean relative standard deviation of 11.0%. The detection limit was evaluated from the calibration curve constructed for the range of 1–105 ng of iodine in 1 ml of the calibration solution, using a standard statistical procedure (Meloun and Militký, 1994) in the Excel software program, and it was found to be 28.71 ng of iodine per 1 g of fresh milk.

## RESULTS AND DISCUSSION

The accuracy of determination was examined using 15 parallel analyses of the certified reference material BCR 063 (skim milk powder). The mean of

Table 1. Iodine content (µg/kg) in the milk of dairy cows during the studied period

Farm	Range of values	Mean	Coefficient of variation (%)	Median
Farm 1	94.4–268.2	167.4 ± 41.4	24.73	159.7
Farm 2	128.2–323.9	210.0 ± 48.4	23.05	207.1
Farm 3	127.0–303.5	194.5 ± 39.7	20.41	191.7
Farm 4	140.7–292.3	224.4 ± 42.5	18.94	222.2
Farm 5	299.6–605.0	425.0 ± 74.1	17.44	418.1
Farm 6	89.3–403.4	231.8 ± 96.9	41.80	204.3
Farm 7	34.8–109.5	59.0 ± 31.5	53.39	41.1

Table 2. Iodine content ( $\mu\text{g}/\text{kg}$ ) in the milk of dairy cows in the summer season

Farm	Range of values	Mean	Coefficient of variation (%)	Median
Farm 1	94.4–268.2	153.1 $\pm$ 44.6	29.13	152.2
Farm 2	128.2–323.9	202.2 $\pm$ 53.3	26.36	203.7
Farm 3	127.0–303.5	192.5 $\pm$ 45.1	23.43	187.8
Farm 4	140.7–292.3	231.3 $\pm$ 42.3	18.29	227.1
Farm 5	299.6–483.7	410.0 $\pm$ 77.9	19.00	418.1
Farm 6	89.3–307.4	152.7 $\pm$ 65.2	42.70	161.5
Farm 7	34.9–109.6	59.0 $\pm$ 31.5	53.39	41.1

experimental values was  $0.814 \pm 0.036 \mu\text{g}/\text{g}$  (certified value  $0.81 \mu\text{g}/\text{g}$ , uncertainty  $0.05 \mu\text{g}/\text{g}$ ).

Two-way analysis of variance (ANOVA) with and without replications, including Scheffe's comparison, was used for statistical evaluation of experimental data (Meloun and Militký, 1994). Changes in the iodine content of milk in three dairy cow breeds from seven farms located in Central and Northern Bohemia were studied over a period of 17 months (Figure 1). The mean iodine concentration in all samples of milk was  $225 \pm 109 \mu\text{g}/\text{kg}$  during the studied period, with the values ranging from 35 to  $605 \mu\text{g}/\text{kg}$  (Table 1). The wide range of iodine concentrations is connected with differences in iodine saturation of dairy cows and it is a result of multiple factors.

Mean iodine concentrations in the milk from farms F2, F4, F5, and F6 were higher than those reported in the literature:  $150 \mu\text{g}/\text{kg}$  (Lee et al., 1994),  $205 \mu\text{g}/\text{kg}$  (Fiedlerová, 1998) or  $215 \mu\text{g}/\text{kg}$  (Řehůrková et al., 2000), but except farm F5 lower than were determined by Kursá et al. (2005) after iodine supplementation of feed ( $310 \pm 347 \mu\text{g}/\text{l}$ ). The mean iodine concentration in milk from the studied farms amounts to 72.5% of the value detected by Kursá et al. (2005).

The presence of goitrogenic substances in feed for dairy cows is a very important factor influencing the iodine content of milk. Statistically significantly lower ( $P < 0.05$ ) iodine contents were found in the milk of dairy cows fed a diet with the addition of rapeseed cake compared to dairy cows receiving feed without rapeseed cake. Farm F5 had a statistically significantly higher ( $P < 0.05$ ) iodine content of milk than the other farms. The mean iodine content of milk from farm F5 was  $425.0 \pm 74.1 \mu\text{g}/\text{kg}$  during the study. This relatively high value may be explained by the fact that the diet contained no rapeseed cake. A decrease in iodine content in sheep's milk after the addition of rapeseed meal into the diet was also observed by Kursá et al. (2000). Both studies identically support the conclusion about the important role of goitrogenic substances in dairy cows' diet.

