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SOLID participatory research from UK: Iodine concentrations in milk of organic dairy farms

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Summary

Organic milk normally contains less iodine than conventional milk but the concentrations of iodine in organic milk are well within the optimal levels for human nutrition as it is evidenced by a number of European studies. Nevertheless, the relatively lower iodine concentrations in organic milk trigger discussions amongst stakeholders, farmers and researchers in view of the recent evidence that iodine intake has decreased due to a decrease in milk consumption in the UK.

The aim of the current project was to investigate the relationship between iodine concentrations in bulk milk samples with iodine concentrations in forage on organic dairy farms in view of farm practices. Ten organic dairy farms located in the west-south of England were selected and farmers agreed to participate in this study. The farms were categorised in terms of milk iodine concentrations as “low” (i.e below 60 μg/L), “optimal” (i.e between 60 to 120 μg/L) or “high” (i.e above 120 μg/L) and farmers agreed to a monitoring protocol that allowed data collection on iodine and other mineral concentration in milk, blood, urine and forage samples.

The results show that the monthly milk iodine concentrations averaged over the farms remained within optimal levels, but, in some farms milk iodine concentrations were systematically low through the monitoring period. Urine iodine concentrations were significantly higher in the farms with high (i.e. 1.5 mg/kg) or optimal (i.e. 0.5 to 0.8 mg/kg) forage iodine concentrations compared to the farms with low forage iodine (i.e < 0.5 mg/kg). This outcome reflects the well-established evidence that urine iodine is indicative of dietary iodine intake. With regards to milk iodine, this was not the case: farms with low or average forage iodine concentrations had higher milk iodine compared to the farms with high forage iodine concentrations.

Although this outcome is surprising, it reflects the fact that milk iodine concentrations are affected by the use of iodine-based teat disinfectants. Indeed, six out of the 10 case-study farms use iodised post-dip teat disinfectants, while the remaining 4 farms do not. Comparison between the two groups of farms indicated that milk iodine concentrations were 2.3 times higher in the farms that use iodised post-dip teat disinfectants (mean average 195 ± 13 μg/L) compared with the farms that do not use iodised post-dip teat disinfectants (mean average 85 ± 8.9). This outcome indicates that iodised post-dip teat disinfectants have a major positive effect on milk iodine concentrations and can wipe-out any effect that dietary iodine intake might have on milk iodine concentrations.

In conclusion, this study show that the use of iodised post-dip teat disinfectant is the most important influencing factor for the iodine concentration in milk and that where post-dip teat disinfectant is used the iodine concentrations in milk do not serve as a robust indicator in identifying shortfalls in iodine intake.

However, forage iodine concentration is an important factor in maintaining milk iodine concentrations at optimal levels, in addition to its importance in maintaining animal health and performance at optimum levels. Milk iodine concentrations fluctuated within farms across samplings but in some farms they were systematically low. This outcome deserves further attention in order to alleviate recent concerns that organic milk contains less iodine than conventional milk and to avoid that the health status of the animals might be negatively affected by low iodine intake. Where doubts about the iodine supply of animal exist, urine samples can be used to monitor the cow’s iodine status.

Table of content

1 Aims and research question

To investigate the relationship between iodine concentrations in bulk milk samples with iodine concentrations in forage on organic dairy farms in view of farm practices. We hypothesise that low levels of iodine and other minerals in milk are related to nutritional shortfalls due to the farm management practices on organic dairy farms.

2 Background

2.1 Research background

2.1.1 Iodine in dairy cows

Iodine is an essential trace element for animals and humans because it is necessary for the synthesis of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) which have multiple functions in energy metabolism, growth and brain development. The iodine requirement for dairy cows is estimated to be about 0.33 mg/kg DM or about 0.6 mg dietary iodine/100 kg of body weight (NRC, 2001). Pregnancy does not increase the requirement for iodine for thyroxine production to any significant degree (Miller et al., 1988). Late gestation cows incorporate about 1.5 mg iodine/day into thyroid hormone while during lactation thyroid hormone production is increased, especially in high producing cows and iodine incorporation into thyroid hormones may reach 4 to 4.5 mg iodine/day (NRC, 2001). The percentage of the dietary iodine that is incorporated into the thyroid gland is inversely related to the iodine content of the diet. In diets with adequate iodine content, about 20 percent of the dietary iodine is incorporated into the thyroid gland; when intake of dietary iodine is marginal the thyroid gland will incorporate about 30 percent of the dietary iodine and up to 65 percent of the dietary iodine in iodine deficient diets (Miller et al., 1988). Dietary iodine that is not taken up by the thyroid gland is excreted in urine and milk making the iodine content of milk a possible indicator of iodine status (Berg et al., 1988).

Table 1*: Average iodine concentration of bulk milk (µg/l) in some European countries measured during summer (outdoor, grazing) and winter (indoor) periods

Country	Season	
	Summer	Winter
Norway	88	232
Norway‡	60	127
Czech Republic	212	251
Czech Republic	351	494
Slovakia	155	127
Poland	100	147
Germany	108	134
Spain	247	270
Spain‡	35	73
Norway	92	122
Germany	87	110

**Source EFSA Journal 2013 and Flachowsky et al., (2014)*

‡Organic milk

Milk normally contains from 30 to 300 µg iodine per litre (Berg et al., 1988). These values are confirmed by recent studies which report average values from bulk sample analysis between 100 and 200 µg Iodine /l milk (table 1) with the exception of data from the Czech Republic which have higher values (means of 351 and 494 µg Iodine/l milk, respectively for summer and winter) due to a specific feed supplementation program (EFSA, 2013; Flachowsky et al., 2014).

Iodine deficiency reduces production of thyroid hormones slowing the rate of oxidation of all cells. In adult cattle, iodine deficiency can cause enlarged thyroid glands, reduced fertility (in both males and females), increased pregnancy loss and foetal mortality. Under conditions of marginal or deficient dietary iodine the maternal thyroid gland becomes extremely efficient in the removal of iodine from the plasma resulting in slight iodine availability for the foetal thyroid gland and the foetus becomes hypothyroid (Hemken, 1970). Hypothyroid calves may be born hairless, weak, or dead while foetal death can occur at any stage of gestation, while the cow appears normal (Hemken, 1970). Iodine toxicity can occur in adult dairy cows with dietary intakes of just 50 mg/day (about 5mg/kg DM). Symptoms included excessive nasal and ocular discharge, salivation, decreased milk production, coughing and dry, scaly coats (Olson et al.,1984).

