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**Influencing Factors on Iodine Content of Cow Milk**

--Manuscript Draft--

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| Abstract:          | Purpose
Iodine is an essential trace element for humans and animals and it is incorporated into the thyroid hormones thyroxine and triiodothyronine, which have multiple functions in energy metabolism and growth, but also as transmitter of nervous stimuli and as an important factor for brain development. Because of the small range between iodine requirements and the upper level for humans (between 1 : 2.5-3), the requirements should be met, but excesses should be avoided. One of the most important iodine sources for humans is milk of ruminants. Therefore, various influencing factors on the iodine content of milk of ruminants should be analysed in the paper.

Results
The iodine content of milk depends on many factors, such as iodine content and level of iodine supplementation of feed, iodine source, iodine antagonists such as glucosinolates in the feed, farm management, teat dipping with iodine containing substances, milk processing in the dairy, etc. The effects of some factors on the iodine content of milk are demonstrated and discussed. Feed iodine supplementation has the main effect on milk iodine. However, the iodine content of milk may vary considerably depending on many other influencing factors.

Conclusions
As a consequence of preventive consumer protection, the European Food Safety Authority (EFSA) proposed a reduction of the I-upper level for lactating ruminants from 5 to 2 mg/kg complete feed.
Dear Prof. Rechkemmer, dear Dr. Oberreuther-Moschner,

Thank you very much for the evaluation of our manuscript and your helpful suggestions for improving the quality. During the last weeks we revised the manuscript taking into account all the remarks given by you and the reviewers. Now we really hope that the revised manuscript is clearly structured and meets all your requirements.

Please find our responses to the comments of the reviewers below.

Yours sincerely

Ulrich Meyer and coworkers

Reviewers’ comments:

Reviewer #1: This review is on an important topic, the factors that determine iodine content of milk. As milk is the major source of iodine in the diets of many Western countries, this subject deserves a thorough review. The current paper has a wealth of good information, but the beginning needs to be organized into a more logical framework.

I would suggest a few additional subheadings (and a bit more material/discussion) in the beginning section, such as:
Subheadings have been added.
- Brief background and importance of iodine for humans, current prevalence of deficiency
Comment has been taken into account.
- Percentage of human iodine intake that comes from dairy products in different countries
To our knowledge there is reliable information available.
- Milk iodine content in different countries
We summarized the data available to us in Tables 6 and 7.
I would move the section 'Method of iodine analysis in milk', which is currently the last section, up into this front material.
Comment has been taken into account.

The material should be organized throughout the review into distinct paragraphs of appropriate length. Single sentences do not constitute a paragraph.
Comment has been taken into account.

The abstract should be rewritten to reflect the major findings and conclusion of the review. The 'purpose' section of the abstract should be shortened and more length/information/emphasis be written into the results section. The conclusion of the abstract is unclear and needs to be rewritten.
Comments have been taken into account.

Reviewer #2: A very timely, informative and thoughtful review of the recent literature. The paper is concise and well written with only a few very minor grammatical or terminological slip-ups. As you might wish to eradicate these from your otherwise excellent manuscript, I have listed the following suggestions:

1. Page 1, Introduction, Para. 2 line 2: replace "in dependence on" with "depending on".
Changes have been done.
2. Page 2, Para. 2, line 5: insert "the" before the word "elderly".
Changes have been done.
3. Page 3, Iodine intake of cows, Para. 2, line 2: insert "or" between "animal yield" and "any" (i.e. "animal yield or any....")

Changes have been done.

4. Page 5, Para 3, line 1: replace "will go" with "goes".

Changes have been done.

5. Page 6, Farm management (organic - conventional, Para 1, line 4: replace "less frequently" with "the less frequent ....".

Changes have been done.

6. Page 7, line 1: replace "Less" with "Little". (Or change the construction to "Information on .......is scarce."

Changes have been done.

7. Page 7, Para 2, line 4: delete "given with".

Deleted.


Changes have been done.

9. Page 7, Para 4, line 2: replace "some" with "a few".

Changes have been done.

10. Page 7, Para 5, lines 4 and 5: delete "indicates" and "value", then rearrange to: "a very high milk iodine concentration of 520 ug iodine/L [134]"

Changes have been done.

11. Page 7 Para 6, line 7: replace "as" with "to". However, I think the construction "same .... as" sounds better than "similar .... to" in this sentence.

Changes have been done.

12. Page 7 Para 7, line 1: reverse order to "still is", replace "usual" with "common" and "because of" with "for" (i.e."...or still is common in many countries for..." then in line 2: replace "to prevent" with "preventing".

Changes have been done.

13. Page 8, Method of iodine analysis, Para 1, line 3: to most people "the speciation of iodine" may be a more familiar term than "the specification of iodine compounds".

Changes have been done.

14. Page 8 Method of iodine analysis, Para 2, line 4: replace "fasten" with "accelerate".

Changes have been done.

15. Page 8, Method of iodine analysis, Para. 3, line 1: replace "at most" with "mostly" or better "usually". Then line 4: replace "in preanalytics" with "prior to analysis".

Changes have been done.

16. Page 9, Line 3: Either inser "a" after "by using" or better, replace "by using" with "with a.....".

Changes have been done.

17. Page 9, Para 2, line 1: replace "at" with "on". Line 7: replace "distributed" with "used".

Changes have been done.

18. Page 9. Para 3, Line 2: there is an accidental carriage return after "German Agricultural......" and the rest of the title should read "...Analytical and ...."

Line 4: replace "by using" with "with"; Line 6: delete "by this procedure".

Changes have been done.

19. Page 9, Para 5, Line 1: replace "for low iodine matrices" with "matrices of low iodine content" and replace "compared with" with "than". Line 2: replace "Contrasting with that" with "In contrast, ....". I think this complete sentence should be re-formulated to: "In contrast, previous investigations of feed and meat with the SK method overestimated the iodine contents and these overestimates were then taken over in feed and food tables".

Changes have been done.

20. Page 9. Para. 6, Line 1: A carriage line return too many. Line 6: replace "demonstrated high agreement by the ...." with "...correlated well with the ...".

Changes have been done.
21. Page 9. Para. 7, Line 2: rearrange "has been not" to "has not been" and replace "up to now" with "so far".
Changes have been done.

Changes have been done.

I hope you agree with the suggestions and that they are a help.
Review

Influencing Factors on Iodine Content of Cow Milk

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Abstract

Purpose
Iodine is an essential trace element for humans and animals and it is incorporated into the thyroid hormones thyroxine and triiodothyronine, which have multiple functions in energy metabolism and growth, but also as transmitter of nervous stimuli and as an important factor for brain development. Because of the small range between iodine requirements and the upper level for humans (between 1 : 2.5-3), the requirements should be met, but excesses should be avoided. One of the most important iodine sources for humans is milk of ruminants. Therefore, various influencing factors on the iodine content of milk of ruminants should be analysed in the paper.

Results
The iodine content of milk depends on many factors, such as iodine content and level of iodine supplementation of feed, iodine source, iodine antagonists such as glucosinolates in the feed, farm management, teat dipping with iodine containing substances, milk processing in the dairy, etc. The effects of some factors on the iodine content of milk are demonstrated and discussed. Feed iodine supplementation has the main effect on milk iodine. However, the iodine content of milk may vary considerably depending on many other influencing factors.

Conclusions
There is no simple solution to declare the iodine concentration of milk and milk products on the food label. As a consequence of preventive consumer protection, the European Food Safety Authority (EFSA) proposed a reduction of the I-upper level for lactating ruminants from 5 to 2 mg/kg complete feed.

