Geographical and seasonal variation in Iodine content of cow's milk in the UK and consequences for the consumer´s supply.

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Abstract.

Background. Dairy products provide a crucial source of dietary iodine for the majority of the UK population, contributing approximately 30-40 % of daily intake. Fluctuations in the iodine content of purchased milk both seasonally and annually implies potential fragility of iodine supply likely through fluctuating supplementation practices in cow herds. We set out to establish the level of national variation in herds and identify factors which might impact milk iodine content.

Methods. Milk samples were obtained from 98 herds across the UK via the National Milk Laboratories in August and December 2016. Iodine concentration of samples was measured using ICP-MS. Milk samples and feed intake data were additionally taken from 22 cows from the University of Nottingham (UON) dairy herd.

Results. There was considerable variation in milk iodine content from < 0.012 (Limit of Detection) to 1558 $\mu g\ L^{-1}$, with a summer median of 197 $\mu g\ L^{-1}$ and winter median 297 $\mu g\ L^{-1}$. Overall, winter values were higher than summer counterparts ($P<0.001$) and this held true for samples taken from the North West ($P=0.002$) and South West ($P=0.006$) but not for other regions studied. Data from the UON herd showed a negative relationship between iodine content and milk yield ($P=0.03$) and we found that milk iodine content varied considerably despite apparently similar iodine intakes.

Conclusions. Regional differences in milk iodine concentration between summer and winter suggests that feeding practices are far from uniform across the country. The negative association observed between iodine concentration and milk yield in UON samples, suggests that reduced summer values may be influenced by dilution in addition to seasonal differences in concentrate feed provision.

Introduction

Iodine is an essential dietary micronutrient required for the production of the thyroid hormones [1] which participate in a broad array of functions including regulation of growth, physical development, cardiac function, cholesterol homeostasis, metabolic regulation, thermogenesis, carbohydrate metabolism, cognitive development and adult cognitive function [2-4]. Both iodine deficiency and iodine excess disrupt the thyroid hormone axis resulting in developmental, cognitive and metabolic pathologies.

The prevalence of iodine deficiency in the global population was estimated at almost 2 billion in 2011 [5] and remains present in school age children in 20 countries and in pregnant women in 39 countries today despite the success of global salt iodisation [6]. Conversely, 11 countries are now recognised as consuming excessive levels of iodine [6]. In the UK it has recently been highlighted that iodine intake by pregnant women and school children remains inadequate [6, 7].

Iodine is solely acquired from the diet. Whilst the best source is seafood [8], the principal route of iodine intake for omnivores in the UK is from milk and dairy products [9]. The iodine content of cow's milk is provided through feed in the form of fortified feed concentrate or mineral supplements, in addition to iodinated teat disinfectants [10]. The concentration of milk iodine varies greatly with different levels of provision, from <20 μg L^{-1} to >1500 μg L^{-1} depending on feed concentration [11]. Iodine derived from teat disinfectants may contribute ~30 μg L^{-1} to 150 μg L^{-1} [12-14] and whilst the iodine content of milk has become less significantly influenced by iodine concentrations in pasture in recent years, it remains heavily influenced by season [15].

Bovine health issues related to iodine deficiency, including stillbirth, calf goitre and low first service conception rate highlight the necessity to maintain a minimum iodine intake for dairy cows to ensure their health and that of their offspring [16]. Such health issues, whilst problematic in their own right, additionally impact milk yield and future breeding success [17]. The dietary iodine requirement for dairy cows is 0.2 – 0.33 mg kg^{-1} dry matter (DM) dependent upon lactation state, but an intake of 0.6 mg kg^{-1} DM is recommended to overcome the impact of any dietary goitrogens [18, 19]. Many farms, however, ration up to 2 mg iodine kg^{-1} DM due to a lack of knowledge of iodine content of other feed components. As overconsumption of iodine is also of concern, European legislation [20, 21] indicates an upper limit of consumption via iodine supplements (potassium iodide/calcium iodate) of 5 mg iodine kg^{-1} of complete feed with a moisture content of 12 % (5.7 mg iodine kg^{-1} DM) with a recommended maximum of 2 mg iodine kg^{-1} (2.3 mg iodine kg^{-1} DM) for dairy cows. Since the principal dietary source of iodine in the UK is cow's milk, it is important to ensure consistency of iodine supply from primary production.

The main objectives of this study were to determine if there are regional differences in milk iodine concentration for UK herds and whether the seasonal change described in assessments of processed milk samples is true for all herds. We additionally set out to establish the degree of variation in iodine concentration at the herd level to gain insight into the composition of the milks which contribute to the final product supplied to the consumer.

Methods

Sample collection and analysis.

Farms which provided milk samples to the National Milk Laboratories (NML) for screening were randomly selected for inclusion by workers at the NML. In total 98 herds were selected representing 6 regions of the UK including South West (SW), South East (SE), Midlands (M), East (E), North West (NW) and North East (NE). Milk samples were acquired for both summer (August 2016) and winter (January 2017) periods (Table 1). Samples were stored at 2-6°C for 5 days for routine laboratory analyses at the NML prior to freezing and storage at -70°C before shipping to the School of Biosciences, University of Nottingham for analysis. Samples were anonymised with only the first 5 letters/digits of the postcode provided to enable regional but not specific identification.

We additionally sampled milk from the University of Nottingham (UON) dairy herd. Over a single day, milk samples were taken from 24 cows as they were milked. Aliquots (~20ml) were sampled and stored at -80°C prior to determination of iodine concentration. Data regarding milk yields were extracted from the farm database. Samples were excluded from cows which were not milked at least 3 times in the preceding 24 hours to avoid analysis of samples which may have accumulated excessive iodine over an extended period of retention (2 cows).