2.1.2 Concentrations of Iodine in milk and implications for iodine intake in humans

The iodine requirements for humans are related to age, body weight, physiological stage, and gender and can vary from 40 to 290 µg per day. However, differences in quoted human demand levels for iodine exist between various scientific committees such as the World Health Organization (WHO), Food and Agricultural Organization (FAO), and the German, Austrian, and Swiss Societies for Nutrition (Flachowsky et al., 2014). According to the WHO iodine sufficiency is defined by median urinary iodine concentrations of 100–299 µg /l in school-aged children (i.e. 6 – 12 years-old) and ≥150 µg /l in pregnant women. According to the Scientific Advisory Committee on Nutrition (SACN, 2014) dietary iodine intake in the UK for the adult is 140 µg while for toddlers it is 70 µg per day. The maximum iodine intake level is only three times higher than the required which means there is also a risk of overdosing, especially when supplementing iodine after previous iodine deficiency (Zimmermann et al., 2005). Both, excessive and deficient iodine intake should be avoided as it can cause alterations in thyroid function, may increase the risk of thyroiditis, hyperthyroidism, or hypothyroidism (Pennington 1989; Zimmermann et al., 2005; Pearce et al., 2013).

Table 2*: Average iodine concentration of bulk organic and conventional milk (µg/l) in some European countries

Author(s)	Country	Type of farming	
		Organic	Conventional
Rey Crespo et al. (2012)	Spain	78	157
Bath et al. (2012)	UK	144	250
Payling et al. (2015)	UK	404	595
Johner et al. (2012)	Germany	58	112
Jahreis et al. (2007)	Germany	112	169
Köhler et al. (2012)	Germany	92	143
Rozenska et al. (2011)	Czech Republic[1]	302	350
Dahl et al. (2003)	Norway	72	199

**Source EFSA Journal 2013 and Flachowsky et al., (2014)*

[1]*Sheep milk*

Iodine deficiency has historically been considered an issue for developing countries rather than industrialized countries. However, there is some concern that iodine intake in the UK has decreased due to a decrease in milk consumption (Vanderpump, 2012). Milk and milk products are an important source of dietary Iodine in the UK in view of the lack a of salt-iodization programme in the UK unlike in other European countries (Vanderpump, 2012). Based on a number of European studies (see table 2) organic milk normally contains less iodine than conventional milk, but, concentrations of iodine in organic milk are well within the optimal levels that have been reported for many decades (Berg et al., 1988; Holland et al., 1995; Franke, 2009; Haug et al., 2012; Borucki et al., 2012; EFSA, 2013; Flachowsky et al., 2014).

In a recent study, Bath et al., (2012) compared the iodine concentration of retail organic and conventional milk and evaluated regional influences in iodine levels in the UK. The authors report that organic milk was 42.1% lower in iodine content than conventional milk (median iodine concentration 144.5 v. 249.5 ng/g). However, it is notable that the iodine concentration of organic milk in the UK, as reported in the study of Bath et al., (2012), is one of the highest in Europe compared with that of organic milk in other EU countries (Table 2), with the exception of the Czech Republic, where a specific feed supplementation program was applied (EFSA, 2013; Flachowsky et al., 2014). According to the results of Bath et al., (2012) there is no difference in iodine concentrations between organic and conventional milk in Northern Ireland. Scottish organic milk had the highest geometric mean (i.e. 276.5 ng/g of iodine) of all the samples tested (both organic and conventional) and was significantly higher in iodine than organic milk from the West Country and organic milk of unknown origin. The authors did not test differences in iodine concentration between Scottish organic milk and Scottish conventional milk. The recent study of Payling et al., (2015) reports that organic winter milk has 32.2% lower iodine concentration than conventional milk (i.e. 404 vs. 595 μg/L). According to the literature (Berg et al., 1988; Holland et al., 1995; Franke, 2009; Haug et al., 2012; Borucki et al., 2012; EFSA, 2013; Flachowsky et al., 2014), the iodine concentrations reported in the study of Payling et. al., (2015) are exceptional high (see table 2) even for the "low-in-iodine" organic milk. The results of Payling et. al., (2015) imply that ½ litre of organic or conventional milk provides 1.4 or 2.1 times, respectively, more iodine than the dietary iodine requirements for adults according to the UK Dietary Reference Values (DRVs) for iodine intake (SACN, 2014; Department of Health, 1991). According to Borucki et al., (2012), to preserve milk safety, the milk iodine concentration should be to maintained below 400 μg/L given the fact that at this concentration, a 3-yr-old child would have to consume more than 0.5 L/d of milk to exceed the upper tolerable intake of iodine by 2.8 fold (IOM, 2001). Nevertheless, Payling et. al., (2015) propose that replacement of conventional milk by organic milk will increase the risk of sub-optimal iodine status especially for pregnant/lactating women.

A recent publication from the European Food Safety Authority (EFSA, 2013) reviewed the safety and the efficacy of calcium iodate anhydrous and potassium iodide as iodine feed additives for all animal species. According to this scientific report, the use of sodium iodide (NaI), calcium iodate anhydrous ($Ca(IO_3)_2$) and potassium iodide (KI) as sources of iodine is considered safe for all animal species when used up to the currently authorised maximum content of total iodine in complete feed (EFSA, 2013). High concentrations of dietary iodine increase iodine concentrations in milk, and because humans are much more sensitive to iodine thyrotoxicosis than cows, the danger of excess dietary iodine fed to cattle is also a public health issue (Hetzel and Welby, 1997). According to Franke et al., (2009) the high transfer of iodine from feed into milk may cause the upper tolerable level (UL) in human nutrition to be exceeded at the maximum level of iodine currently permitted in feed in Europe (5 mg/kg) and thus, the maximum level of iodine in dairy cow feed needs to be re-evaluated. The EFSA report predicts that the iodine content of milk, if produced taking into account the current authorised maximum content of iodine in animal feed, would represent a substantially high risk to consumers. The UL for adults (i.e. 600 µg/day) would be exceeded by a factor of 2, and that for toddlers (i.e. 200 µg/day) by a factor of 4. The EFSA report proposes a reduction in the maximum allowed iodine concentrations for dairy cattle from 5 to 2 mg/kg feed in the EU. This reduction would help to lower the exposure of consumers to high iodine intake from food of animal origin to optimal levels. Nonetheless, iodine intake in high-consuming toddlers would remain above the UL (1.6-fold). With regards to the UK, data from the National Diet and Nutrition Survey shows that currently there is no excessive dietary iodine intake as the upper 2.5 percentile of daily intakes of iodine from food sources were 440 µg for men and 290 µg for women (Bates et al., 2011).

2.1.3 Factors affecting the iodine concentration in milk

The levels of micro and macro elements in milk depend largely upon the content of these elements in soil (which affects levels in pasture) and animal feed, which varies considerably among and within countries. In general, the mineral content of milk is not constant through the lactation period of a cow and can be influenced by both genetic and environmental factors. Variation in the reported concentrations of many minerals in milk can also be due to analytical errors and contamination from milk collection and processing equipment and procedures (Cashman, 2006, Flachowsky et al., 2014). Representative values for the average mineral content of milk in the UK are presented in Table 3.