Keywords: Iodine, milk, iodine sources, iodine levels, antagonists, teat dipping

Introduction

Iodine is a trace element that is essential for humans and animals and was discovered as a novel element in 1811\(^1\). As part of the thyroid hormones triiodothyronine (T3) and thyroxine (T4), iodine is highly important for key processes in the body. For example, a lack of iodine is known to cause a loss of viability in embryos. Furthermore, the development of the human brain may be influenced unfavourably. A severe iodine deficiency during pregnancy is known to cause mental retardation up to cretinism. Goiter, the enlargement of the thyroid gland is another clinical sign of an insufficient supply with iodine. Further health consequences may be hypothyroidism and iodine induced hyperthyroidism. Nutritional, biochemical, pathological and therapeutical aspects of iodine in humans and animals were recently summarized \([2-4]\). Iodine is almost completely absorbed in the form of iodide from the gastrointestinal tract \([5,6]\). Iodate and most organic iodine compounds are converted into iodide by reducing agents prior to absorption.
The iodine requirements for humans are quoted as a function of age, body weight, physiological stage and gender as shown in Table 1. They vary in dependence on the influencing factors between 40 and 290 µg per day. Differences in human demand levels also exist between various scientific committees such as the World Health Organisation (WHO), Food and Agricultural Organisation (FAO) and the German, Austrian and Swiss Societies for Nutrition (DACH). The iodine requirements for food producing animals vary between 0.16 and 0.5 mg/kg dry matter (DM; [10-16]); those for lactating cows are given with 0.5 mg/kg DM [12,16]; for lactating goats with 0.8 mg/kg DM [17].

Table 1:

The iodine requirements for food producing animals vary between 0.16 and 0.5 mg/kg dry matter (DM; [10-16]); those for lactating cows are given with 0.5 mg/kg DM [12,16]; for lactating goats with 0.8 mg/kg DM [17].

Iodine in human nutrition

In various regions of the world people still suffer from iodine deficits [18-22]. In 2001, the WHO estimated that about 800 million people suffer from iodine undersupply [7]. But there is a decrease in the number of iodine deficient countries (from 54 to 32 from 2003 to 2011; [18]). However, significant regional differences in iodine supply still remain. For example, Ethiopia shows by far the highest number of children with insufficient iodine intake followed by Sudan, the Russian Federation, Afghanistan and Algeria. More than 50% of children with deficient intakes are living in Africa and southeast Asia [18]. Subclinical iodine deficiency may be observed in some specific sub-groups of the population such as pregnant and lactating women, preschool and schoolchildren, the elderly and consumers of organic products [23-25]. As a result of this situation iodine is classified in supply category 1, as the risk of a deficiency is relatively high [26,27].

This results in extensive efforts to improve iodine supplies of humans, for example by supplementing iodine in table salt in the food industry and household [28,29,21], and enriching foods of animal origin through iodine supplements in the feedstuffs extending beyond the animals requirements [30,31] or intramuscular injection of iodised oil [32]. According to European law, the present maximum level for iodine in feed of dairy cows is 5 mg/kg diet with 88% DM [33] while in the United States (US) a supplementation (EDDI) of just 10mg/d (approximately 1mg/kg DM) is recommended [16]. The recent EFSA publication [34] proposed a further reduction of the iodine upper level for dairy cattle and small lactating ruminants in the EU from 5 to 2 mg/kg feed.

The iodine upper levels in human nutrition vary between 200 (1 – 3 years old kids; toddlers) and 1100 µg per day (adults; USA [8]). In Europe [9] proposed a limit at 500 and the Scientific Committee on Food [35] at 600 µg I/day for adults. The differences between various scientific committees should not be discussed here. The margin between demand level and maximum level for iodine in humans is slight (e.g., demand for adults under European conditions: about 200 µg; upper level (UL): 500 – 600µg I/day; see Table 1), so that the maximum level is only three times higher than the demand. That means there is also a risk of overdosing, especially when supplementing iodine after previous iodine deficiency [36,37]. Therefore iodine was classified in the risk category “high” [26,27].

Milk as an important iodine-source in different countries

Apart from iodized table salt, milk is one of the most important I-sources in many countries as demonstrated by various authors (e.g., [38-47]). One third of the total iodine intake (30 to 40%) and more (about 60% in the Norwegian winter; [41]) comes from milk and milk products. Recently, [48] analysed the iodine status in preschool children in Germany and estimated 42% of I-intake from milk and dairy products. In another study [49] milk and dairy products contributed up to 38% of the iodine intake in German schoolchildren.

Studies show that up to half of the iodine supplemented in dairy cow feed is excreted via milk [50-52]. The predominant amount of iodine in milk is inorganic [53,4], the amount of organically-bound iodine in milk varies by various authors between 3 [54], 10% [55], 11-22% [56,57,53] and 30% [58], [59] found about 95% of iodine of milk in milk plasma. A nearly complete absorption of iodine from milk is considered [60].

Average values from bulk sample analysis of various European studies were predominantly between 100 and 200 µg I/L milk [61]. These values are generally confirmed by recent studies [34]. Data between 100 and 240 µg
iodine/L milk [62] are also given in various Food Tables of North European countries (Norway: 190; Denmark: 243; Sweden: 140; Finland: 170; Iceland: 112 µg iodine/L).

[63] confirmed previous data from Germany (Thuringia). The authors analysed 135 samples of cow’s milk from 2007 to 2011 and found a mean iodine concentration of 122 µg/L. Similar data have been described by Johner et al. [64]. The authors purchased 112 milk samples from 2004 to 2010 in the same food markets. During this time the iodine content increased from 97 (2004) to 110 µg/L (2010). Only data from the Czech Republic [65,66] are higher (mean values 324 and 489 µg iodine/L milk, respectively), likely due to a specific feed supplementation program. In contrast, much lower values (20 to 60 µg iodine/L cow’s milk) are still given in the Food Composition and Nutrition Tables [67].

The type of farming, animal keeping, animal feeding (supplementation of iodine containing mineral feed) and many other factors may influence the iodine concentration in milk. The objective of the present paper is to summarize the most important influencing factors on the iodine content of milk and to discuss various factors on the basis of recent studies (later than 2000). Older data were summarized by previous reviews (e.g. [2,61,68]) and are only considered in some special cases.

**The objective of the present review is to summarize influencing factors on the iodine concentration of milk, such as iodine intake of ruminants, iodine species, goitrogens in the rations, animal keeping and management, cattle breed, lactation stage, teat dipping and milk processing. The method of iodine analysis may also influence the iodine concentration of milk. Therefore, the review will start with a chapter on iodine analysis.**

**Method of iodine analysis**

Target for the analysis is the milk iodine concentration, however, in a dose-effect approach also the iodine supply, i.e. the content of native feed and of the iodine supplement, has to be determined with a valid method. Additionally, under biochemical and physiological aspects the specification of iodine, mainly in the milk, may be of interest. The ionic iodine, e.g. the iodide and iodate salts, approved as feed additives, and also the iodide in urine and milk, as well as the organic iodine compounds, e.g., thyroxine and further iodothyronines of blood and tissues, are characterized as more or less stable. However, elemental iodine is at least in parts volatile and during preparation and analysis of biological sample a risk for iodide oxidation with a subsequent loss of the analyte has to be avoided. Also in the context of prevention of oxidation a complete digestion of organic part of the sample and a full mineralization, resp., is the prerequisite for minimizing each iodine loss during analysis.

At present, in most feed and food laboratories and in clinical laboratory diagnostics measurement of total iodine is carried out either by a “traditional” method, the so-called Sandell-Kolthoff (SK) method [150], or by the newer method of inductively coupled plasma-mass spectrometry (ICP-MS) [151]. The first uses the iodide catalysed reduction of yellow Ce(IV) by As(III) to colorless Ce(III). Without the catalyst the reaction is normally very slow. Only trace amounts of iodide accelerate the bleaching reaction according to the following scheme:

$$2 \text{Ce}^{4+} + 2 \text{I}^- \rightarrow 2 \text{Ce}^{3+} + \text{I}_2$$

$$\text{As}^{3+} + \text{I}_2 \rightarrow \text{As}^{5+} + 2 \text{I}^-$$

The rate of disappearance of the yellow color in a Ce(IV)-As(III) mixture, usually measured as absorbance at 405 to 420 nm, represents the iodine content. Iodate acts also as catalyst, however, indirectly, because it is converted to iodide in the presence of arsenite in an acidic medium [152]. Organiodine compounds do not react without decomposition which illustrates the importance of mineralization in prior to analysis.