Prior to analysis a test to establish the best method of sample preparation was undertaken. Semi-skimmed and full fat milks were purchased and prepared in triplicate by (i) dilution with MQ water (1 in 20), (ii) dilution with 1 % tetramethylammonium hydroxide (TMAH) (1 in 20), (iii) extraction with 10 % TMAH with heating (90°C) for 3 hours followed by dilution with MQ water (1 in 10) and (iv) microwave extraction with TMAH followed by dilution with MQ water (1 in 10). All dilutions were undertaken immediately prior to analysis. Samples were analysed by inductively coupled plasma mass spectrometry (ICP-MS; Thermo-Fisher Scientific iCAP Q) operated in standard mode before and after filtration (5µm; Sartorius, Surrey, UK). Internal standards included Rh and Re in 1 % TMAH. The milk standard ERM®-BD150 (Sigma-Aldrich; measured concentrations were within the certified range: 1.73 ± 0.14 mg kg^{-1}) was used to validate milk analyses. Tests of sample preparation gave similar iodine concentrations when the different methods were compared and therefore defrosted samples from the NML were simply diluted in MQ water (1 in 100) immediately prior to analysis.

Analysis of silage and feed concentrate iodine content.

Silage samples from different sources were obtained, comprising rye grass (n = 4), whole crop triticale silage (n = 3) and maize silage (n = 3). Samples were dried in an oven at 60°C until weight ceased to decline before homogenisation and microwave (Multiwave, Anton Paar) digestion in 5 % TMAH in duplicate. Digested samples were diluted to 1 % TMAH before ICP-MS analysis as described above. The powdered hay standard BCR129 was used in silage analysis digestions to validate the digestion procedure (Sigma-Aldrich; measured concentrations were within the certified range: 0.167 ± 0.024 mg kg^{-1}).

Statistical Analyses.

Milk iodine concentrations showed a considerable right-sided skew in both summer (skewness = 1.88; kurtosis = 4.34) and winter (skewness = 1.32; kurtosis = 1.86), necessitating non-parametric analyses to be employed. Samples containing iodine at < Limit of detection (LOD; 0.012 μg L^{-1}) were treated as 0.5 X LOD and included in the analyses. For comparisons of milk iodine content between different regions the Kruskal–Wallis test by ranks was adopted. The Dunn test was used for post hoc analyses of Kruskal-Wallis tests. For paired comparisons within regions between summer and winter values Wilcoxon signed-rank tests were used. Summary data are presented either in tabular or graphical format as ranges of data with median values, maxima, minima and first and third quartiles where relevant. Correlation analysis of UON samples was carried out using Spearman's non-parametric rank correlation test. All statistical analyses and graphical rendering was carried out using the R software [22].

Results

Milk iodine content varies widely across all regions and shows region specific seasonal variation.

The mean iodine content of milk samples during the summer from all regions was 262 µg L^{-1} (median - 197 µg L^{-1}) whilst the mean of the winter samples was 365 µg L^{-1} (median - 297 µg L^{-1}). We observed considerable variation in the iodine content of milk from different herds in each region in both summer and winter samples (Figure 1; Table 1). The greatest variation in concentration was observed in the winter samples which, for each region, included samples with negligible iodine content (0.09 µg L^{-1}) and those with the greatest concentrations (1558 µg L^{-1}).

Comparing the seasonal iodine contents of the two milk samples from each farm, summer values were weakly correlated with winter values (correlation coefficient = 0.49, R^2 = 0.24; Figure 1A; Table 1). This indicates that several farms produced milk with approximately comparable iodine concentrations throughout the year. However, the low R^2 means that 76 % of the variation was not based on similarities in the iodine content of the milk from the summer and winter originating from the same farms. We saw a seasonal change in milk iodine content increasing during winter compared to summer ($P < 0.001$; Figure 1A – indicated by 1:1 gradient solid line). This difference, whilst apparent in samples from most regions, only showed significant differences in those from the NW and SW. Winter milk iodine content was significantly greater in herds from the NW ($P = 0.002$) and the SW ($P = 0.006$) compared to their respective summer values whilst those from all other regions had less variation in iodine content with season (Figure 2).

Analysis across regions within seasons, showed no significant differences in iodine content of milk from different regions in both summer ($P = 0.325$; Figure 1B) and winter ($P = 0.099$; Figure 1B). Our data included samples from 4 organic farms which produced milk with summer values ranging from 89 - 239 µg L^{-1}. Winter milk iodine concentrations were elevated in 3 of the 4 to 358 - 561 µg L^{-1}, whilst the concentration in the other organic farm dropped to an undetectable level as was seen for a number of the conventional farms. Comparison of milk iodine concentrations between summer and winter excluding those from organic farms did not differ from that obtained by their inclusion ($P<0.001$).

A subgroup of farms appears to avoid supplementation at specific times.

A number of samples were found to have little or no iodine (Table 1 & Table 2). We found that 17 % of farms produced milk with an iodine concentration <20 µg L^{-1} and 19 % below 50 µg L^{-1} in the winter. None of the summer samples were lower than 20 µg L^{-1} however, there were 5 farms which produced milk below 50 µg L^{-1}, only one of which showed a similarly low winter iodine concentration whereas all the other 4 provided winter milk with iodine at >100 µg L^{-1}.

Concentration of iodine in silage samples.

Samples of rye grass (n=4), maize (n=3) and whole crop triticale silage (n=3) were obtained and concentration of iodine determined (Table 3). For all crops, silage iodine concentration was low, ranging from 0.09 mg kg^{-1} to 0.30 mg kg^{-1} and we observed no significant differences between groups ($P=0.556$).

Iodine concentration in milk is negatively associated with yield.