Table 3*: Optimal mineral concentration of bulk milk samples (µg/L)

Mineral	Guide Values
Manganese	20 – 25
Zinc	3000 – 4000
Copper	50 – 60
Molybdenum	40 – 50
Iodine	**60 – 100**
Selenium	15 – 20

**According to Thomson & Joseph Ltd, Albion Laboratory Services, Hoveton, Norwich, NR12 8QN, UK*

In a recently published study, Flachowsky et al., (2014) reviewed key factors that influence the iodine content of cow milk. It is well documented that high concentrations of dietary iodine in the diet proportionally increase iodine concentrations in milk but also in urine and faeces. Franke et al., (2009) tested the effect of six dietary iodine supplementation levels (between 0.5 and 5 mg/kg DM) and found that iodine content in the milk increases in a dose-dependent manner. This is in accordance with earlier studies that show that there is a linear increase in the iodine concentration of milk with increasing iodine intake of the cows (see Figure 1). The types of iodine used as nutritive additives do not seem to affect the iodine content of the milk differently (Franke et al., 2009; Flachowsky et al., 2014) but simultaneous use of different iodine sources must be avoided because of the possible oxidation reactions that can occur under the acidic conditions of the stomach (EFSA, 2013).

In addition to the iodine intake the most important influencing factor seems to be the use of iodine-containing teat dips (Flachowsky et al., 2007; Borucki et al., 2012). Pre-milking teat preparations aim to remove bacteria and other contaminants, particularly the pathogens which can cause environmental mastitis. The aim of post-milking dipping is to remove any contagious mastitis-causing pathogens from the teat surface and just inside from the opened teat canal that are transferred during milking from infected milk residues. Pre-dips and sprays tend to be formulated differently to post-dip treatments but the types of chemicals used for pre-milking treatments are generally the same as those used for post-milking treatments (AHDB Dairy, 2015). The content of iodine in iodine-based teat dips varies between 1-10 g/L and the use of these disinfectants can increase milk iodine concentration by 11 to 150 µg /kg (Flachowsky et al., 2007). Iodine-based teat-dipping spraying solutions increase milk iodine concentrations more than pre-dipping iodine-based sanitizers and according to Borucki et al., (2012) their use should be avoided to maintain milk safety (i.e. <400 µg iodine /L). There is some debate in the literature about how the stage of lactation influences the iodine content of the milk, but it was shown that colostrum generally features considerably higher milk iodine contents than later milk (see Franke, 2009).

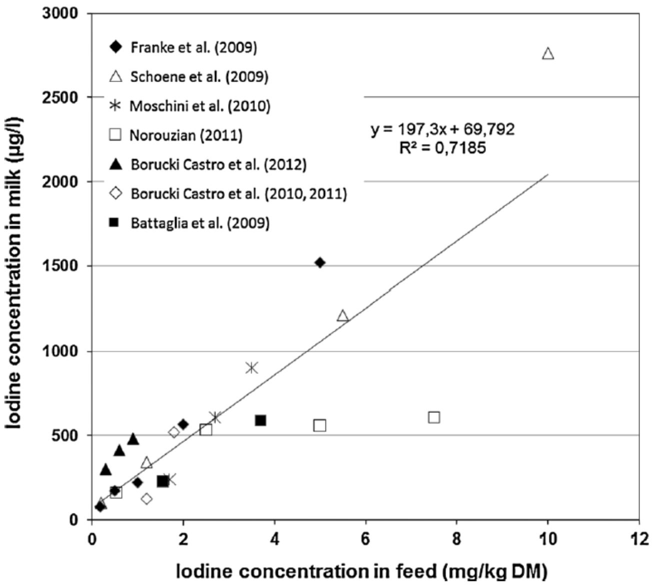

Figure 1: Influence of iodine concentration in the feed of dairy cows (mg/kg DM) on the iodine concentration of milk (µg /L) by various authors [Source: Flachowsky et al., (2014)]

The iodine content of the milk can also be influenced by breed, as at the same level of dietary iodine, there are differences in milk iodine between breeds. However, breed differences, although significant, cannot be used to control iodine concentrations of milk (Franke et al., 1983).

Feeds containing goitrogens or glucosinolates, when fed to cows negatively affect the iodine concentrations of the milk (Flachowsky et al., 2014). Goitrogens are substances that suppress the function of the thyroid gland by interfering with iodine uptake, which can, as a result, cause an enlargement of the thyroid (i.e. a goiter). Plants that contain goitrogenic substances are those 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 (Castro et al., 2011). Glucosinolates are secondary plant metabolites which occur in almost all plants of the order Brassicales such as rape, mustard and cabbage. Glucosinolates inhibit iodine accumulation from the blood to the thyroid and the mammary gland (Franke, 2009).

The current report provides information about dietary factors and farm management practices that influence the iodine content of milk in organic dairy herds based on data collected from case study farms during 2014/2015. The study was undertaken in close collaboration with OMSCo which is an SME partner of the SOLID project.

2.2 Farmers' background

During 2013 and 2014 the Organic Milk Suppliers Cooperative (OMSCo) collected data on iodine concentrations in organic milk from hundreds of farms throughout the UK. Preliminary analysis of these data showed that iodine concentration in bulk milk samples collected in January 2014 (winter) were significantly higher (P <0.0001) than in those collected in September 2013 (136 *vs.* 47.5 µg/L). In the summer, iodine concentrations in the collected samples ranged from as little as 1 µg/L up to 806 µg/L with the 25% and 75% percentiles being 14.9 µg/L and 56.2 µg/L, respectively. Iodine concentrations in bulk milk samples collected in January 2014 ranged from 5.6 µg/L up to 1004 µg/L with 25% and 75% percentiles being 57 µg/L and 162 µg/L, respectively. Although average iodine concentration in winter samples fall within the optimal values, iodine concentrations in milk samples collected during summer were below optimal levels (i.e. 60 µg/L) in 75% of total farms sampled.

3 Methodology and data collection

Twelve case-study farms were selected and farmers agreed to a monitoring protocol that allowed data collection on iodine and other mineral concentration in milk, blood, urine and forage samples. The study lasted from June 2014 to January 2015.

3.1 Selection of the farms

Determination of trace elements in milk samples from more than 800 organic dairy herds throughout the UK have been carried out on behalf of the Organic Milk Suppliers Cooperative (OMSCo) during September 2013 – January 2014. This initial data set was analysed and results were used as a basis to identify participating farms for the current project. Based on these data, the farms were categorised as L (low), O (optimal) or H (high) in milk iodine when milk iodine concentrations were below 60 µg/L, between 60 to 120 µg/L or above 120 µg/L, respectively. To facilitate farm visits and regular contact with the farmers, those farms that were located more than 200 miles from the ORC (Elm Farm, Newbury, RG20 0HR, Berkshire) were excluded from the selection. From the remaining farms, 4 farms from each one of the L, O or H groups were selected randomly for participation in the study. All the selected farmers (i.e. 12 in total) agreed to participate. However, two farms out of the twelve, one from the O and one from the L group, voluntarily withdrew from the study shortly after the start of the monitoring and no data were collected from these farms.