Today inductively coupled plasma mass spectrometry (ICP-MS) is often used as a very sensitive method for the quantitative determination of the total iodine content in feed and food. An induction coil generates a very hot argon plasma (4,500 to 8,000 K) which splits the sample into the atoms according to their number of valence electrons. Although the relatively high first ionization potential of iodine (10 eV) caused only a partial ionization in the plasma, very low determination limits are achievable.

**Particular attention must also be paid to the complete extraction of iodine from the sample and also to avoiding losses. The iodine concentration of fresh milk samples can be determined by direct measurement of the diluted samples with a standard addition calibration method.**
For digestion procedures focusing on iodine preservation up to the determination, it is consensus by operators in different laboratories that ashing at 600°C and dilution in acids, as routinely used for mineral analyses are not practicable without iodine losses. In laboratories for clinical diagnostics, particularly for urine and blood serum, acid digestion procedures dominate, e.g., with perchloric acid or its combinations with nitric acid or with nitric acid and sulphuric acid [152]. Benotti et al. [153] established a chloric acid digestion procedure which was adopted in many investigations of urine, feces, tissues and food. On the other hand, starting with the approach of Barker et al. [154] the alkaline digestion is widely distributed.

In food and feed analytics, there are two standard methods for iodine extraction. The VDLUFA(Association of German Agricultural Analytic an Research Institutes)-method 2.2.2.3 [155] can be used to determine the amount of iodide and iodate added to the animal feed. The dried and homogenized sample material is extracted with 0.5% ammonia solution at room temperature. However, this method does not allow analysts to assess whether all natural iodine has been recovered. The method is used for checking supplemented iodine amount in feed samples taken by official feed surveillance in Germany. The limit of quantitation is 0.4 mg/kg.

The European standard EN 15111:2007 [156] specifies an extraction method for the determination of iodine compounds in foodstuffs by ICP-MS extracted with the stronger alkaline reagent tetramethylammonium hydroxide (TMAH = (CH3)4N+OH-) at elevated temperature (90°C). In a comparison of both digestion methods, the TMAH digestion adopted from 22 food laboratories showed significantly higher native iodine contents for a range of feed than the ammonia digestion [157].

A comparison was made between the SK method and the ICP-MS method in milk. Eleven milk lyophilisates over a broad iodine concentration range from a dose response experiment [52] were analyzed by both these methods (Remer et al., Schöne and Leiterer 2006, unpublished results). The analysis results, means (and minimum maximum ranges) agreed satisfactorily: 9.2 (0.8-25.9) mg iodine/kg lyophilisate for the ICP-MS method versus 11.1 (0.7-30.1) mg iodine/kg lyophilisate for the SK method. In the regression/correlation analysis the milk iodine determinations correlated well with the equation: y = 1.24 x − 0.19 (R² = 0.999, P <0.001), where x represents iodine concentration determined by ICP-MS and y represents it determined via the SK reaction.

The many approaches using ion sensitive electrodes (IES) – at most silver iodide precipitated by a silver-anodic surface is measured – has not been established in laboratory routine up to now. Reasons for the lacking success might be interferences with halides and pseudohalides, a lower lifespan of the IES and as comparison with the ICP MS method a lower precision and sensitivity, i.e., higher LOD [152]. Despite these limitations the development of IES for fast and reliable iodine determination [159] preferably in milk, urine and blood serum, should be continued.

Iodine concentration in milk and other samples is also discussed under consideration of the methods of iodine analysis. The SK reaction was mainly used for iodine determination in previous studies. More recent studies use the inductively coupled plasma-mass spectrometry (ICP-MS) as described by Benkhedda et al. [160]. The method by Benotti et al. [161] was also used in some recent studies (e.g., Soriguier et al. [117]). Some authors compared the SK method with the ICP-MS - method. Johner et al. [64] compared both analytical methods and found a good agreement of results (r=0.99).

**Influencing factors on the iodine content of milk**

**Iodine intake of cows**

Iodine intake is the most important influencing factor on the iodine content of milk. The iodine concentration of concentrates except fish meal and milk products is less than 0.1 mg/kg DM [69,43]. Forages represent a higher iodine concentration of up to 0.2 mg/kg DM. The iodine content of plants reflects passive uptake from contamination or diffusion of iodine in the air, water and soil. Therefore distance from sea [70]; iodine content of soil [4] and vegetation stadium of plants [70] may mainly influence the forage iodine contents. The iodine
concentration in German and Austrian soils is around 1 to 3 mg/kg [71-73] and soil contamination of grass silages, shown as higher ash content increased the iodine content [74]. The iodine concentration of water for drinking fell from nearly 10 to about 1 µg/L as the distance from the coast increased from less than 50 to more than 400 km; [70]). The average is given with about 5 µg iodine/L [75,26,76] and its contribution to iodine intake can be neglected.

Commonly used unsupplemented feeds and water do not meet the iodine requirements of food producing animals. Therefore iodine supplementation of feed depending on animal species/categories, animal yield or any other influencing factors (see below) is necessary to meet the iodine requirements and to influence the iodine content of food of animal origin.

Binnerts [77] and Alderman and Stranks [78] deduced regression formulas to calculate the expected milk iodine content (see [61]). It was expected that a decreasing percentage of iodine will be excreted via milk with increasing iodine supply. These results are not in accordance with recent findings. Franke [30] summarized previous studies on the influence of various iodine intakes and species in the milk iodine concentration.

Furthermore, Franke et al. [50,51] carried out a representative dose response study with 32 dairy cows testing six iodine dosages between 0.5 and 5 mg/kg DM and additional influencing factors (e.g. two iodine sources, rape seed meal as source of glucosinolates; see Figs. 1 and 2). A linear increase of the iodine concentration of milk with increasing iodine intake of the cows was derived and supported by some other studies (see Fig. 1).

**Fig. 1**

A more or less linear increase of iodine concentration of milk after iodine supplementation of feed (see Fig. 1) was also measured by other authors in previous (e.g., [41,86-90]) and recent studies [79,81,82,52], but non-linear response curves were calculated by another group of authors (e.g., [83,84]; see Fig. 1). The highest milk iodine concentration was measured by Schöne et al. [52] after supplementation of dairy cow diets with 10 mg iodine/kg DM (see Fig. 1). Similar values (2,160 µg I/L) were found by Hillman and Curtis [88] after supplementation of about 10 mg I/kg DM from EDDI.

Moschini et al. [82] carried out two studies with dairy cows and applied up to 87.3 mg iodine per cow per day (3.54 mg iodine/kg DM). They calculated the following linear regression for the iodine concentration in milk; 
\[ y = -323.5 + 13.98 \times \text{x; R}^2 = 0.90 \]

where \( y \) is the iodine concentration in milk (µg/L) and \( \text{x} \) is the iodine intake (mg/d).

The study by Battaglia et al. [79] showed no influence of milk yield on milk iodine concentration. Castro et al. [85,81] grouped 30 farms out of 200 farms with a low (1.20 mg/kg DM) and 30 farms with a higher (1.81 mg/kg DM) iodine concentration in the feed and measured 103 and 554 µg iodine/L milk in 2007 as well as 146 and 487 µg iodine/L milk in 2008, for the low and high supplementation respectively.

Castro et al. [81] calculated a linear relationship between dietary iodine concentration and milk iodine level as follows:
\[ y = 145 \pm 67 + 113 \pm 39 \times \text{x; R}^2 = 0.15 \]

where \( y \) is the iodine concentration in milk (µg/L) and \( \text{x} \) is the iodine concentration of the diet (mg/kg).

However, the low coefficient of determination (0.15) and the high variation of the values indicate that other factors, such as the presence of glucosinolates, milking management and keeping of animals may have affected the concentration of iodine in milk. Some years earlier a similar equation was deduced by Swanson et al. [90].
\[ Y = 98.6 + 155.9 \times \text{x; R}^2 = 0.525 \]

where \( y \) is the iodine concentration in milk (µg/L) and \( \text{x} \) is the iodine concentration in feed (mg/kg DM).