We finally attempted to establish whether there was intra-herd variation in milk iodine content. We examined milk samples taken from a herd of barn housed dairy cows, all of which were supplied with a precisely regulated feed ration that varied according to yield. All cows (more than one lactation) and heifers (first lactation cows) were supplied a partial mixed ration at 45.3 kg day^{-1}. This mixed ration included a number of iodine containing feed components including grass silage, maize silage, whole crop triticale silage and a commercial mineral mix at regulated quantities (Table 4). The provision of iodine from the partial mixed ration was 22.45 mg animal^{-1} day^{-1}. Cows and heifers were additionally provided concentrate feed at a quantity that was proportional to their yield. This was delivered individually to cows during milking. Concentrate feed contained iodine at a concentration of 1.72 mg kg^{-1} DM. Cows in the UON dairy are self-milked requiring them to visit a milking robot when they deem it necessary. Each cow is identified and provided an amount of feed concentrate determined by recent yield volume. The quantity of concentrate provided for cows was according to a rising relationship of 2 kg feed for a yield of 25 L with an additional 0.45 kg L^{-1} up to a maximum of 12 kg. Heifers were provided 2 kg concentrate for a yield of 21 L plus 0.45 kg L^{-1} up to a maximum of 10 kg.

Iodine content of samples varied between 221 µg L^{-1} and 1393 µg L^{-1} (>6 fold difference; median = 743 µg L^{-1}) despite animals being identically housed. Single samples were taken and data regarding yield at each milking was recorded by the milking robot (n=22). The range of yields for single milking events was 6.4 - 14.5 L and over the course of the entire day (29/07/2016) yield for the cows sampled was 29.5 – 50.4 L. The number of milking visits contributing to the total day's yield differed for each cow. Consequently, the proportion of the total yield which comprised the sample volume varied considerably, from 18 % to 43 % with a median of 26 % and an interquartile range of 21 % - 32 %. We saw a significant relationship between sample yield and iodine content. As single sample yield increased, the milk iodine content decreased (Figure 3A; P=0.03; spearman correlation = -0.46) suggesting a potential dilution effect.

Since concentrate feeding increases with yield, the concentration of iodine in milk should be fairly static. An iodinated teat steriliser was used post-milking, which will have had an impact on the iodine concentration of the milk. However, the considerable degree of fluctuation in the concentration of milk iodine implied variation in the passage of iodine from feed to milk and suggested a potential for individual variability in the ability of cows to absorb dietary iodine and transfer it to their milk. We established precise feed intakes for each cow/heifer and examined the association between total iodine intake, excluding that derived from teat disinfectant, and milk iodine concentration. Milk iodine differed greatly within and between feed boundaries (Figure 3B) despite animals consuming equal amounts of iodine within their lactation category. We calculated the expected day milk iodine output using values of day milk yield and the single sample iodine concentration described above (Figure 3C). There was no significant relationship between consumed iodine and calculated iodine output (P=0.53).

Discussion

Seasonal and regional influences on milk iodine concentration.

The current study provides the first analysis of milk iodine at the herd level with nationwide coverage in the UK. We have shown that considerable variation exists in milk iodine concentration from herds across the UK. We found an overall seasonal change in milk iodine content as indicated by the predominance of points above the 1:1 line in Figure 1 and this was significant for herds from both the NW and SW regions. The values we report here are in line with published herd values from the UK and Europe which range from ~20 µg L^{-1} to ~500 µg L^{-1} [11, 12, 14, 23, 24] and those from combined creamery milk samples [25]. The range of published values reflects the level of variation that we have indicated in our study, highlighting the breadth of iodine exposure to which dairy cows are subject.

The seasonal variation in milk iodine content, which is generally attributed to cows being supplied different diets in the winter months as they are brought into barns, is well established [12, 26, 27] and our data lend further support to this. However, we additionally noted that in our study the relationship was far from universal and in fact, a number of farms (38 %) generated milk with a lower iodine concentration in winter compared with summer (Figure 2). Cows are bred all year round in order to maintain a continuous milk supply to the market [28] and feeding practices are mostly dependent on stage of lactation and milk yield rather than time of year. This should result in a stable milk iodine concentration, but it is clear that farms do not universally adhere to this process. Our data indicate that there are a number of farms (up to 19 %) which do not appear to provide any additional iodine beyond that acquired from the low iodine feed materials produced in the farms. This would suggest that these farms may be supplying limited additional supplements or that they may not be providing commercial feed concentrate in a yield dependent manner.

In addition to varying feed supply, there may also be an influence of dilution on milk iodine concentration. Milk production follows an annual cycle with the greatest yield produced in May with the preferences for spring calving, and the lowest in November aligning with the majority of cows entering the last lactation period [29]. If farms do not rigidly control the provision of feed according to yield, then the elevated yield may be contributing to reduced iodine concentration in the milk from dilution, during the high yield summer months [12, 30].

We explored this with samples taken from our own dairy herd. Cows kept in the UON dairy were barn housed and provided a standard mixed feed ration which included mineral supplementation in addition to iodine supplemented feed concentrate. The feed concentrate was provided strictly according to yield, stage of lactation and parity. An iodine containing teat treatment was applied post-milking, representing an additional route of iodine entry. We found a significant negative association between yield and milk iodine concentration, albeit from a relatively small sample size (Figure 3A). When we plotted milk iodine concentration as a function of total iodine intake over the previous 24hrs however (Figure 3B), we found no clear relationship. Similarly, there was no relationship between iodine supplied and the calculated quantity of iodine excreted in milk (Figure 3C). For the large majority of cows (19 out of 22) the quantity of iodine excreted into the milk represented more than 50 % of that ingested, with an average of approximately 80 %

apparent carry-over of iodine into milk. This, however, remains an estimate only since it was contributed to by the teat steriliser and the transfer from this source was not measured.