3.2 Location of the farms

Two farms were located in Devon, 5 in Wiltshire, 1 in West Sussex, 1 in Oxfordshire, 2 in Gloucestershire and 1 in Herefordshire (see Table 5). Data collection and Sampling

3.3 Data collection and Sampling

3.3.1 Milk, forage and feed samples for iodine and mineral determination

Bulk milk samples were collected via OMSCo's routine farm milk collection from the participating farms from May 2014 to January 2015. These samples were analysed for iodine and other minerals every 35 – 45 days. In addition, the farmers were asked and agreed to provide a representative sample of the grazed forage or diet (TRM, silage) once every month for iodine and mineral analysis (Freepost sample bags, and input sheets were provided). Laboratory analyses on the milk, forage and feed samples were carried out by Thomson & Joseph Ltd, Albion Laboratory Services, Hoveton, NR12 8QN, UK.

3.3.2 Blood and urine samples for iodine and mineral analysis

For each farm, blood and urine samples from 10 milking cows were obtained under normal vet visits in three occurrences over the study period in 2014. Table 4 summarises the sampling schedule and the type of laboratory analysis carried out in the samples obtained. The first sampling took place during August/September, the second during October/November and the third during December/January. In each occurrence the cows with the highest milk yield (measured over the previous week) were selected for sampling. This was because milk Iodine was analysed in bulk samples: it was anticipated that the cows with the highest milk yield will have the greater contribution to the total milk yield, hence the selection of the cows with the highest milk yield for further testing.

Table 4: Summary of the sampling schedule

Type of laboratory analysis	Sampling period		
	August/September	October/November	December/January
Urine Iodine[1]	Yes	Yes	Yes
Glutathione Peroxidase (GSHPx)[2]	Yes	Yes	Yes
Full Trace Element[3]	Yes	-	-

[1]Urine samples were collected from 10 cows by free catch
[2]Blood samples were collected into lithium heparin (LH) vacutainers from the same 10 cows on the same day in each farm
[2]Blood samples were collected into plain (z) or clot activator tubes from 4 of the 10 cows sampled for urine iodine and GSHPx

On all occasions, blood samples were analysed for glutathione peroxidase (GSHPx), which is a selenium dependent enzyme. Selenium is required for the conversion of the thyroid hormone triiodothyronine (T3) to thyroxine (T4), hence the measure of selenium status. In the first sampling occasion only (i.e. August/September), supplementary blood samples from 4 of these cows were analysed for full trace element profile.

Urine samples obtained from the same 10 cows were analysed for Iodine concentrations. The results are reported in µg/L and are standardised to a creatinine concentration of 5000 µmol/L to account for the different dilution of urine samples collected. All samples were dispatched from the farms within 48 hours of collection. These laboratory analyses were carried out by the School of Veterinary Medicine and Science, University of Nottingham, Loughborough.

3.3.3 Farmer's Questionnaire

To obtain an overview of the management and practices of the case-study farms, the farmers were asked to fill in a questionnaire. This was developed specifically for the purposes of the study and aimed to collect information primarily in relation to the provision of iodised rock salt and the use or not of iodine-based pre- or post- dip teat disinfectants. A according to the literature, these practices are related to or can affect the iodine concentration in the milk (Borucki et al., 2012). The questionnaire also collected information on farm topography, livestock, crop production, health, fertility and housing (these data are beyond the scope of the study and are not presented).

4 Time scale

The project lasted from February 2014 until August 2015. Specific dates and key periods are listed below:

- February 2014 - April 2014: Developing of research question and project planning with SME partner OMSCO.
- May 2014: Dataset analysis, identification of participating farms and farm visits.
- June 2014 to January 2015: Monitoring of the case study farms and data collection
- March 2015: The laboratory analyses finishes
- June to August 2015: Analysis of data and production of final report.

5 Results and discussion

Table 5 indicates the geographic location of the farms that participated in the study, as well as the group in which they have been assigned based on their average milk iodine concentrations measured in September 2013 and January 2014. It is notable that all the farms that do use iodine-based disinfectants (either pre- or post-dip) belonged to the H group and those that do not use iodine-based disinfectants belonged to L group. Group O included farms that practiced either approach. None of the farms uses iodine-based chemicals for cleaning the parlour or milk tanks.

Table 5: Overview of the farms' practices regarding the provision of iodised rock salt and the use of iodine-based pre- or post- dip teat disinfectants.

Farm[1]	location	Group[2]	Farm Practice		
			Iodised Rock Salt	Iodine-based Pre-dip	Iodine-based Post-dip
1	Oxfordshire	O	Yes	No	Yes
2	Wiltshire	O	No	No	No
3	Wiltshire	O	Yes	No	Yes
4	Wiltshire	H	Yes	No	Yes
5	Devon	L	No	No	No
6	Gloucestershire	H	Yes	No	Yes
7	Wiltshire	L	Yes	No	No
8	Devon	L	No	No	No
9	West Sussex	H	No	Yes	Yes
10	Gloucestershire	H	No	No	Yes

[1]Two farms voluntarily withdraw from the study shortly after the start of the monitoring, not shown in the table
[2]Farm categorised as "L; Low", "O; Optimal" or "H; High" had milk iodine concentrations that were below 60 µg/L, between 60 to 120 µg/L or above 120 µg/L, respectively based on bulk milk samples obtained during September 2013 and January 2014.

5.1 Iodine and mineral concentrations in milk

Mineral analysis of milk samples from dairy herds is widely recognised to be a useful indicator of the status of trace elements, particularly selenium, iodine and molybdenum.

The iodine and other mineral concentrations of bulk milk samples obtained from May to December 2014 are shown in Tables 6 and 7, respectively. Calculated mean iodine concentrations over the sampling period (i.e. May to December 2014) varied considerably across the farms; in four farms (i.e. 3, 5, 7, 8) the mean iodine concentrations were below the optimal levels (<60 µg/L; see Figure 2, panel a), in two farms (i.e. 1 and 10) they were within optimal levels (60 to 120 µg/L) and in four farms mean iodine concentrations were above optimal levels (>120 µg/L; see Figure 2, panel a). The highest iodine concentration of 1025 µg/L was observed in a sample collected from Farm 6 (average milk iodine of 576 ± 104.1 µg/L) in September 2014. The lowest iodine concentrations were observed in Farms 8 and 5 with 7 µg/L and 10 µg/L, and mean iodine concentrations of 26 ± 15.3 µg/L and 23 ± 5.3 µg/L, for each farm respectively. Farm 6 had significantly higher milk iodine concentrations compared with Farms 3 ($P \leq 0.05$), 5 ($P \leq 0.001$), 7 ($P \leq 0.01$) and 8 ($P \leq 0.001$); milk iodine concentrations in Farm 4 were significantly higher than Farm 8 ($P \leq 0.05$).

Table 6: Iodine concentrations of bulk milk samples obtained from May to December 2014 in the study farms and average farm iodine concentrations over the same period. Dara are reported as µg/L.

