

Influence of an iodine depletion period and teat dipping on the iodine concentration in serum and milk of cows

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(Received 14 September 2006; accepted 2 February 2007)

ABSTRACT

The objectives of the study were to investigate the influence of an iodine depletion period (from 10 to 0.2 mg I per kg dry matter, DM) on the iodine concentration of serum and milk and the effect of an iodized udder disinfectant solution on the iodine concentration of milk from dairy cows. Five late-lactating cows (milk performance: 22.1±2.0 kg per day) were used for the study. At the end of the dose-response study (up to 10 mg I supplementation per kg DM) without teat dipping, the basal diet (0.2 mg I/kg DM) was given for 16 days. The serum iodine concentration decreased from 290 to ≈ 60 µg kg⁻¹, that of milk, from 2762 to ≈ 100 µg kg⁻¹. In the second part of the study the teats were separately dipped twice a day after milking in a disinfectant providing a free available iodine concentration of 3 g per l through nonoxinol(9)-iodine. The diet fed had a native iodine content of 0.2 mg/kg DM. Dipping was compared over 18 days with non-dipping. The dipping procedure significantly increased the mean iodine concentration from 100±23 to 154±42 µg kg⁻¹ milk.

KEY WORDS: dairy cow, teat dipping, iodine concentration, serum, milk

INTRODUCTION

Iodine is an essential trace element for humans and animals. More than 95% of total iodine is accumulated in the thyroid gland. The only known roles of iodine in metabolism are its incorporation into the thyroid hormones, thyroxine (T₄)

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and triiodothyronine (T_3), and into the precursor iodotyrosines. Both hormones have multiple functions in the energy metabolism of cells, in the growth, as a transmitter of nervous stimuli, and as an important factor in brain development (Underwood and Suttle, 2001; Delange and Hetzel, 2003). Iodine deficiency reduces the production of thyroid hormones in humans and animals, leading to morphological and functional changes of the thyroid gland and reduction of the formation of thyroxin (ICCIDD, 2001). A high proportion of the population in Western and Central Europe is still at risk of iodine deficiency (Delange, 2002; Vitti et al., 2003; Delange and Dunn, 2004).

Numerous measures have, therefore, been undertaken to improve the iodine supply to human diets, e.g., using iodized salts (e.g., Lind et al., 2002; Zimmermann, 2004), other vehicles for iodine (e.g., Dunn, 2003) supplementation of foods of plant or animal origin (e.g., Zimmermann et al., 2005), or supplementing iodine to animal feed in order to increase the iodine content of food of animal origin (e.g., Kaufmann and Rambeck, 1998; Flachowsky et al., 2006; Schöne et al., 2006). Until 2005, 10 mg I per kg feedstuff were authorized for most food producing animals in the EU. There are, however, people for whom a higher iodine intake is harmful. Tolerable upper intake levels for iodine have therefore been proposed (WHO, 1994; SCF, 2002). These are the highest levels of iodine intake that are likely to pose no risk of adverse health effects.

During the last few years the status of iodine nutrition in some European countries has improved (Lind et al., 2002) thanks to the use of various possibilities of adding iodine to human diets. Because of the supplementation of feed, cow milk is one of the most important iodine sources for human nutrition (e.g., Dahl et al., 2003; Lindmark-Mansson et al., 2003; Großklaus and Jahreis, 2004). The iodine concentration of milk depends not only on the iodine content of feed (e.g., Kaufmann and Rambeck, 1998; Launer and Richter, 2005; Flachowsky et al., 2006; Schöne et al., 2006) but also on other influencing factors, such as the glucosinolate content of feeds (e.g., Böhme et al., 2005) or that of the disinfectant for teat dipping (e.g., Amount, 1987; Falkenberg et al., 2002; Galton, 2004). The iodine concentration of disinfectant can vary between 0.1 and 0.8%; 20 to 70% of cows are dipped with such disinfectants. A recent EFSA-study (2005) dealt with the use of iodine in feedstuffs and concluded that more studies are necessary to assess the factors influencing the iodine concentration of foods of animal origin. Apart from dose-response studies with food-producing animals to assess the transfer of iodine, further investigations to evaluate influencing factors are necessary. The monitoring of iodine fortification of salt for human consumption and the use of iodized minerals for food-producing animals should be combined with studies of relevant population groups (Lauerberg, 2004) to see if iodine intake of humans is within the optimal levels (100-200 $\mu\text{g}/\text{day}$; DACH, 2000). The present study should contribute to this aim and has two objectives:

- to trace the iodine concentration in serum and milk of cows during a depletion phase after a period of highest iodine concentration permitted by the EU until 2005 (10 mg I/kg feed DM)
- to investigate the influence of an iodine-containing teat dip solution on the iodine concentration of milk.