Published estimates of iodine contribution from teat disinfectant ranged from 27 - 150 µg L^{-1} [14, 31-33]. When these values are subtracted from our own measurements for individual cows, they indicate that the milk iodine concentration derived from feed alone may range from 658 ± 298 µg L^{-1} to 781 ± 298 µg L^{-1} (mean ± SD). For the highest level of iodine carry over (150 µg L^{-1}) the proportion of iodine derived from feed entering the milk drops to 68%. This exceeds previously published values of up to 47 % [24] and 56 % [11] however, this is a closer estimate than that generated without correcting for the potential contribution from the disinfectant. In addition, the value generated for the calculated iodine excretion was based on the concentration measured in single samples multiplied by the total milk volume over the course of 24hrs derived from up to 5 separate visits to the milking robot. It has been shown for other milk components (e.g. fat – [34]) that there is diurnal variation in their milk concentration. This may also be the case for iodine, although we have not measured it in the current study, so the elevated carry over that we have observed may also be influenced by the fact that it is derived from calculated values rather than measurement from each milking event.

The mode of husbandry at the UON dairy is necessarily rigidly controlled in order to enable scientific study, but it also needs to be reflective of broader practice. In general, farms adopt different levels of feeding control which vary in their rigor. A proportion of farms will feed in the manner described for the UON dairy, whilst others will have capacity for provision of additional feed using computerised concentrate feeders located outside the parlour [35]. Additionally, a number of farms feed according to groups separated by yield (high and low) and stage of lactation whilst some remain which supply mixed ration to all [36]. We do not have specific data regarding feeding practice but we would expect a range of practices to be present among our cohort.

We saw significantly higher concentrations of milk iodine in winter compared with the respective summer values from herds in the NW and SW of the country. These regions have historically been areas of which have experienced higher rates of still born calves. Evidence from the Animal and Plant Health Agency Cattle Dashboard [37] indicates that the majority of thyroid hyperplasia cases over the past 6 years have occurred in these regions. Whilst this is probably a reflection of the fact that the NW and SW regions have the most dairy cows, it may help to explain local perceptions of potential iodine deficiency. It was notable that from the herds we sampled, of those with a winter milk iodine concentration higher than 600µg L^{-1}, 72 % were from either the NW or the SW.

Mode of production and influence on milk iodine content.

Milk iodine content is dependent on dietary intake, clinical administration and teat sterilisation. Dietary sources of iodine are derived from drinking water [7], pasture, grass silage, corn/maize silage and hay, feed concentrate and mineral supplements [12]. Pasture/silage sources generally contribute ~0.2 – 0.4 mg kg^{-1} DM (Table 3; [38]), although much of this may not be bioavailable as it is likely provided by contaminating soil rather than from the vegetation itself [39]. Cows exclusively fed via these sources generate milk at low concentrations (~20 µg L^{-1}). Teat sterilisation by dipping in iodinated germicide has been shown to increase milk iodine to 27 - 150 µg L^{-1} [14, 31-33], however, the most controlled approach to increasing milk iodine content is feed supplementation.

The iodine content of organic milks has consistently been reported to be lower than that of milk produced via conventional farming methods [9, 15, 27]. Restrictions imposed on the diet of dairy cows influence the ability of organic farms to provide iodine at the levels achieved in conventional production. At least 60 % of the diet must be derived from forage with an expectation that this comes directly from the farm itself or the immediate vicinity [40]. Feeding of concentrate is permitted, however this must be 100 % organic and feeding of mineral supplementation is only permitted where requirements cannot be met by husbandry practices. In addition, organic farming necessitates grazing on pasture with a high clover content to enable adequate protein intake. This poses an additional burden of cyanogenic glycoside consumption, which reduces uptake of iodine because of competition of thiocyanate (generated by the organism during cyanide detoxification) with the sodium iodide symporter [11, 41]. Our data set only included 4 organic farms and they did not show any notable difference in iodine content compared to conventionally produced milk samples.

Studies examining specific supplementation levels show a stepwise increase in milk iodine content with increased feed provision [11, 12, 24]. Cows producing baseline milk containing ~20 – 80 µg L^{-1} showed proportional increases in milk in the order of ~200 – 300 µg L^{-1} with each 1 mg kg^{-1} DM increase in dietary iodine [11, 42]. Clear supplementation boundaries were shown which indicate that cows supplied iodine at 1, 2 and 5 mg iodine kg^{-1} DM produce milk containing iodine at approximately 200-300, 500-600 and 1400-1500 µg L^{-1} respectively [12, 42]. Our data comprise herds from each of these concentrations, with the greatest number in the lower range. This would suggest that the majority of herds are supplemented to some degree, with a few (exclusively from the NW and SW) supplementing to the maximum level during the winter months. Excluding the 4 organic farms did not affect seasonal differences. Winter values for 3 of the organic farms were >350 µg L^{-1} (368 µg L^{-1}, 561 µg L^{-1} and 554 µg L^{-1}), which are at levels indicating supplementation to between 1 mg kg^{-1} DM and 2 mg kg^{-1} DM. These data are roughly in line with recent findings for processed organic milks [15].

Consequences of a variable milk iodine content for the UK consumer.

The failure of some farms to supplement with iodine indicates that there is the potential for significant ramifications for human iodine intake. Some published reports indicate that the iodine content of unfortified feed may be sufficient for general bovine health [16] and that under these conditions, the iodine content of the milk generated will be ~20 µg L^{-1} or less. However, cows left unsupplied with additional iodine remain at significant risk of deficiency, particularly if exposed to glucosinolate containing feeds such as rape seed meal or clover [11, 43]. The lack of supplementation becomes of particular importance for human health, however, as the acquisition of iodine from milk and dairy products provides 34-62 %, depending on age group, of the dietary iodine for the milk consuming population [44]. It would seem likely that, if cow feed in these farms is not supplemented with iodine, then they are also potentially, failing to supplement with other micronutrients, which has further potential impacts for the consumer.