Farm	*n*	Month[1]							Mean ± SE
		M	J	J	A	S	N	D	
1	6		103	13	40	44	89	134	71 ± 18.6
2	6	174		40	42	62	312	279	152 ± 50.0
3	6	18	28		32	11	142	83	52 ± 20.7
4	6	407	121	55		79	144	401	201 ± 65.4
5	6	23	27	10	10		25	45	23 ± 5.3
6	6	645	601	481	289	1025		412	576 ± 104.1
7	6	29	38	56	13	19	63		36 ± 8.2
8	7	7	17	7	5	13	17	117	26 ± 15.3
9	5	143		90	77	73		276	132 ± 38.2
10	6		71	35	72	95	71	277	104 ± 35.6
Farms Sampled		8	8	9	9	9	8	8	

[1]Samples were collected from May 2014 to December 2015 every 35 – 45 days; No sample collection occurred in October; Monthly values are raw data as reported from the lab based on which mean mineral content and SE were calculated for each farm; Laboratory analyses were carried out by the Thomson & Joseph Ltd, Albion Laboratory Services, Hoveton, NR12 8QN, UK

Monthly averaged milk iodine concentrations over the 10 study farms indicate that iodine concentrations in organic milk drop from early spring to late summer and increased again from autumn towards winter (Figure 2, Panel b). Providing that the farmers do not change farm practices in terms of the use of iodine-based teat disinfectants (this effect will be discussed later), this outcome is in accordance with the literature which suggests that winter milk normally contains higher iodine than summer milk, likely because animals spend more time indoors during winter and have access to diets with higher mineral content (Flachowsky et al., 2014). The monthly milk iodine concentrations averaged over the 10 study farms remained within optimal levels (60 to 120 µg/L) or above (>120 µg/L). The lowest average was observed in August with 64 ± 27.7 µg/L and the highest in December with 225 ± 42.6 µg/L (Figure 2, Panel b). Calculated average milk iodine concentrations in all farms from May to August and from September to December 2014 were 112 ± 28.6 µg/L and 166 ± 40.7 µg/L, respectively and the overall average milk iodine concentrations was 135 ± 24.2 µg/L (data not shown). These data suggest that despite milk iodine concentrations being systematically low in some study farms (i.e. Farms 5, 7 and 8), the overall milk iodine concentration in organic milk remains within or above optimal levels defined in the literature for cows that are fed with about 0.33 mg iodine/kg of dietary DM (Berg et al., 1988; Holland et al., 1995; Franke, 2009; Haug et al., 2012; Borucki et al., 2012; EFSA, 2013; Flachowsky et al., 2014).

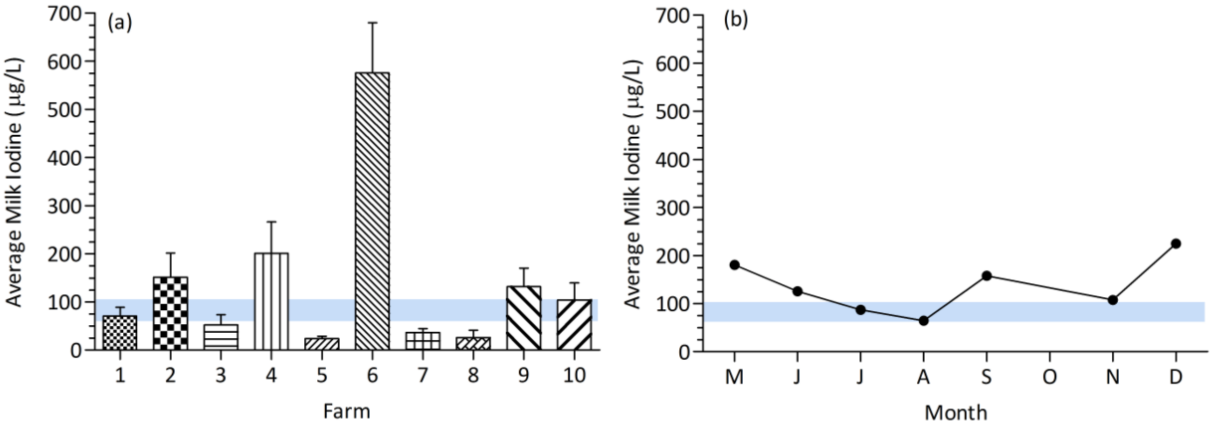

Figure 2: Panel (a), Milk iodine concentrations in each farm averaged over the sampling period (i.e. May to December 2014); Panel (b), fluctuation of the average milk iodine concentrations collected from 10 organic farms from May to December 2014.

Table 7: Average mineral concentration in bulk milk samples in 10 organic dairy farms

		Micro-element (µg/L)[1]					
Farm	*n*	Manganese (Mn)	Copper (Cu)	Zinc (Zn)	Iodine (I)	Molybdenum (Mo)	Selenium (Se)
1	*6*	21 ± 1.5	35 ± 1.5	3531 ± 82	71 ± 18.6	54 ± 5.7	24 ± 3.3
2	*6*	19 ± 1.0	38 ± 4.8	3295 ± 27	152 ± 50.0	56 ± 4.8	22 ± 1.4
3	*6*	23 ± 3.8	35 ± 2.7	3776 ± 78	52 ± 20.7	54 ± 1.1	16 ± 1.8
4	*6*	28 ± 5.8	35 ± 4.5	3340 ± 111	201 ± 65.4	43 ± 2.4	17 ± 1.1
5	*6*	27 ± 1.2	45 ± 1.2	3365 ± 53	23 ± 5.3	47 ± 1.2	23 ± 1.8
6	*6*	25 ± 1.5	44 ± 1.9	3998 ± 80	576 ± 104.1	48 ± 1.4	15 ± 0.3
7	*6*	23 ± 1.0	38 ± 5.1	3211 ± 126	36 ± 8.2	47 ± 4.8	17 ± 3.4
8	*7*	64 ± 4.0	47 ± 3.0	4139 ± 48	26 ± 15.3	47 ± 0.8	18 ± 0.9
9	*5*	20 ± 1.7	54 ± 16.3	3327 ± 292	132 ± 38.2	66 ± 10.1	20 ± 1.2
10	*6*	16 ± 1.1	35 ± 3.1	3939 ± 85	104 ± 35.6	49 ± 3.2	15 ± 2.2
Optimal levels		20 - 25	50 – 60	3000 - 4000	60 - 100	40 - 50	15 - 20

[1]*Six milk bulk samples were collected from May 2014 to January 2015, with exception of farms 8 and 9 from which 7 and 5 samples were collected, respectively. Results are expressed as mean mineral content ± SE; Laboratory analyses were carried out by the Thomson & Joseph Ltd, Albion Laboratory Services, Hoveton, NR12 8QN, UK*

Selenium concentrations in bulk milk samples remained within optimal levels if not slightly higher on all study farms (Table 7). Given the fact that the concentration of selenium in milk is dependent on selenium intake (Walker et al., 2010) the data show no nutritional shortfalls in selenium in the case-study farms. It should be mentioned that increased concentrations of selenium in milk may have positive effects on calf and human health (NRC, 2001). Molybdenum concentrations in milk samples (Table 7) averaged from optimal to high which reflects the high Molybdenum concentrations in the forage (see section below). The current status of molybdenum does not indicate a practical concern, however when dietary molybdenum levels reach as little as 5mg/kg DM they can inhibit absorption of copper (NRC, 2001).