The mean iodine concentration in all samples of milk obtained during the summer season (April through September) was  $212 \pm 104 \mu\text{g}/\text{kg}$  with the values ranging from 35 to  $484 \mu\text{g}/\text{kg}$  (Table 2). The mean iodine content in all samples of milk obtained during the winter season (October through March) was  $251 \pm 110 \mu\text{g}/\text{kg}$  with the values ranging from 147 to  $605 \mu\text{g}/\text{kg}$  (Table 3). Although the effect

Table 3. Iodine content ( $\mu\text{g}/\text{kg}$ ) in the milk of dairy cows in the winter season

Farm	Range of values	Mean	Coefficient of variation (%)	Median
Farm 1	147.4–220.4	186.8 $\pm$ 28.5	15.26	193.5
Farm 2	189.3–274.5	224.2 $\pm$ 39.5	17.62	208.7
Farm 3	165.0–230.0	198.4 $\pm$ 28.5	14.36	213.3
Farm 4	153.5–282.0	210.7 $\pm$ 44.5	21.12	211.0
Farm 5	404.9–605.0	475.1 $\pm$ 77.9	16.40	435.2
Farm 6	204.3–403.4	310.0 $\pm$ 68.3	22.03	329.6

of the year season on iodine content in milk was confirmed in accordance with other studies (Lee et al., 1994; Fiedlerová, 1998 – the mean iodine content during the winter season was determined to be by 72% and 133%, respectively, higher than in the summer season), this trend was statistically insignificant on all farms, except for farm F6. The mean iodine content of milk was 18% higher during winter than during summer. Only the dairy cows kept on farm F6, receiving maize silage without added rapeseed cake during winter, had a statistically significantly higher ( $P < 0.05$ ) iodine content in milk during winter than during summer, when they were fed fresh fodder. It can be assumed that fresh fodder contains goitrogenic substances that significantly reduce milk iodine levels. This opinion is supported by experimental findings that the date of milk sample collection has no statistically significant influence ( $P < 0.05$ ) on milk iodine levels if the dairy cows are fed a diet containing added rapeseed cake (farms F1, F2, F3, and F4) all the year round. In accordance with Lee et al. (1994) it seems very probable that differences in the milk iodine contents between the summer and winter season are not caused by the year season itself and its specific characteristics, but primarily by the way of feeding that can be changed during the year.

Although the iodine concentration of milk in farm F5 is higher than the acceptable upper daily intake of 0.3 mg iodine (Bürgi et al., 1982), the human health is not threatened because the high iodine level in milk is eliminated by delivering the milk from all 7 farms to the same dairy (the mean value is within the acceptable daily intake 0.1 to 0.3 mg). The same opinion concerning this problem was shared by Kursá et al. (2005), even though the iodine concentration in milk above 500 µg/l was detected in 16.8% of the studied farms.

## CONCLUSION

Based on the acquired data, it can be concluded that the iodine content in the environment, especially in dairy cows' diet, has a crucial effect on milk iodine levels. The presence of goitrogenic substances in feed for dairy cows also plays an important role. The milk of dairy cows fed maize silage and haylage without the addition of rapeseed cake had a statistically higher ( $P < 0.05$ ) iodine content of milk than the milk from the summer season, when the dairy cows were fed fresh fodder, or milk

from dairy cows receiving a diet with the addition of rapeseed cake throughout the year. The results of this study confirmed that milk is an important source of iodine given the high content of this important microelement. On average, 700 ml of milk or equivalent milk product can be considered sufficient to cover the daily requirement for iodine with the absence of other iodine-containing foods.

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