MATERIAL AND METHODS

Five late-lactating cows (average milk yield: 22.1 ± 2.0 kg/day) of the German Friesian breed with an initial body weight of 580 ± 23 kg were used for the experiments. The cows were fed with a roughage, (grass 40 %; maize silage 60% on dry matter (DM) base) to concentrate mixture based on 2/3 roughage and 1/3 concentrate (DM base) and 150 g mineral mix/d. The concentrate mixture consisted of, %: wheat 27.3, peas 25.3, dried sugar beet pulp 23.3, oats 11.3, soyabean meal 6.4, barley 5.4 and soyabean oil 1. The DM-intake amounted to 13.3 kg per day. Feed intake had been restricted to this low level to avoid any feed refusals. The iodine content of the basal diet amounted to 0.2 mg/kg DM, which is lower than the iodine requirements of lactating cows (GfE, 2001). After a dose-response study with a high-iodine mineral mix at the end (10 mg I/kg feed DM) containing $\text{Ca}(\text{IO}_3)_2 \times \text{H}_2\text{O}$, (Schöne et al., 2006) and without teat dipping; the cows were fed with the unsupplemented control diet (0.2 mg I/kg DM) for 16 days. Water was freely available. All cows were tethered in a tie stall. Each cow had an individual crib. The animals were fed and milked twice daily.

Blood from the *vena jugularis externa* and milk samples from morning and evening milking were taken 1, 2, 4, 8 and 16 days after the start of the depletion period. Blood (10 ml) was sampled two h after the morning feeding and centrifuged, the serum was deep frozen for iodine analysis. After the depletion period the teat dip treatment was started.

After milking, the teats were dipped in a teat disinfection solution containing Nonoxinol(9)-Iodine with 3 g/kg of available iodine. Representative milk samples were taken after 2, 3, 5, 10 and 18 days of treatment from the morning and evening milkings.

The I content of the feed ingredients and of the lyophilized milk samples was measured by intracoupled plasma-mass spectrometry (ICP-MS *SCIEX ELAN® DRC-e*, Perkin-Elmer) after matrix disintegration. An ammonia solution (DIN) was used for analysis of the feed and a solution of tetra-methylammonium-hydroxide, TMAH, for the milk lyophilizate (Fechner et al., 1998; Leiterer et al., 2001). To 500 mg of a finely ground feed sample 100 ml of a 0.5% aqueous ammonia solution (made from 25% ammonia, Merck, Darmstadt, Germany, and

distilled deionized water) were added in a vessel that was tightly closed and kept overnight. Thereafter the sample was shaken and the filtrate was used for the determination.

For analysis of the milk, 1 ml TMAH (TAMA Chemicals, Kawasaki Lab., Osaka, Japan) was added to 500 mg of a finely ground lyophilized sample and 5 ml distilled deionized water in a 50 ml polypropylene tube with a gas-tight closure. After disintegration for 3 h at 90°C and cooling to room temperature, 19 ml distilled deionized water were added and centrifuged for 15 min at 4000.

A standard addition calibration method was used to determine the iodine concentrations in the samples. A sample (4 × 1 ml) was spiked with three different amounts of iodine as KI (ultrapure, Johnson Matthey ALFA products, Karlsruhe, Germany): 5, 10 and 20 µg iodine/L, made up with distilled deionized water to 5 ml and 1 ml tellurium (200 µg, Merck, Darmstadt, Germany) added. The fourth sample, unspiked, was measured after the highest calibration sample signalling the start of analysis for the software.

The method had a recovery of 95 to 109%, a detection limit of 2.2 µg iodine/kg lyophilized milk and a determination limit of 6.6 µg iodine/kg lyophilized milk = 0.3 and 1 µg/kg fresh matter. The results were confirmed using the certified standard BCR N 151 (Community Bureau of Reference, Brussels, Belgium). In this “Spiked Skim Milk Powder” the recovery corresponded with the certified I concentration (103% from spiking).

In the case of serum, freshly prepared KI standards were added to the liquid samples and these were directly injected into the plasma of ICP. Tellurium (Spex, Grasbrunn, Germany), 100 µg/l, was used as the internal standard. Further details of the serum iodine measurement have been described (Schöne et al., 2001). The chemical composition of feedstuffs was determined according to the methods of VDLUFA (Bassler, 1976). Data obtained in the studies were analysed using analysis of variance, regression calculation and Student’s t-test.

RESULTS AND DISCUSSION

The chemical composition of feedstuffs is shown in Table 1.

Table 1. Analyses of the feeds and the ration

Feeds	DM %	% of DM				
		ash	CP	EE	ADF	NDF
40% grass + 60% maize silage	30.2	7.5	11.6	3.3	24.8	44.9
Concentrate	88.6	4.6	16.5	3.0	9.2	20.6
Ration (incl. 150 g mineral mix)	39.1	7.5	13.1	3.1	13.3	36.3

The iodine concentration in serum and milk amounted to 290 and 2762 $\mu\text{g kg}^{-1}$ at the end of the high dosage study (Schöne et al., 2006; see Table 2).