5.2 Urine iodine and mineral concentrations in blood samples in view of milk iodine

Average urine iodine concentrations in each farm and for each sampling occasion are shown in Figure 3 and average blood plasma mineral concentration in Table 8.

As expected there was variation in urine iodine concentrations between farms but urine iodine also fluctuated considerably within farms across samplings. Determination of iodine in urine is a reliable parameter for the assessment of the iodine supply and reflects nutritional shortfalls of iodine intake. Urine iodine concentrations were above optimal levels (i.e >100 μg/L) in most of the farms but in Farms 7 and 8 urine iodine was marginal or below optimal levels in the first two samplings (Figure 3, panels (a) and (b); Farm 6 there were below optimal levels in the last sampling (Figure 3, panel (c), which may suggest some dietary losses of iodine intake. However, the average urine iodine concentrations over the sampling period (i.e. May to December 2014) were above optimal levels on all farms (Figure 4, panel (b).

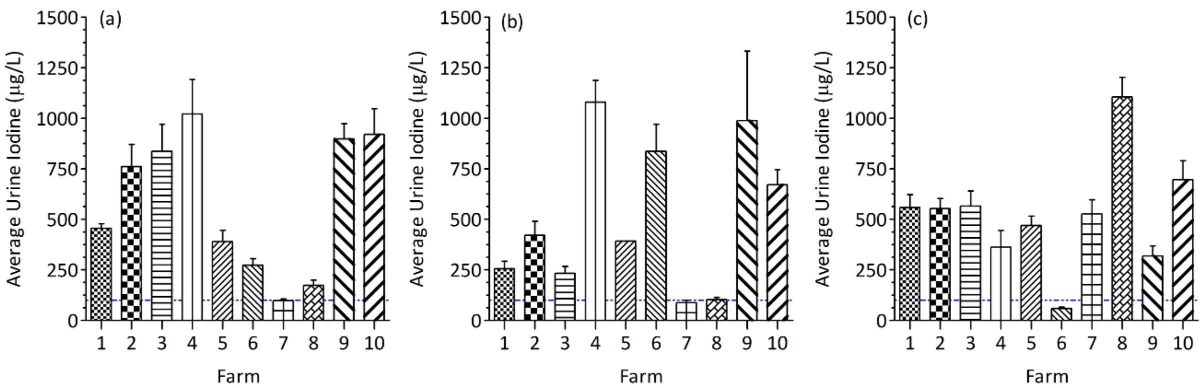

Figure 3: Average urine iodine concentrations in each farm during August/September (Panel a), October/November (Panel b) and December/January Panel (c). The results reported are standardised to a creatinine concentration of 5000 μmol/l.

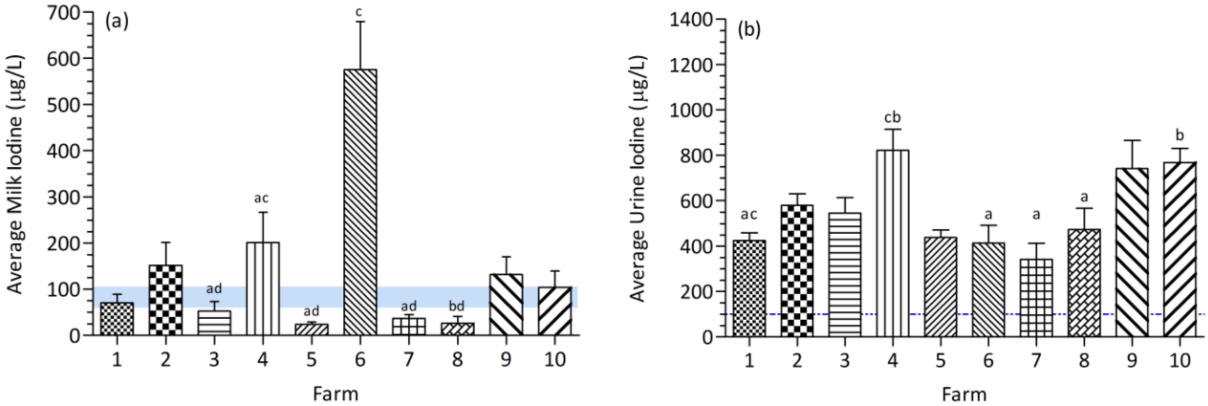

Figure 4: Milk iodine (panel a) and urine iodine (panel b) concentrations in each farm averaged over the sampling period (i.e. May to December 2014). Urine iodine is standardised to a creatinine concentration of 5000 µmol/l. (Means with different letters differ significantly by Kruskal-Wallis H test)

Because urine iodine was determined in individual cow samples on three occasions and milk iodine concentrations were determined in bulk milk samples every 35 – 45 days, correlation analysis between the two variables is not possible. Farm comparisons in urine iodine concentrations showed that cows in farm 10 had significantly higher urine iodine compared to those in farms 1 ($P \leq 0.05$), 7 ($P \leq 0.01$), 8 ($P \leq 0.01$) and 6 ($P \leq 0.001$); urine iodine concentrations in farm 4 were significantly higher compared to farm 6 ($P \leq 0.01$), 7 ($P \leq 0.05$) and 8 ($P \leq 0.01$). These results show that the differences between farms in milk iodine (as described in Section 5.1) do not follow the same pattern as the farm differences in urine iodine (Figure 4). Unlike urine iodine, milk iodine concentrations are affected -in addition to iodine intake- by farm practices and in particular by the use of iodised-based teat disinfectants (discussed later). In view of that the present results suggest that urine iodine concentrations are not indicative of milk iodine concentrations.

Blood plasma mineral analysis did not show mineral deficiency in any of the farms studied as average plasma concentrations of minerals were within optimal levels (Table 8). It should be noted that plasma selenium concentrations of 0.5 to 1 µmol/L is required to maintain target GSHPx concentrations and these were marginal in Farms 10 and 7.