In Northern Ireland (NI) it has recently been shown that levels of consumption of milk in children may result in a seasonal mild deficiency in some areas and a seasonal excess in others [26]. These observations were based on milk data provided by the Food Safety Authority of Ireland [45] which showed wide variation in NI milk iodine across the year with a very substantial increase in the winter. These data were for processed milk and so reflect fluctuations in products themselves potentially implying there may be as much variability in herd data as that seen in our study. We do not see such clear seasonal variation in our own UK data although these were only measured at two time-points in the year. Published values of iodine in purchased milk from mainland UK show a substantial range of values for conventional milk (202 µg L^{-1} to >750 µg L^{-1}) and organic milk (85 µg L^{-1} to ~500 µg L^{-1}; [9, 15, 27]). According to recent NDNS data, approximately 87 % of the population consume milk either as whole, semi-skimmed or skimmed but for a large number of these consumption is at a very low level. The proportion of the population consuming 100ml or more per day is 55 %, whilst only 28 % consume 200 ml or more per day [44]. Consumption of 200 ml of organic milk at the lower concentration would provide only 16 µg iodine whilst the same volume of conventional milk at the greatest concentration would yield 150 µg iodine from the milk alone. This suggests that there is scope, depending on season and type of milk, for individuals to consume either too little or too much iodine. When we consider that ~45 % of the population are consuming less than 100 ml milk per day, the impact of such variations may be even greater.

Dairy farmers are not required to supplement with iodine to benefit public health. They do so exclusively in order to support the health and therefore the productivity of their cows. If any cows exhibit symptoms of iodine deficiency, they will be treated, either directly (by injection or dermal administration), or via dietary supplementation. For many farmers, there may be a historical memory of iodine deficiency within the region or the farm itself and for others, supplementation is routinely adopted as a preventative measure. However, there is no mandatory requirement to supplement iodine in dairy cows, there is only an upper limit placed on the degree of supplementation [20, 21].

Whilst there is a clear necessity for some farms to supplement, it is apparent from our data that this is not a practice universally employed, presumably due to a lack of deficiency associated pathologies on these farms. Additional factors which may influence supplementation include commercial pressures such as milk and feed prices. The overall median milk iodine concentration in summer samples was considerably lower than those reported for processed milk in recent studies (Table 1; [15]). Summer values from the Stevenson study showed milk iodine concentration at almost 500 µg L^{-1}. These samples were taken during the summer of 2015, whilst our own were from the following year. The considerably lower values from our own measurements just 12 months later (197 µg L^{-1}), although from primary milk rather than final product, suggests that supplementation behaviour may have changed in this time. The price of milk dropped to its lowest level for around a decade in June 2016, just two months before our first samples were taken [46]. This price was approximately the same as that which was received 20 years previously. The commercial pressure this placed on farmers would have been considerable and many were losing money. The impact of such pressures on husbandry decisions may have influenced a number of farms to reduce their provision of compound feed with the consequent outcome of reducing iodine supplementation. We do not have the data to confirm this, but the evidence would support such conclusions. A more thorough survey of herd outputs over an extended period would be necessary to fully clarify the degree of responses to such fluctuations and help to predict behaviour. This would be of significance for public health planning as broader adoption of concentrate reductions might have a considerable impact on the iodine available through milk and consequently the iodine intake of the population.

We estimate that provision of iodine at a concentration of 1 mg iodine kg^{-1} DM would potentially enable consistent production of milk containing iodine at 100 - 200 µg L^{-1} in the absence of additional dietary goitrogens, such as feeds including rapeseed meal. For those consuming a portion of 200 ml milk day^{-1}, this would provide approximately a quarter of the daily iodine requirement. In the more likely scenario that dietary goitrogens will be present to some extent, this concentration of milk iodine might require supplementation to 2 mg iodine kg^{-1} DM as has been suggested previously [39, 47].

Limitations of the study.

Our study focussed on accessing samples with a broad geographical coverage. As a consequence, the numbers of samples for some regions, notably from the east, were few and diminished the power of the study. Additionally, we were only able to access samples from two specific times during the year and so the values we report are snapshots. Future work will attempt to expand the number of farms, increase the sampling frequency and obtain specific information regarding the herds, daily yield information, modes of production and feeding regimes. Samples of feed including silage and pasture will also enable provision of greater accuracy in our estimates of milk transfer from feed to milk and ultimately the consumer.

Estimation of iodine content in milk over the course of 24hrs were generated by determining the total daily milk yield and assuming a consistent concentration of iodine in each milk sample. Several milk components (e.g. protein, fat, urea) have been shown to vary significantly over 24hrs [34] and therefore this may also be the case with iodine. Hence the calculations may produce inaccurate assessments of daily iodine output in the absence of concentration measurements for all samples provided during one day. This would likely be exacerbated by the degree to which each sample yield contributed to the day's yield.

In addition, we were unable to determine the contribution of the teat disinfectant to iodine input. Since an iodinated teat disinfectant is used in the UON dairy, this will have provided an unmeasured quantity of iodine into the milk. More detailed studies confirming the level of transmission of iodine from teat disinfectant into milk within the UON dairy herd will be necessary to more precisely quantify the contribution of dietary iodine supplementation to milk iodine concentration in future studies.

Conclusions.

The study demonstrated a considerable range in the milk iodine concentration produced by herds from farms across the UK. Whilst an increase of milk iodine concentration in the winter vs. summer sampling was observed, the pattern was not universal. We found a number of herds which appeared to be un-supplemented in the winter, suggesting a reduced supplementation of other micronutrients also. The considerable milk iodine concentration range observed suggests that the stability of the iodine supply through dairy routes is potentially fragile, particularly if economic pressures result in altered feeding habits among UK dairy farmers.

Tables and figures.

Figure 1.
Milk iodine content of 98 herds was measured in summer and winter. (**A**) Summer values for milk iodine are plotted against winter values from the same herd. The line of best fit for the entire dataset is indicated (dotted line). Line indicating 1:1 gradient is shown by a solid line. E = East, Mid = Midlands, NE = North East, NW = North West, SE = South East & SW = South West. (**B**) All values for summer and winter milk iodine content are shown separated by region. No significant differences were observed between regions in either summer (P = 0.325) or winter (P = 0.099). For significance levels of differences between each region in summer vs. winter see Table 1.