The total excretion of iodine via milk (carry-over of iodine from feed into milk) varied between about 10 % (with rape seed meal; [50] and up to 55 % [51,90] of iodine intake.

**Iodine species**

According to the EU-Legislative sodium iodide (NaI), potassium iodide (KI), calcium iodate hexahydrate (Ca(IO\(_3\))\(_2\) x 6H\(_2\)O) as well calcium iodate anhydrous (Ca(IO\(_3\))\(_2\)) are approved as nutritive additives for feed supplementation in the EU [33].

Iodides are powerful reducing substances, but iodates are a powerful oxidising agents [91]. Their common use in feed may result in redox reactions which in turn may influence feed characteristics, shelf life and stability.
Therefore [34] recommended avoiding the simultaneous use of different iodine sources because of the possible comproportionation reaction under the acidic conditions of the stomach.

Some authors compared the effect of various iodine sources in dairy cattle feed on the transfer into milk. Franke [30] reviewed data about the influence of iodine species on the iodine concentration of milk. Most studies comparing iodate and iodide showed no clear differences in the iodine concentrations of milk after daily application [54,92]. In contrast, Leskova [93] described higher milk iodine concentrations as well longer excretion times following oral application of potassium iodate compared to potassium iodide. Franke et al. [51] detected similar tendencies for higher iodine concentrations in milk when applying iodate compared to iodide (Table 2; Fig. 2). However, this effect was not related to a higher iodine amount transferred into milk, but due to the lower milk yield in the iodate groups. On the average the iodine transfer from feed into milk amounted to 31.2% in the case of iodide and to 31.9% in the case of iodate [50].

Table 2

It is discussed that iodate may lead to higher iodine concentrations in milk, since higher storage losses are expected for iodide due to its instability in the presence of oxygen, excessive aeration, sunlight and ultraviolet light [94,95]. However, in other studies [50,51] feed iodine concentrations throughout the whole experiment did not differ between the feed mixtures with added potassium iodide and calcium iodate hexahydrate which does not point to higher storage losses of iodide.

Goitrogens in the rations

Goitrogens are agents that may cause thyroid enlargement by interference with the thyroid hormone synthesis and secretion including feed-back mechanisms of thyroid stimulating hormone (TSH) and TSH releasing factor. They either influence the iodine uptake into the thyroid, the oxidation of iodide to elemental iodine with the subsequent transfer into the thyroglobulin, the synthesis of thyroid hormones or the proteolysis or release of the thyroid hormones [96]. Apart from plants of the cruciferous family, including rape, canola and kale, as well as raw soybean, beet pulp, millet, linseed, cyanogenic strains of white clover and sweet potato may contain goitrogen substances [81].

Glucosinolates contained in cruciferous plants liberate isothiocyanates, oxazolidinithiones and further degradation products into the animal organism which inhibit the sodium iodide symporter and thereby diminish the iodine uptake by the thyroid and also the mammary gland [97]. In consequence feedstuffs with glucosinolates and its degradation products, like co-products from oil production such as rapeseed and other feed of the genus Brassica lead to a lower iodine excretion via milk, as already described by Piironen and Virtanen [98]. Indeed, solvent extracted rapeseed meals (RSM), or rapeseed press cakes (RPC) from old conventional rapeseed varieties with a glucosinolate concentration in a magnitude of 50 to100 mmol/kg depressed the milk iodine content by one half [99] up to three quarters [100] as compared with a ration free of rapeseed feed, but, with the same iodine addition. Since the 1970s and 1980s, in comparison with high glucosinolate conventional rapeseed varieties the glucosinolate content of rapeseed was decreased by at least four fifths in Canada and Europe as a result of successful plant breeding. Farm animals tolerate higher amounts of newer low glucosinolate varieties and up to 4.5 kg rapeseed meal for a cow are fed daily compared with 1 to 1.5 kg high glucosinolate rapeseed meal in the older dairy cow feed regimes. Considering the different feeding recommendations for high versus low glucosinolate rapeseed meal the glucosinolate intake per cow and day does not differ greatly between the former and the more recent studies.

Testing rapeseed feeds in recent German cow experiments in comparison with rapeseed-free protein sources, mainly soybean meal, diets consisting primarily of silages, hay and grain were supplemented in a range from 0.7 to about 1 mg iodine/kg DM, dosages typical for dairy cows on farm level (Table 3). In older Scandinavian and recent Czech experiments the iodine supplementation level was higher – in the Czech Republic according to the strategy mentioned for higher milk iodine concentrations as a contribution to a better consumer iodine supply.

Table 3

By inclusion of rapeseed feeds, glucosinolates diminished the milk iodine concentration in a range from one third to two thirds. In comparison of the trials already conducted between the controls (without rapeseed feeds) the milk iodine concentration differed, presumably due to differences in iodine supply and differently filled thyroid iodine stores prior to the experiment. The extent of the rapeseed mediated milk iodine decrease does not seem to depend on the amount of glucosinolates ingested: At 12 mmol glucosinolates per cow and day [51] a...
milk iodine content reduction of 2/3 in comparison with the control agreed with the reduction extent at 102 mmol glucosinolates per cow and day in the trial of Koch et al. [107].

The experiment of Franke et al. [51] will go beyond the further experiments cited in Table 3, because it demonstrates feed dose effect relations between feed and milk iodine with two diets either with or without rapeseed. Seven iodine dosages including a variant without added iodine were tested: 0, 0.5, 1, 2, 3, 4, 5 mg iodine per kg feed DM.

Diet I was without RSM, Diet II contained 16.5% of RSM on DM-basis and 0.6 mmol glucosinolates/kg DM, respectively.

Reductions of the milk iodine concentration of 51 up to 78 % [50,51] and 54 % [105] were observed at glucosinolate intakes between 11.0 and 13.7 and 9.2 mmol/d, respectively compared to a glucosinolate free ration (see Fig. 2).

Fig. 2

The following regression equations were calculated:

\[ y = 342.2 - 73.1; \text{(} R^2 = 0.98 \text{)}, \]

\[ y = 298.3 - 64.0; \text{(} R^2 = 0.97 \text{)}, \]

\[ y = 136.5 - 67.1; \text{(} R^2 = 0.94 \text{)}, \]

\[ y = 112.0 - 24.3; \text{(} R^2 = 0.96 \text{)}, \]

where \( y \) is the iodine concentration of the diet(mg/kg DM) and \( x \) is the iodine concentration in milk (µg/kg).

In the study by Franke et al. [51], the milk iodine depression was observed at various iodine supplementations. Since the percentage of milk iodine reduction by rapeseed stayed constant, it was shown that the inhibitory effect of the glucosinolates will not be overcome by higher iodine dosages. The carry-over of iodine decreased from 46.9% after feeding of diets without rapeseed meal to 16.3% after supplementation of rapeseed meal [50].

Based on Fig. 2 and the regression equations by Franke et al. [50] EFSA [34] calculated the safety of the consumer on the basis of iodine concentrations in milk a shown in Table 4.

Table 4

These results agree with findings by Trinacty et al. [108] who determined 595 µg iodine/L milk of cows and after supplementation of 27% rapeseed meal to the diet a reduction to 209 µg iodine/L. Hejtmankova et al. [109] also found a significantly reduced iodine content in milk when cows were fed with rapeseed-enriched diets (181 µg iodine/L) in comparison to cows without rapeseed cake (425 µg iodine/L milk). The authors also postulated that fresh fodder may contain more goitrogenic substances than dry products.

In accordance with present monitoring studies milk iodine concentrations in a magnitude of 100 to 300 µg /kg should be targeted. Diets without iodine antagonists realize this desired milk iodine concentration range of 0.5 to 1.5 mg iodine/kg diet DM. This iodine addition falls between the recommendations of animal nutrition societies (e.g., [12]) and the practices of feed compounders [110]. Regarding concentrations of less than 100 µg iodine/kg milk caused by rapeseed feeds in three of the eight cited experiments (Table 3) the iodine supplementation in the presence of GSL should be in a range of 2 to 3 mg/kg diet DM. This iodine dosage level of feed containing iodine antagonists corresponds with the 4 to 6fold of the recommended supplementation of dairy cow diets free of glucosinolates and presumably free of further iodine antagonists.