Table 8: Average blood plasma mineral concentration

Farm	Micro-element (µg/L)[1]						
	Selenium (µmol/l)	Zinc (µmol/l)	CP Activity (mg/dl)	Copper (µmol/l)	CP:Pl Cu	SOD (U/g Hb)	GSHPx (U/ml PCV)
1	1.1 ± 0.08	11.1 ± 0.7	29.6 ± 1.1	13.1 ± 0.7	2.3 ± 0.1	2533 ± 53	118 ± 3.8
2	1.2 ± 0.05	12.3 ± 0.4	19.8 ± 2.3	10.1 ± 0.4	2 ± 0.2	2373 ± 104	109 ± 3.5
3	0.8 ± 0.02	16.5 ± 1.3	30.3 ± 4.1	12.1 ± 0.7	2.5 ± 0.3	2416 ± 80	141 ± 3.7
4	1 ± 0.07	11.8 ± 0.4	19.8 ± 1.3	11 ± 0.5	1.8 ± 0.1	2764 ± 103	121 ± 2.9
5	1.3 ± 0.03	12.6 ± 1.5	35.8 ± 1.2	11.5 ± 0.4	3.2 ± 0.2	2606 ± 107	120 ± 3.6
6	1 ± 0.07	13.6 ± 0.3	26.6 ± 1.5	12.2 ± 1.0	2.3 ± 0.3	2754 ± 609	102 ± 3.9
7	0.6 ± 0.03	13.7 ± 0.6	23.4 ± 1.1	10.2 ± 0.6	2.3 ± 0.1	2425 ± 57	51 ± 3.3
8	0.7 ± 0.09	11.7 ± 1	29.7 ± 1.1	12.8 ± 0.5	2.3	2342 ± 182	70 ± 2.6
9	1.1 ± 0.03	16.3 ± 1.1	29 ± 4.1	14 ± 0.2	2.1 ± 0.3	2340 ± 99	117 ± 2.8
10	0.5 ± 0.07	11.9 ± 0.4	30.3 ± 3.7	11.3 ± 0.8	2.8 ± 0.6	2524 ± 137	65 ± 3.4
Optimal levels	Norm>0.2	12.3–18.5	Norm>15	9.4-19	Norm>1.7	Norm >2000	Norm > 40

[1]Blood samples were collected from 4 cows in each farm during August/September. GSHPx data are based on 30 samples per farm. Results are expressed as mean mineral content ± SE; Laboratory analyses were carried out by the Thomson & Joseph Ltd, Albion Laboratory Services, Hoveton, NR12 8QN, UK

5.3 Iodine and mineral concentrations in forage samples

Data on forage/diet mineral concentrations are missing from three farms as samples were not collected. Over the study period only two farms submitted four samples and three farms submitted one sample for mineral analysis. Table 9 shows the average mineral concentration of the forage/diet fed to the animals over the study period in each farm for those farms where it was possible to calculate.

Calcium in forage is an indicator of the soil pH conditions. In all studied farms the average forage calcium concentrations were above optimal levels to high (>0.8%; Table 9). Although calcium tends to rise as the plant matures due to its association with the fibre fraction, alkaline soil, over liming or naturally calcaerous soil remain the major influence. Calcium is important for optimal trace element uptake but at high concentrations can reduce trace mineral absorption (especially zinc) in animals (NRC, 2001). The forage analysis results (Table 9) show that across the study farms, copper, zinc, cobalt, iodine and selenium were relatively low but molybdenum levels were above optimal levels.

Iodine as an element is essential for animals, but plants have no requirement for iodine. On three farms (i.e. Farms 2, 5 and 6) the average iodine concentrations in forage were below optimal levels (< 0.5 mg/kg) while in another three farms (i.e. Farms 4, 7 and 9) average iodine concentrations were marginal or optimal (0.5 to 0.8 mg/kg). In one farm average forage iodine concentrations were relatively high (1.5 mg/kg). Across farms the average iodine concentration in the forage samples was 0.63 ± 0.2 mg/kg DM. The relatively low average iodine concentrations of the forage samples in the case-study farms can be seen as reflecting the notion that British soils are low in iodine. The transfer ratio of iodine from soil to plant is low and, with the exception of coastal zones, it is suggested that most of the land surface is actually low in iodine (Johnson, 2003). It is important to note that, when animals are given a choice, they will select for forages that are high in protein, calcium, and phosphorus. However, a study that evaluated clipped pasture samples and steer selected forage showed that this is not the case for trace elements (Corah, 1995). This outcome can partially explain nutritional shortfalls in terms of trace elements in grazed cattle.

A comparison both in milk iodine and urine iodine concentrations was performed between farms with low, average and high iodine concentrations in forage. Results show that urine iodine concentrations were significantly higher in the farms with average or high forage iodine compared with the farms with low forage iodine ($P \leq 0.001$), adding to the existing body of evidence that iodine excreted in urine is indicative of dietary iodine intake (Figure 5, panel b). With regards to the milk iodine concentrations this was not the case as farms with low or average forage iodine concentrations had higher milk iodine compared to those with high forage iodine values (Figure 5, panel a). This outcome is not surprising in view of the data presented in Section 5.2 and reflects the notion that milk iodine concentrations are affected by farm practices such as the use of iodine-based teat disinfectants which is discussed in the next section.

Table 9: Average mineral concentration of forage/diet in each participating farm.

Mineral[1] *Macro-elements (% DM Basis)*		Optimal levels	Farm									
			1	2	3	4	5	6	7	8	9	10
Calcium	Ca	0.5 - 0.7	-	0.9	-	0.8	1.1	0.9	0.9	-	1.0	1.5
Phosphorus	P	0.3 - 0.4	-	0.1	-	0.3	0.4	0.3	0.3	-	0.4	0.4
Magnesium	Mg	0.15 - 0.25	-	0.1	-	0.2	0.2	0.2	0.2	-	0.3	0.3
Potassium	K	1.5 - 2.5	-	2.3	-	3.0	3.2	2.3	2.4	-	1.8	2.4
Sodium	Na	0.2 - 0.3	-	0.1	-	0.2	0.1	0.1	0.2	-	0.1	0.4
Chloride	Cl	0.6 - 1.4	-	0.7	-	1.4	0.9	0.7	0.8	-	0.6	1.0
Sulphur	S	0.15 - 0.25	-	0.2	-	0.3	0.2	0.2	0.2	-	0.2	0.2
Micro-elements (mg/kg DM)												
Manganese	Mn	75 - 125	-	32	-	72	102	92	80	-	258	100
Copper	Cu	08 - 12	-	6.8	-	13.3	8.9	8.3	8.3	-	21.0	61.2
Zinc	Zn	40 - 80	-	31	-	39	26	33	34	-	73	194
Cobalt	Co	0.2 - 0.3	-	0.1	-	0.2	0.1	0.1	0.1	-	0.1	0.9
Iodine	I	0.5 - 1.5	-	0.3	-	0.8	0.4	0.2	0.5	-	0.7	1.5
Selenium	Se	0.1 - 0.2	-	0.0	-	0.1	0.1	0.1	0.0	-	0.3	1.0
Iron	Fe	100 - 200	-	59	-	561	252	296	377	-	197	551
Molybdenum	Mo	0.35 - 1.25	-	1.5	-	1.4	2.5	1.6	2.0	-	1.3	1.5
Forage samples submitted for analysis			0	1	0	4	3	2	4	0	1	1
Farm Iodine in milk[2]			O	O	O	H	L	H	L	L	H	H

[1]Laboratory analyses were carried out by the Thomson & Joseph Ltd, Albion Laboratory Services, Hoveton, NR12 8QN, UK except for farm Number 2 for which samples were analysed by Sciantec Analytical Services, Stockbridge Technology Centre, Selby YO8 3SD
[2]L=Low, O=Optimal and H=High with milk iodine concentrations below 60 µg/L, between 60 to 120 µg/L or above 120 µg/L, respectively