Figure 2.
Milk iodine values of samples are plotted against each other for each separate region. Samples from North West and South West show a significant increase in milk iodine content from summer to winter (P=0.002 & P=0.006 respectively).

Figure 3.
A. Milk iodine content was measured in 22 cows from the University of Nottingham dairy herd. Values are plotted for sample volume against milk iodine concentration. A significant negative association was identified between volume of milk produced and iodine concentration (P = 0.03, Spearman’s correlation = -0.46). B. Total iodine intake was established for each of the cows/heifers represented in A and plotted against milk iodine concentration. Black circles indicate cows (more than one lactation), grey circles indicate heifers (first lactation). Data points are sized according to daily yield as indicated in the key panel. C. Total milk iodine output was estimated for the day during which the milk sample had been taken. The line of best fit for the entire dataset is indicated (dotted line). There was no significant relationship between iodine intake and calculated milk iodine output (P=0.53).

Table 1: Summary data of milk iodine content from herds across 6 regions of the UK.

Region	**No. Herds**	**Summer Iodine Range ($\mu g\ L^{-1}$)**	**Summer median value ($\mu g\ L^{-1}$)**	**Winter Iodine Range ($\mu g\ L^{-1}$)**	**Winter median value ($\mu g\ L^{-1}$)**	**No. of organic farms.**	**Change between Summer and Winter.**
NE	15	44 - 1178	154	1 - 991	148	1	$P = 0.679$
NW	29	97 – 1075	223	< 0.01 – 1530	359	1	$P = 0.002$
Mid	10	24 - 612	239	1 - 573	465	0	$P = 0.922$
E	5	108 - 347	201	< 0.01 – 625	156	0	$P = 0.625$
SW	19	40 – 590	147	< 0.01 – 1558	307	0	$P = 0.006$
SE	20	79 – 671	204	< 0.01 – 915	269	2	$P = 0.261$
Total	98	24 - 1178	197	< 0.01 - 1558	297	4	$P < 0.001$

NE – North East, NW – North West, Mid – Midlands, E – East, SW – South West and SE – South East.

Table 2: Breakdown of herds exhibiting low (<20 $\mu g\ L^{-1}$), medium (>20 $\mu g\ L^{-1}$ / $\leq$ 500 $\mu g\ L^{-1}$) and high (>500 $\mu g\ L^{-1}$) milk iodine concentrations. Values in brackets refer to the number of organic farms in each category. Concentrations were chosen to encompass groups estimated to be fortified at 0 mg kg^{-1} DM (low), up to 2 mg kg^{-1} DM (medium) and >2 mg kg^{-1} DM (high) assuming no inclusion of rapeseed meal [11].

	Summer			**Winter**		
Region	**No. herds with milk iodine <20 $\mu g\ L^{-1}$**	**No. herds with milk iodine >20 $\mu g\ L^{-1}$ / $\leq$ 500 $\mu g\ L^{-1}$**	**No. herds with milk iodine >500 $\mu g\ L^{-1}$**	**No. herds with milk iodine <20 $\mu g\ L^{-1}$**	**No. herds with milk iodine >20 $\mu g\ L^{-1}$ / $\leq$ 500 $\mu g\ L^{-1}$**	**No. herds with milk iodine >500 $\mu g\ L^{-1}$**
NE	0	13 (1)	2	5	8 (1)	2
NW	0	25 (1)	4	3 (1)	17	9
Mid	0	8	2	2	3	5
E	0	5	0	2	2	1
SW	0	17	2	2	12	5
SE	0	18 (2)	2	3	11	6 (2)
Total	0 %	88 %	12 %	17 %	54 %	29 %

Table 3: Summary of silage iodine composition. Silage samples from the present trial and from alternative sources were analysed to determine their iodine content. Values are presented as mean ± standard deviation. One-way ANOVA indicated that iodine concentration did not differ between silage crops (P=0.791).

Silage	**n**	**Iodine concentration (mg kg^{-1} DM)**
Rye grass Silage	4	0.173 ± 0.047
Maize Silage	3	0.205 ± 0.085
Whole crop	3	0.149 ± 0.051

Table 4: Composition of iodine containing ingredients of total mixed ration provided to cows in the UON dairy with associated dry matter content, iodine concentration and quantity of iodine supplied for each iodine containing component.

Ingredient	**Quantity supplied daily (kg)**	**Dry matter (%)**	**Iodine concentration (mg kg^{-1} DM)**	**Quantity of iodine provided (mg day^{-1})**
Grass Silage	16	27.3	0.22	0.96
Maize Silage	15	44.3	0.27	1.79
Whole crop Triticale Silage	10	65	0.19	1.24
$Ca(IO_3)_2$ (Mineral Mix: Longstaff Nutrition).	0.02835	100	650.99	18.46
Total for all iodine sources				**22.45**