Apart from rapeseed products, other cruciferous plants such as Crambe abessynica also show similar effects on the iodine concentration of milk (Table 5). About 0.8 and 1.45 kg DM of crambe cake or crambe meal per cow and day reduced the iodine concentration of milk from 226 (average) to about 120 and 97 µg/L milk (Table 5).
Animal keeping (Grazing/outdoor; Indoor season)

Some authors compared the iodine concentration of milk from summer (outdoor) with winter (indoor) feeding and keeping of dairy cows (Table 6).

**Table 6**

In summary except for cow milk in the study by Paulikova et al. [113], recent results in Table 6 show a higher iodine content of milk during indoor/winter feeding, probably caused by a higher mineral supplementation and a lower content of antinutritive substances (e.g. glucosinolates) in the winter diet. Similar data were also demonstrated in previous studies (e.g. [119-121]). In his paper, Pennington [120] compared summer and winter milk from 15 reports and found an iodine concentration of winter milk 1.4 to 6 times higher compared with summer milk. Furthermore Prestlekkken et al. [122] found it also noticeable that the average iodine concentration of winter milk (indoor feeding) measured in Norwegian studies by Dahl et al. and Haug et al. [41,62] was reduced by almost half during the last decade.

**Table 6**

Goat milk in the study by Paillikova et al. [113] and sheep milk in the study by Rozenska et al. [116] contained less iodine than cow milk (Table 6, see also Chapter 2.6.). On the other hand, Lengemann [123] and Lengeman and Wentworth [124] informed about an up to six times higher iodine concentration in milk of goats during high environmental temperatures, probably as a consequence of changes in metabolic processes. Thyroid activity was observed to be lower in summer than in winter and spring [55]. Thompson et al. [125] described a lower thyroidal T4 secretion at higher temperatures and less iodine is used for thyroxin production under high temperatures and more iodine would be available for the mammary gland and for incorporation into milk. But recent studies on this topic are lacking.

Farm management (organic – conventional)

Type of farming seems to be a further influencing factor on the iodine content of milk (Table 7).

**Table 7**

Various factors may be responsible for the lower iodine content in milk from organic farming compared with milk from conventional farming. The differences in iodine content between organic and conventional milk can mainly be explained by the variation in feeding practices [41,126]. A reduced use of iodine-containing mineral mixtures (see Table 7) and the less frequently practice of teat dipping (see Table 8) in organic farms could be reasons for lower iodine content in organic milk.

**Table 7**

Trinacty et al. [109] and Travnicek et al. [66] supposed a higher content of goitrogenic substances in fresh fodder compared with dry feed. Considering values observed in bulk milk, mean supplementation is likely not to exceed 2 mg iodine/kg DM. This conclusion is confirmed by analyses of German feed samples (mixed ration), which contained between 0.45 and 3.04 mg iodine/kg DM [110].

Cattle breed, lactational stage and small ruminants

Less Little information is available about the influence of cattle breed on the iodine content of milk. [127] mentioned an influence of breed on milk iodine concentration, but later papers could not confirm this statement.

Regarding the impact of lactation, it was shown that colostrum generally features considerably higher iodine contents than later milk [128,129]. The National Research Council (NRC) [17] reacted with higher iodine
requirements for goats in comparison to cows. The concentration of dietary iodine recommended by the NRC [17] is given with 0.5 mg/kg DM in the diet of growing and non-lactating goats and 0.8 mg/kg DM for lactating goats.

Apart from that, data is controversial. Franke et al. [127] detected increases of milk iodine with a rising stage of lactation while Scherer-Herr [130] showed decreased concentrations and Krupova et al. [131] found no differences. Falkenberg et al. [132] described a negative correlation between the iodine concentration of milk and milk yield of cows.

These results are understandable, if the feed intake did not linearly increase with the milk yield. In the case of a linear correlation between dry matter intake and milk yield, the iodine concentration in milk should not be changed.

Information about the effects of iodine supplementation on milk composition in goats and sheep are rather scarce. Only a few studies with small ruminants are available to assess influencing factors on the iodine content of their milk (see Tables 6 and 7). Travnicek and Kursa [133] investigated the milk iodine concentration in ten sheep flocks and found 105 µg/L. The corresponding value for four farms where sheep had access to mineral licks containing 35 mg iodine/kg was 243 µg iodine/L milk. Similar results were measured for goats.

Goats and sheep showed a similar influence of outdoor (summer) and indoor (winter) keeping compared with dairy cows ([113,116]; see Table 6). Sheep kept under organic farming conditions showed a lower milk iodine concentration than conventionally kept sheep ([116]; see Table 7). However, in samples from eight sheep (East Friesian) kept on an organic farm with access to iodized mineral premix (50 mg iodine/kg) a very high milk iodine concentration of 520 µg iodine/L indicates a very high value was measured [134].

Nudda et al. [135] fed ten goats each of the Sarda population with an unsupplemented hay-concentrate diet containing 0.35 mg iodine/kg DM and supplemented two other groups with 0.45 and 0.90 mg potassium iodide/d. The iodine intake amounted to 0.65, 1.00 and 1.35 mg/d and the mean milk iodine concentrations were 60, 79 and 130 µg/L. The carry-over of iodine from feed into milk varied between 9.4 and 11.3%, without significant differences caused by the amount of iodine supplementation. These figures are lower than the values mentioned for dairy cows (see 2.1). But in general, goats and sheep showed a similar behaviour concerning carry-over of iodine in the milk as dairy cattle. Therefore the upper level for feeding of lactating minor animal species was also reduced from 5 to 2 mg iodine/kg DM (see EFSA [34]).

Teat dipping with iodine containing substances

Teat dipping with iodine-containing solutions was or is still usual-common in many countries because of its use for preventing transmission of contagious mastitis pathogens from cow to cow [136]. Nowadays it has been stopped in some countries and iodine is replaced by other substances (e.g. chlorohexidine, glycerine, panthenol etc.) in the dipping solution. Depending on the iodine content of the disinfectant and the manner of application of solution, the iodine concentration of milk could be significantly increased (Table 8). Conrad and Hemken [137] determined the mode by which the iodine entered the milk. They concluded that the primary mode of increased iodine appears to be the absorption through the skin and entry into the milk by the milk synthesis process rather than by contamination from the teat’s surface. On the other hand, Rasmussen et al. [138] suggested from their studies that iodine residues in milk originate mainly from contamination of the teat surface rather than from iodine absorption through the skin, because iodine residues in milk were insignificant when teats were cleaned with towels.

Table 8

Most authors investigated the influence of iodine after milking, but the available iodine level in disinfectants seems to be more important for iodine transfer into the milk than dipping time (Table 8). On the average, iodine concentration in milk increased between 50 and 60 µg/L, if 3 to 5 g/L available iodine were in the disinfectants used for dipping after milking, but the results vary between no appreciable additional amounts of iodine in the bulk milk and up to more than 100 µg additional iodine/L. The expected effect of teat-dipping with iodine containing dipping solutions on transfer into milk is lower when the milk yield per cow increases. For example, the milk yield in the study by Flachowsky et al. [142] amounted to 20 L/d. Galton [143] used cows with a higher milk yield and recorded a lower iodine transfer into the milk despite a higher iodine concentration in the disinfectants (Table 8). Another influencing factor could be the inaccurate content of iodine in the disinfectants solution (see Wilkens [146]) or the spraying of solution (e.g., Castro et al. [80]).
Milk processing in the dairy

Milk pasteurization is the most important step of milk processing. Norouzian et al. [84] and Norouzian [83] used the high-temperature short time pasteurization method (HTST) in two studies. In the first study they measured an average decrease of iodine concentration in milk by 34% varying between 21.2 and 53.1%. The values for the second study were 27.4%, varying between 17.6 and 37.6% (Table 9). Other authors also describe iodine losses between 20 and 40% during pasteurization [147,148], but Aumont et al. [149] did not find any impact of pasteurization and spray drying on iodine concentration of milk.