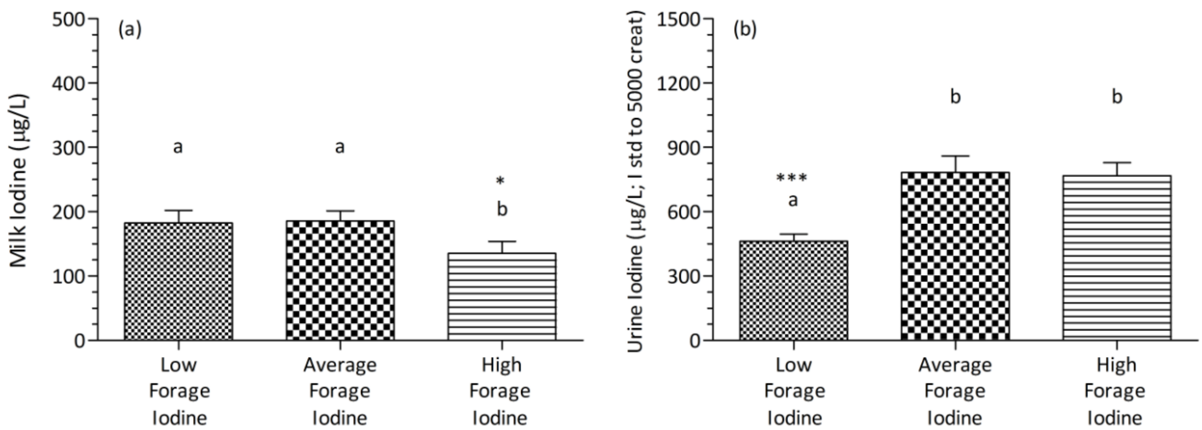

Figure 5: Effect of iodine content of forage on bulk milk iodine (panel a) and urine iodine (panel b) concentrations (means with different letters differ significantly by t-test, * $P \leq 0.05$; *** $P \leq 0.001$)

Half of the farms supplement the animals with iodised rock salt (IRS). Differences in milk iodine and urine iodine concentrations between farms that offer IRS or not were also tested. Analysis of these data showed that there were no differences in urine iodine concentrations between the farms that offer IRS and those that do not (530 ± 35.6 µg/L vs. 612 ± 39 µg/L, respectively; *P*=0.06) but milk iodine concentrations were higher in the farms that supplement the animals with IRS (179 ± 16 µg/L *vs.* 121 ± 8.8 µg/L, respectively; *P*<0.001). However, this comparison does not account for the use or not of iodised teat disinfectants in the farms compared and this result is rather confounded by the effect of iodised teat disinfectants on concentrations of iodine in milk.

5.4 Effect of iodised teat disinfectants

Six out of the 10 case-study farms use iodised post-dip teat disinfectants, while the remaining 4 farms do not follow that practice. Comparison between the two groups of farms indicated that milk iodine concentrations were 2.3 times higher (Figure 6, panel a; *P*<0.0001) on the farms that use iodised post-dip teat disinfectants (mean average 195 ± 13 µg/L) compared with the farms that do not use this practise (mean average 85 ± 8.9). Similarly, urine iodine concentrations were significantly higher on the farms that use iodised post-dip teat disinfectants compared with those that do not (618 ± 34 µg/L *vs.* 481 ± 37 µg/L; Figure 6, panel b, *P* ≤ 0.01).

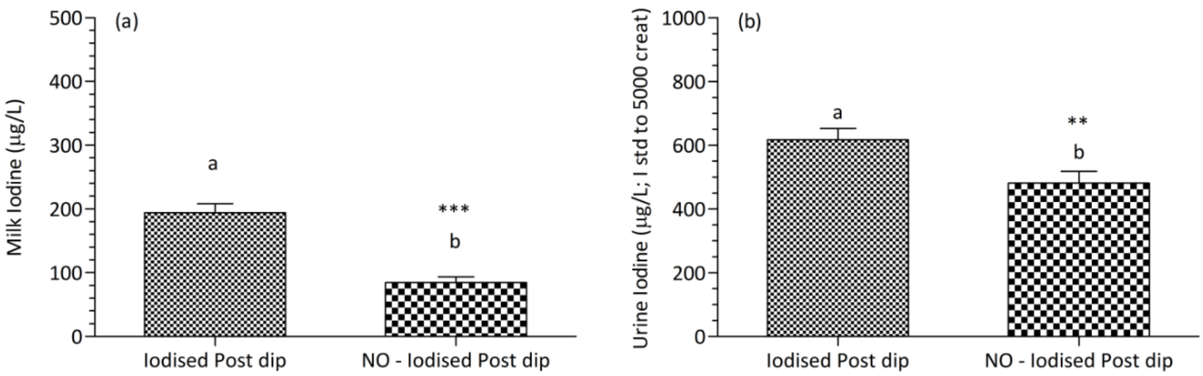

Figure 5: Effect of iodised post-dip teat disinfectants on bulk milk iodine (panel a) and urine iodine (panel b) concentrations (means with different letters differ significantly by t-test, ** *P* ≤ 0.01; *** *P* ≤ 0.0001)

The results show that the use of iodised post-dip teat disinfectants has a major effect on milk iodine concentrations, which is in accordance with the current literature. According to the review of Flachowsky et al. (2014) there are different views about the mode by which the iodine enters into the milk; some authors suggest that iodine residues in milk originate mainly from the contamination of the teat surface (Rasmussen et al., 1991) but earlier studies proposed that iodine enters into the milk by the milk synthesis process due to the absorption through the skin (Conrad and Hemken, 1978). The content of iodine in the disinfectants is more important factor than the timing of the application (i.e. pre- or post- dip) in the increase of iodine concentration in milk (Flachowsky et al., 2014).

It has been discussed earlier that absorption of dietary iodine is a key factor affecting urine and milk iodine concentrations. The finding that urine iodine concentrations were also significantly higher in the farms that use iodised post-dip teat disinfectants is of particular importance as it can reflect the fact that iodine can be also absorbed in the lungs or via the skin (Conrad and Hemken, 1978; Flachowsky et al., 2014).

6 Conclusions/Recommendations

The results show that the use of iodised post-dip teat disinfectant is the most important factor influencing the iodine concentration in milk. In this respect, the iodine concentrations in milk does not serve as a robust indicator in identifying shortfalls in iodine intake or dietary iodine deficiencies especially on farms that use iodised post-dip teat disinfectants. Forage iodine concentration is an important factor in maintaining milk iodine concentrations at optimal levels, in addition to its importance in maintaining animal health and performance at optimum levels. Therefore, dietary iodine supplementation is recommended to the farms in which iodine concentrations in forage are below 0.5 mg/kg. Over the course of the study average iodine concentrations in organic milk remained within or above optimal levels defined in the current literature (i.e. >120 µg/L). Milk iodine concentrations fluctuated within farms across samplings but in some farms they were systematically low. This outcome deserves further attention in order to alleviate recent concerns that organic milk contains less iodine than conventional milk.

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