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Figure 1

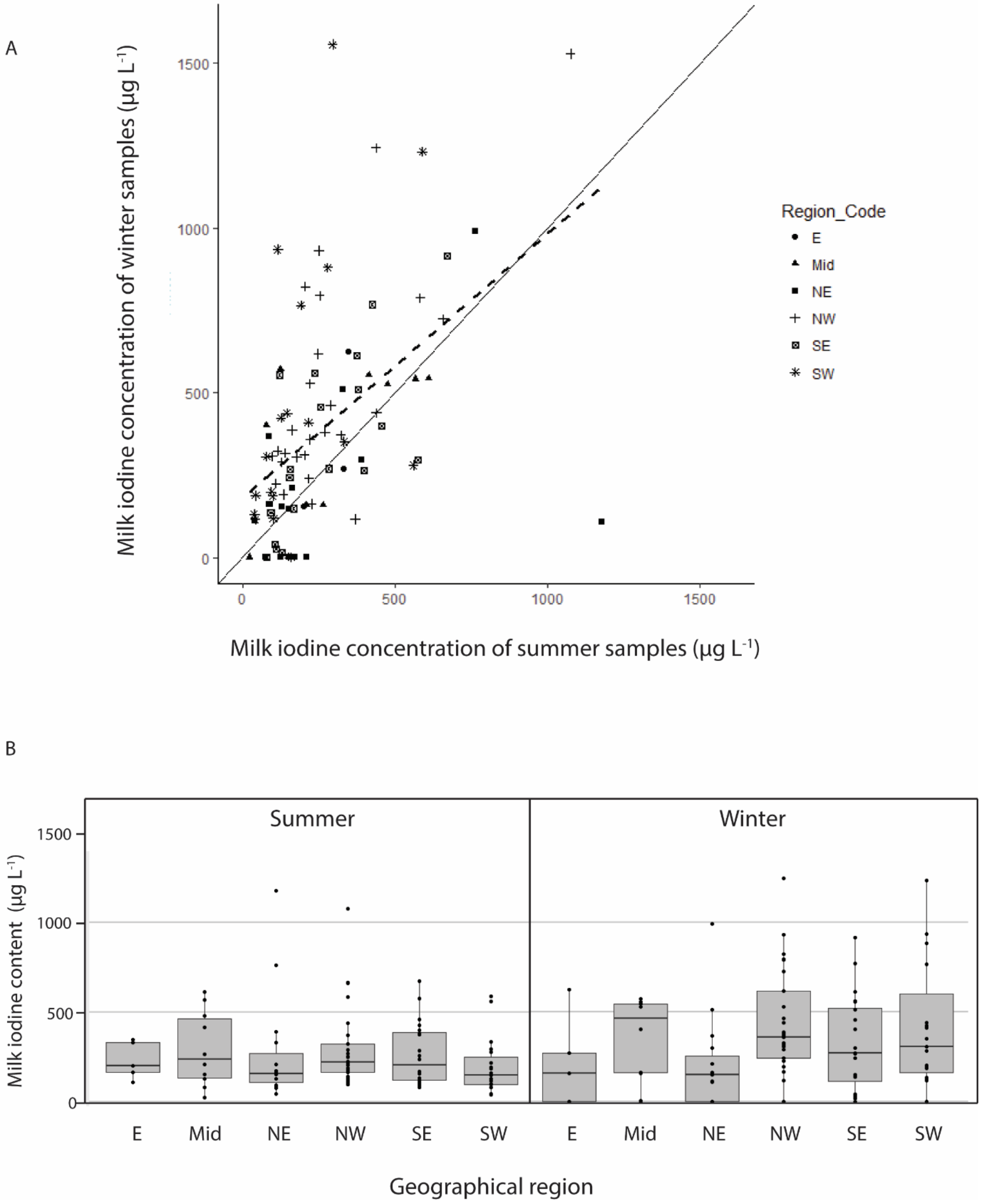
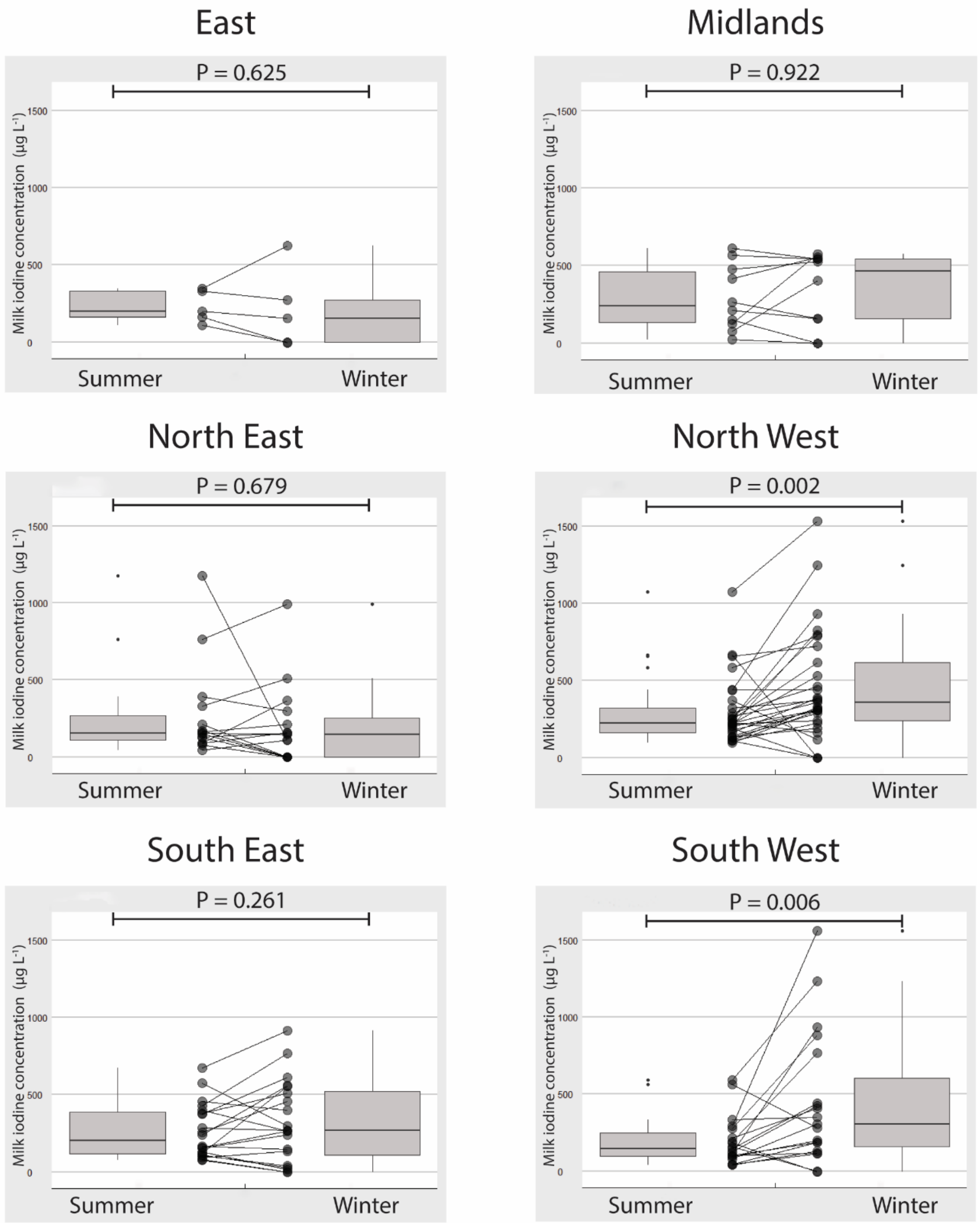