The reason for iodine losses during treatment is the sublimation characteristic of the element, because more than 90% of iodine in milk is inorganic [83,84]. The iodine losses during pasteurization could also be one reason for the differences in iodine concentration in raw milk (see Fig. 1 and Tables 2 and 3) and in bulk milk or in milk samples from the food retail sector (see Tables 6 and 7), if adequate amounts of iodine were supplemented to the diets. Small differences in iodine concentration of milk have been also described between whole milk (251 µg/L), semi-skimmed milk (254 µg/L) and skimmed milk (273 µg/L; [117]). More research seems to be necessary to understand the behaviour of various iodine species depending on the feed and milk processing [91].

Table 9

<table>
<thead>
<tr>
<th>Method of iodine analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target for the analysis is the milk iodine concentration, however, in a dose-effect approach also the iodine supply, i.e. the content of native feed and of the iodine supplement, has to be determined with a valid method. Additionally, under biochemical and physiological aspects a specification of iodine compounds, mainly in the milk, may be of interest. The ionic iodine, e.g. the iodide and iodate salts, approved as feed additives, and also the iodide in urine and milk, as well as the organic iodine compounds, e.g. thyroxine and further iodothyronines of blood and tissues, are characterized as more or less stable. However, elemental iodine is at least in parts volatile and during preparation and analysis of biological sample a risk for iodide oxidation with a subsequent loss of the analyte has to be avoided. Also in the context of prevention of oxidation a complete digestion of organic part of the sample and a full mineralization, resp., is the prerequisite for minimizing each iodine loss during analysis.</td>
</tr>
</tbody>
</table>

At present, in most feed and food laboratories and in clinical laboratory diagnostics measurement of total iodine is carried out either by a “traditional” method, the so-called Sandell-Kolthoff (SK) method [150], or by the newer method of inductively coupled plasma mass spectrometry (ICP-MS) [151]. The first uses the iodide catalysed reduction of yellow Ce(IV) by As(III) to colorless Ce(III). Without the catalyst the reaction is normally very slow. Only trace amounts of iodide fasten the bleaching reaction according to the following scheme:

\[
2 \text{Ce}^{4+} + 2 \text{I}^- \rightarrow 2 \text{Ce}^{3+} + \text{I}_2
\]

\[
\text{As}^{3+} + \text{I}_2 \rightarrow \text{As}^{5+} + 2 \text{I}^-
\]

The rate of disappearance of the yellow color in a Ce(IV)-As(III) mixture, at most measured as absorbance at 405 to 420 nm, represents the iodine content. Iodate acts also as catalyst, however, indirectly, because it is converted to iodide in the presence of arsenite in an acidic medium [152]. Organoiiodine compounds do not react without decomposition which illustrates the importance of mineralization in preanalytics.

Today inductively coupled plasma mass spectrometry (ICP-MS) is often used as a very sensitive method for the quantitative determination of the total iodine content in feed and food. An induction coil generates a very hot argon plasma (4,500 to 8,000 K) which splits the sample into the atoms according to their number of valence electrons. Although the relatively high first ionization potential of iodine (10 eV) caused only a partial ionization in the plasma, very low determination limits are achievable.

Particular attention must also be paid to the complete extraction of iodine from the sample and also to avoiding losses. The iodine concentration of fresh milk samples can be determined by direct measurement of the diluted samples by using standard addition calibration method.

For digestion procedures focusing at iodine preservation up to the determination, it is consensus by operators in different laboratories that ashing at 600°C and dilution in acids, as routinely used for mineral analyses are not
practicable without iodine losses. In laboratories for clinical diagnostics, particularly for urine and blood serum, acid digestion procedures dominate, e.g., with perchloric acid or its combinations with nitric acid or with nitric acid and sulphuric acid [152]. Benotti et al. [153] established a chloric acid digestion procedure which was adopted in many investigations of urine, feces, tissues and food. On the other hand, starting with the approach of Barker et al. [154] the alkaline digestion is widely distributed.

In food and feed analytics, there are two standard methods for iodine extraction. The VDLUFA (Association of German Agricultural Analytic and Research Institutes) method 2.2.2.3 [155] can be used to determine the amount of iodide and iodate added to the animal feed. The dried and homogenized sample material is extracted by using 0.5% ammonia solution at room temperature. However, this method does not allow analysts to assess whether all natural iodine has been recovered by this procedure. The method is used for checking supplemented iodine amount in feed samples taken by official feed surveillance in Germany. The limit of quantitation is 0.4 mg/kg.

The European standard EN 15111:2007 [156] specifies an extraction method for the determination of iodine compounds in foodstuffs by ICP-MS extracted with the stronger alkaline reagent tetramethylammonium hydroxide (TMAH = (CH₃)₄N⁺OH⁻) at elevated temperature (90°C). In a comparison of both digestion methods, the TMAH digestion adopted from 22 food laboratories showed significantly higher native iodine contents for a range of feed than the ammonia digestion [157].

As described above for low iodine matrices the ICP-MS method seems to be more sensitive compared with the SK method. Contrasting with that, in former investigations by the SK method for feed and meat samples too high iodine concentrations were determined and laid down in feed and food tables [158,67]. Overestimations of iodine content point to possible interferences of halides and pseudohalides (e.g. the thiocyanate ion).

A comparison was made between the SK method and the ICP-MS method in milk.

Eleven milk lyophilisates over a broad iodine concentration range from a dose response experiment [52] were analyzed by both these methods (Remer et al., Schöne and Leiterer 2006, unpublished results). The analysis results, means (and minimum maximum ranges) agreed satisfactorily: 9.2(0.8–25.9) mg iodine/kg lyophilisate for the ICP-MS method versus 11.1 (0.7–30.1) mg iodine/kg lyophilisate for the SK method. In the regression/correlation analysis the milk iodine determinations demonstrated a high agreement, by the equation: $y = 1.24x - 0.19 (R^2 = 0.999, P < 0.001)$, where $x$ represents iodine concentration determined by ICP-MS and $y$ represents it determined via the SK reaction.

The many approaches using ion sensitive electrodes (IES)—at most silver iodide precipitated by a silver-anodic surface is measured—has been not established in laboratory routine up to now. Reasons for the lacking success might be interferences with halides and pseudohalides, a lower lifespan of the IES and as comparison with the ICP-MS method a lower precision and sensitivity, i.e., higher LOD [152]. Despite these limitations the development of IES for fast and reliable iodine determination [159] preferably in milk, urine and blood serum, should be continued.

Iodine concentration in milk and other samples is also discussed under consideration of the methods of iodine analysis. The SK reaction was mainly used for iodine determination in previous studies. More recent studies use the inductively coupled plasma mass spectrometry (ICP-MS) as described by Benkhedda et al. [160]. The method by Benotti et al. [161] was also used in some recent studies (e.g., Soriguer et al. [117]). Some authors compared the SK method with the ICP-MS method. Johner et al. [64] compared both analytical methods and found a good agreement of results ($r=0.99$).

Conclusions

A linear correlation exists between dietary iodine concentration and milk iodine concentration, but other factors, such as milking management and the presence of goitrogens (glucosinolates) or milk processing, may also affect the concentration of iodine in milk. Therefore, the iodine content of milk and milk products may vary considerably depending on many influencing factors.

There is no simple solution to declare the iodine concentration of milk and milk products on the food label. For this purpose and taking into account the low range between requirements and upper levels for various human age groups, iodine supplements should be used with caution in the feed of lactating cows and other lactating ruminants and high iodine concentrations in milk should be avoided.
Because of this, the EFSA [34] proposed a reduction of the upper level for iodine concentration in feed for dairy cattle and minor dairy ruminant species from 5 to 2 mg iodine/kg feed. On the other hand, iodized salt is the most important source of iodine in human nutrition. Against the background of the recommendations for reductions in salt intake as a public health measure worldwide [162], an increment of the iodine concentration in salt should be considered.