Such high concentrations in milk exceed the tolerable upper intake level (600 $\mu\text{g/day}^{-1}$; SCF, 2002) by more than four times. Similar maximal values were reported by Kursa et al. (2004) and were one reason that EFSA (2005) - recommended a reduction of the maximum iodine content in dairy cattle nutrition from 10 to 4 mg/kg DM. Later the EU (2005) followed the EFSA (2005) proposal and lowered the maximum level for dairy cows from 10 to 5 mg per kg feed.

After eight days of depletion the initial level of the previous study (steady state) was achieved (Schöne et al., 2006). The decline of the iodine concentration in milk (y) follows the exponential equation:

$$y = 4002.6 \times^{-1.3533} \text{ (R}^2=0.86; \times = \text{ days of sampling)}$$

These values are in agreement with measurements obtained under farm conditions during the summer (Flachowsky et al., 2006). The higher mineral and iodine supplementation during the winter resulted in an increased iodine concentration in milk (EFSA, 2005). Similar tendencies could be observed for the serum iodine concentration (Table 2), which follows the exponential equation:

$$y = 279 \times^{-0.5901} \text{ (R}^2 = 0.78; \times = \text{ days of sampling)}$$

and is similar to that in milk, but on a lower level. From 1997 to 2003 Launer and Richter (2005) analysed 3334 blood serum samples and observed an increase of the median from 51 to 120 $\mu\text{g I kg}^{-1}$. About 40% of the values were below 80 μg , indicating suboptimal iodine supplementation, and are in agreement with the results from the present study (Table 2).

Table 2. Iodine concentration of serum and milk after high iodine supplementation (10 mg/kg DM) and during the phase of depletion (0.2 mg/kg DM, without teat dipping; n = 5)

Iodine concentration of feed, mg/kg DM	Days of sampling	Iodine concentration, $\mu\text{g kg}^{-1}$	
		serum	Milk
10	average	290 ^a \pm 75	2762 ^a \pm 852
0.2	1	210 ^{ab} \pm 58	1867 ^{ab} \pm 464
	2	130 ^{bc} \pm 49	920 ^b \pm 209
	4	80 ^c \pm 23	292 ^c \pm 88
	8	62 ^c \pm 15	109 ^d \pm 27
	16	59 ^c \pm 10	90 ^d \pm 27

^{a,b,c} means in the same row with different superscripts differ significantly (P<0.15)

Teat dipping with an iodine-containing disinfectant significantly increased the iodine concentration of milk (P<0.05; Table 3) and contributed essentially to concentration of this element in milk.

Table 3. Iodine concentration of milk without and with teat dipping (n=5; 18 days)

Item	Iodine concentration $\mu\text{g kg}^{-1}$
Without	100 ^a ± 23
With dipping after milking	154 ^b ± 42

^{a,b} different letters show significant differences (P<0.05)

Similar results have described by others (Table 4).

Table 4. Influence of iodine concentration of teat-disinfectant on the increase of iodine concentration of milk after various references

Available iodine in disinfectants g l^{-1}	Application of disinfectants b: before, a: after milking	Increase of iodine in milk $\mu\text{g kg}^{-1}$	References
1	a	35	Galton et al. (1986)
2	a	11-60	Ryssen et al. (1985)
2.7	b	30	Falkenberg et al. (2002)
3	a	54	present data
5	a	27-32	Galton (2004)
5	a	36	Galton et al. (1984)
5	a	120	Hamann and Heeschen (1982)
10	a	46	Swanson et al. (1990)
10	a	76	Galton et al. (1986)
10	a	90	Galton et al. (1984)
10	b, a	110	Galton et al. (1986)
10	b, a	150	Galton et al. (1984)

Most authors dipped after milking, but the available iodine level in disinfectants seems to be more important for iodine transfer into the milk (Table 4) than dipping time. On average, the iodine concentration in milk increased between 50 and 60 μg , if 3-5 g l^{-1} available iodine was in the disinfectants used for dipping after milking. Dipping before and after milking increased the iodine concentration in milk much more than dipping only once (Table 4). Another important factor influencing iodine transfer is milk yield. The higher the milk yield, the lower the iodine transfer per litre of milk. The milk yield in the present study amounted to ≈ 20 kg per day; Galton (2004) used cows with a higher milk yield and recorded a lower iodine transfer despite more iodine in the disinfectants (Table 4).

CONCLUSIONS

The iodine concentration in serum and milk depends on the iodine intake of cows and iodine content of disinfectants.

After 10 days of feeding there exists a steady-state of iodine concentration in serum and milk in relation to the iodine supply in the diet. The iodine transfer from the teat disinfectant to milk depends on the concentration of available iodine in disinfectants, milk yield, and dipping frequency. Three to 5 g available iodine per litre and dipping after milking increased the iodine content in milk by 50-60 $\mu\text{g kg}^{-1}$.

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