A
Milk iodine concentration of winter samples (µg L-1)
1500
1000
500
0
0
500
1000
1500
Milk iodine concentration of summer samples (µg L-1)
Region_Code
E
Mid
NE
NW
SE
SW
B
Summer
Winter
Milk iodine content (µg L-1)
1500
1000
500
0
E
Mid
NE
NW
SE
SW
E
Mid
NE
NW
SE
SW
Geographical region

Figure 2


East
P = 0.625
Midlands
P = 0.922
North East
P = 0.679
North West
P = 0.002
South East
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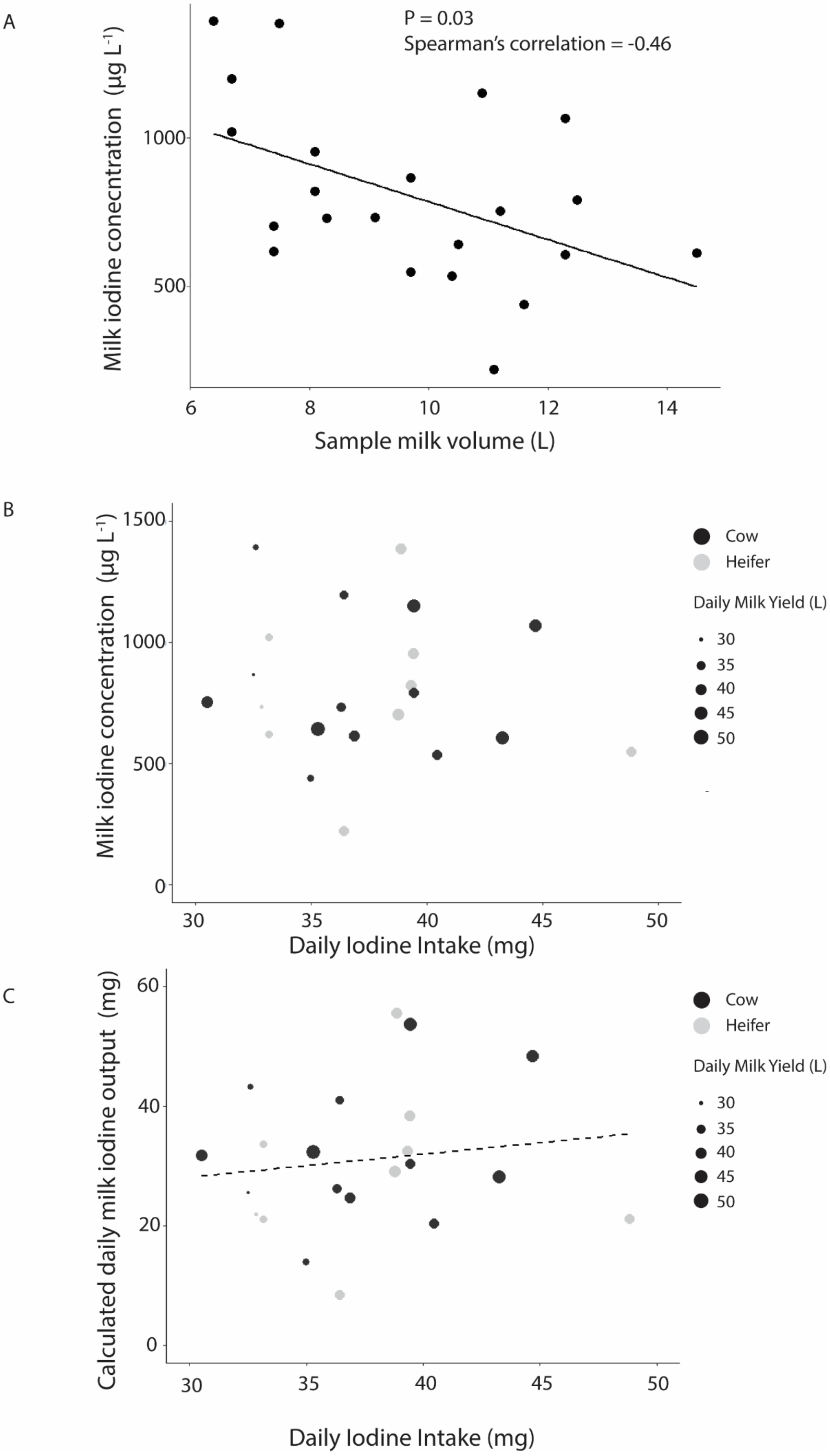
South West
P = 0.006
Milk iodine concentration (µg L⁻¹)
1500
1000
500
0
Summer
Winter

Figure 3


A
P = 0.03
Spearman's correlation = -0.46
Milk iodine conecntration (µg L⁻¹)
1000
500
6
8
10
12
14
Sample milk volume (L)
B
Milk iodine concentration (µg L⁻¹)
1500
1000
500
0
30
35
40
45
50
Daily Iodine Intake (mg)
Cow
Heifer
Daily Milk Yield (L)
30
35
40
45
50
C
Calculated daily milk iodine output (mg)
60
40
20
0
30
35
40
45
50
Daily Iodine Intake (mg)
Cow
Heifer
Daily Milk Yield (L)
30
35
40
45
50