Recently, further research need to improve knowledge on iodine metabolism and status in humans and animals was identified by Swanson et al. [163] and the EFSA [34].

Conflict of interest
On behalf of all authors, the corresponding author states that there is no conflict of interest.

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158. DLG (1973) Mineralstoffgehalte in Futtermitteln. DLG-Verlag, Frankfurt am Main
Table 1: Iodine requirements of humans (µg/d) by various Organizations and Scientific Societies (WHO (World Health Organization), ICCIDD (International Council for the Control of Iodine Deficiency Disorders), UNICEF (United Nations Children’s Fund); FNB (Food and Nutrition Board), IOM (Institute of Medicine’s); DACH (Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, Schweizerische Vereinigung für Ernährung))

<table>
<thead>
<tr>
<th>Age/physiological stage</th>
<th>[215]</th>
<th>[816]</th>
<th>[917]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 1 year</td>
<td>110 - 130</td>
<td>40 - 80</td>
<td></td>
</tr>
<tr>
<td>0 – 6 years</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – 8 years</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – 15 years</td>
<td></td>
<td>100 – 200</td>
<td></td>
</tr>
<tr>
<td>6 – 12 years</td>
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<tr>
<td>9 – 13 years</td>
<td>120</td>
<td></td>
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<tr>
<td>14 – 18 years/adults</td>
<td>150</td>
<td>150</td>
<td>180 - 200</td>
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<td>Pregnancy</td>
<td>220</td>
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<td>200 - 230</td>
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<tr>
<td>Pregnancy/Lactation</td>
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<td>290</td>
<td>260</td>
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<tr>
<td>Lactation</td>
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</tbody>
</table>
Table 2: Influence of iodine source potassium iodide and calcium iodate hexahydrate on iodine concentration of milk as µg/L (Franke et al. [50])

<table>
<thead>
<tr>
<th>Supplementation (mg iodine/kg DM)</th>
<th>Unsupplemented control (0.2 mg iodine/kg DM)</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>potassium iodide</td>
<td></td>
<td>83</td>
<td>158</td>
<td>214</td>
<td>550</td>
<td>638</td>
<td>1085</td>
</tr>
<tr>
<td>calcium iodate hexahydrate</td>
<td></td>
<td>72</td>
<td>188</td>
<td>231</td>
<td>584</td>
<td>930</td>
<td>1188</td>
</tr>
</tbody>
</table>
Table 3: Experiments on dairy cows with solvent-extracted rapeseed meal (RSM) and rapeseed press cake (RPC) at a supplementation level of from 0.7 to more than 2 mg iodine/kg dry matter – Milk yield and iodine concentration in comparison with a control (without rapeseed feed)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Iodine content (addition) mg/kg diet dry matter</th>
<th>Groups, intake rapeseed feeds per cow and day</th>
<th>Milk yield kg/day</th>
<th>Analysis method</th>
<th>Concentration of iodine µg/L milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>[104][14]</td>
<td>1.5 (addition)</td>
<td>without 1.4 kg RSM, 19.9 mmol GSL/kg</td>
<td>19.6 (d)</td>
<td>1</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5 kg RSM, 16.7 mmol GSL/kg</td>
<td>20.9 (d)</td>
<td>2</td>
<td>102*</td>
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<td></td>
<td></td>
<td></td>
<td>19.2 (d)</td>
<td></td>
<td>81*</td>
</tr>
<tr>
<td>[102][15]</td>
<td>1.2 (addition)</td>
<td>without 4.2 kg RPC, 10.0 mmol GSL/kg</td>
<td>32.3 (g)</td>
<td>2</td>
<td>522</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4 kg RPC, 4.5 mmol GSL/kg</td>
<td>32.3 (g)</td>
<td></td>
<td>176*</td>
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<tr>
<td>[103][16]</td>
<td>no information</td>
<td></td>
<td>32.0 (g)</td>
<td>3</td>
<td>180*</td>
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<td></td>
<td></td>
<td></td>
<td>26.3 (g)</td>
<td></td>
<td>156</td>
</tr>
<tr>
<td>[104][17]</td>
<td>2.2</td>
<td>without 2.5 kg RPC, 22 mmol GSL/kg</td>
<td>31.6 (g)</td>
<td>3</td>
<td>108</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>21.1</td>
<td>4</td>
<td>595</td>
</tr>
<tr>
<td>[105][18]</td>
<td>1</td>
<td>without 2.6 kg RSM, 14 mmol GSL/kg</td>
<td>21.7</td>
<td>3</td>
<td>595</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35.0</td>
<td>3</td>
<td>595</td>
</tr>
<tr>
<td>[106][19]</td>
<td>1.8</td>
<td>without 2.0 kg RSM, 5.9 mmol GSL/kg</td>
<td>36.5</td>
<td>4</td>
<td>162*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24.0 (g)</td>
<td></td>
<td>367</td>
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<td></td>
<td></td>
<td></td>
<td>22.7 (d)</td>
<td></td>
<td>197*</td>
</tr>
<tr>
<td>[51]</td>
<td>1.4</td>
<td>without 2.2 kg RPC extruded, 25.3 mmol GSL/kg</td>
<td>34.8</td>
<td>3</td>
<td>222</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.6 kg RSM, 3.5 mmol GSL/kg</td>
<td>33.7</td>
<td>3</td>
</tr>
<tr>
<td>[107][20]</td>
<td>0.8</td>
<td>without 4 kg RPC, 25.5 mmol GSL/kg</td>
<td>28.6</td>
<td>3</td>
<td>182</td>
</tr>
</tbody>
</table>

Rapeseed meal
Glc osinolates
Rapeseed press cake
Fat corrected

Analysis methods: 1 not defined, 2 ion sensitive electrode (IES), 3 inductively coupled plasma mass spectrometry (ICP-MS), 4 Sandell-Kolthoff (SK) method

* Significant difference
Table 4: Milk iodine concentrations (µg/kg) of dairy cows at various concentrations of iodine in complete feed with and without rapeseed meal (Calculations based on regression equations by Franke et al. [51])

<table>
<thead>
<tr>
<th>Diet Type</th>
<th>Iodine (mg/kg feed DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Without glucosinolates</td>
<td>90</td>
</tr>
<tr>
<td>With glucosinolates*</td>
<td>20</td>
</tr>
</tbody>
</table>

*0.58 mmol glucosinolates/kg DM or 11.0 to 13.7 mmol glucosinolates intake per cow and day
**Table 5:** Influence of various amounts of crambe cake (50.4) and crambe meal (77.4 mmol glucosinolates/kg DM) in ration of dairy cows on the iodine concentration of milk (n = 10 cows; 0.8 mg iodine/kg DM; Böhme et al. [111,124])

<table>
<thead>
<tr>
<th>Crambe in concentrate (%)</th>
<th>Crambe cake intake (kg/d)</th>
<th>Glucosinolate intake (mmol/d)</th>
<th>Iodine in milk (µg /L)</th>
<th>Crambe meal intake (kg/d)</th>
<th>Glucosinolate intake (mmol/d)</th>
<th>Iodine in milk (µg /L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>271 ±64</td>
<td>0</td>
<td>0.83</td>
<td>182±30</td>
</tr>
<tr>
<td>15</td>
<td>0.77</td>
<td>39.0</td>
<td>142 ±40</td>
<td>0.83</td>
<td>59.8</td>
<td>95±28</td>
</tr>
<tr>
<td>30</td>
<td>1.35</td>
<td>75.2</td>
<td>117 ±33</td>
<td>1.53</td>
<td>114.7</td>
<td>77±14</td>
</tr>
</tbody>
</table>
Table 6: Influence of summer (outdoor, grazing) and winter (indoor) animal feeding and keeping on the iodine concentration of bulk milk (µg/L) in some European studies.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Country</th>
<th>Type of animal feeding/keeping</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>[41]</td>
<td>Norway</td>
<td>Outdoor 88</td>
<td>Indoor 232</td>
</tr>
<tr>
<td>[41]</td>
<td>Norway</td>
<td>Outdoor 60</td>
<td>Indoor 127</td>
</tr>
<tr>
<td>[409122]</td>
<td>Czech Republic</td>
<td>Outdoor 212</td>
<td>Indoor 251</td>
</tr>
<tr>
<td>[412125]</td>
<td>Czech Republic</td>
<td>Outdoor 351</td>
<td>Indoor 494</td>
</tr>
<tr>
<td>[413126]</td>
<td>Slovakia</td>
<td>Outdoor 155</td>
<td>Indoor 127</td>
</tr>
<tr>
<td>[413126]</td>
<td>Slovakia</td>
<td>Outdoor 56</td>
<td>Indoor 198</td>
</tr>
<tr>
<td>[413126]</td>
<td>Slovakia</td>
<td>Outdoor 48</td>
<td>Indoor 89</td>
</tr>
<tr>
<td>[414127]</td>
<td>Poland</td>
<td>Outdoor 100</td>
<td>Indoor 147</td>
</tr>
<tr>
<td>[415128]</td>
<td>Germany</td>
<td>Outdoor 108</td>
<td>Indoor 134</td>
</tr>
<tr>
<td>[416129]</td>
<td>Czech Republic</td>
<td>Outdoor 38</td>
<td>Indoor 72</td>
</tr>
<tr>
<td>[41781]</td>
<td>Spain</td>
<td>Outdoor 247</td>
<td>Indoor 270</td>
</tr>
<tr>
<td>[418130]</td>
<td>Spain</td>
<td>Outdoor 35</td>
<td>Indoor 73</td>
</tr>
<tr>
<td>[62]</td>
<td>Norway</td>
<td>Outdoor 92</td>
<td>Indoor 122</td>
</tr>
<tr>
<td>[64]</td>
<td>Germany</td>
<td>Outdoor 87</td>
<td>Indoor 110</td>
</tr>
</tbody>
</table>
**Table 7**: Influence of type of farming on the iodine concentration of bulk milk (µg/L) in some European studies.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Country</th>
<th>Type of farming</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Organic</td>
<td>Conventional</td>
</tr>
<tr>
<td>[41]</td>
<td>Norway</td>
<td>93</td>
<td>231</td>
</tr>
<tr>
<td>[41]</td>
<td>Norway</td>
<td>51</td>
<td>167</td>
</tr>
<tr>
<td>[47]</td>
<td>Germany</td>
<td>112</td>
<td>169</td>
</tr>
<tr>
<td>[48,130]</td>
<td>Spain</td>
<td>78</td>
<td>157</td>
</tr>
<tr>
<td>[23]</td>
<td>United Kingdom</td>
<td>144</td>
<td>250</td>
</tr>
<tr>
<td>[64]</td>
<td>Germany</td>
<td>58</td>
<td>112</td>
</tr>
<tr>
<td>[63]</td>
<td>Germany</td>
<td>92</td>
<td>143</td>
</tr>
<tr>
<td>[46,129]</td>
<td>Czech Republic</td>
<td>302</td>
<td>350</td>
</tr>
</tbody>
</table>
Table 8: Influence of iodine concentration of teat-disinfectant on the increase of iodine concentration of milk by various authors

<table>
<thead>
<tr>
<th>Available iodine in disinfectants (g/L)</th>
<th>Application of disinfectants; a: after milking; b: before</th>
<th>Increase of iodine concentration in milk (µg/L)</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>35 [139,151]</td>
<td>[439,151]</td>
</tr>
<tr>
<td>2</td>
<td>a</td>
<td>11-60 [140,152]</td>
<td>[440,152]</td>
</tr>
<tr>
<td>2.5</td>
<td>a</td>
<td>7 [141,153]</td>
<td>[441,153]</td>
</tr>
<tr>
<td>2.5</td>
<td>a</td>
<td>54 [142,154]</td>
<td>[442,154]</td>
</tr>
<tr>
<td>2.7</td>
<td>b</td>
<td>69 [143,155]</td>
<td>[443,155]</td>
</tr>
<tr>
<td>3</td>
<td>b</td>
<td>30 [144,144]</td>
<td>[444,144]</td>
</tr>
<tr>
<td>5</td>
<td>a</td>
<td>54 [145,145]</td>
<td>[445,145]</td>
</tr>
<tr>
<td>5</td>
<td>a</td>
<td>20 [146,146]</td>
<td>[446,146]</td>
</tr>
<tr>
<td>5</td>
<td>b (complete cleaning)</td>
<td>25 [147,147]</td>
<td>[447,147]</td>
</tr>
<tr>
<td>5</td>
<td>a</td>
<td>27-32 [148,148]</td>
<td>[448,148]</td>
</tr>
<tr>
<td>5</td>
<td>a</td>
<td>36 [149,149]</td>
<td>[449,149]</td>
</tr>
<tr>
<td>5</td>
<td>b/a</td>
<td>41 [150,150]</td>
<td>[450,150]</td>
</tr>
<tr>
<td>5</td>
<td>b (incomplete cleaning)</td>
<td>88 [151,151]</td>
<td>[451,151]</td>
</tr>
<tr>
<td>5</td>
<td>a</td>
<td>120 [152,152]</td>
<td>[452,152]</td>
</tr>
<tr>
<td>10</td>
<td>a</td>
<td>7 [153,153]</td>
<td>[453,153]</td>
</tr>
<tr>
<td>10</td>
<td>a</td>
<td>46 [154,154]</td>
<td>[454,154]</td>
</tr>
<tr>
<td>10</td>
<td>a</td>
<td>76 [155,155]</td>
<td>[455,155]</td>
</tr>
<tr>
<td>10</td>
<td>a</td>
<td>88 [156,156]</td>
<td>[456,156]</td>
</tr>
<tr>
<td>10</td>
<td>a</td>
<td>90 [157,157]</td>
<td>[457,157]</td>
</tr>
<tr>
<td>10</td>
<td>b/a</td>
<td>110 [158,158]</td>
<td>[458,158]</td>
</tr>
<tr>
<td>10</td>
<td>b/a</td>
<td>150 [159,159]</td>
<td>[459,159]</td>
</tr>
<tr>
<td>10</td>
<td>a</td>
<td>49 [160,160]</td>
<td>[460,160]</td>
</tr>
<tr>
<td>10</td>
<td>a (spraying)</td>
<td>409 [161,161]</td>
<td>[461,161]</td>
</tr>
<tr>
<td>10</td>
<td>b (complete cleaning)</td>
<td>54 [162,162]</td>
<td>[462,162]</td>
</tr>
<tr>
<td>Iodine concentration (mg/kg feed DM)</td>
<td>0.53</td>
<td>3.03</td>
<td>5.53</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Raw milk (Study 1/Study 2) (µg iodine/L)</td>
<td>143/162</td>
<td>431/534</td>
<td>545/560</td>
</tr>
<tr>
<td>Pasteurized milk (Study 1/Study 2) (mg iodine/L)</td>
<td>67/101</td>
<td>307/381</td>
<td>368/415</td>
</tr>
<tr>
<td>Iodine-loss (Study 1/Study 2) (% of iodine in raw milk)</td>
<td>53.1/37.6</td>
<td>28.8/28.6</td>
<td>32.5/25.8</td>
</tr>
</tbody>
</table>

On the other hand, iodized salt is the most important source of iodine in human nutrition.
Fig. 1: Influence of iodine concentration in the feed of dairy cows (mg/kg DM) on the iodine concentration of milk (µg/L) by various authors [79-81, 50, 82-84, 52, 85-92, 94, 50, 95-97, 52, 98].
Fig. 2: Dependence of the milk iodine concentration on the kind of iodine supplementation in diets without and with iodine antagonists via RSM ($n = 8$, ▲, DDGS/iodide; △, DDGS/iodate; ■, RSM/iodide; □, RSM/iodate). DDGS = distillers dried grains with solubles; RSM = rapeseed meal [50,51].