Stable isotope profile (C, N, O, S) of Irish raw milk: Baseline data for authentication.

Roisin O'Sullivan, Frank J. Monahan, Bojlul Bahar, Laura Kirwan, Karina Pierce, Audrey O'Shea, Shane McElroy, Fionnuala Malone, Brian Hanafin, Sean Molloy, Alexander C.O. Evans, Olaf Schmidt

PII: S0956-7135(20)30559-4

DOI: https://doi.org/10.1016/j.foodcont.2020.107643

Reference: JFCO 107643

To appear in: *Food Control*

Received Date: 2 June 2020

Revised Date: 15 September 2020

Accepted Date: 18 September 2020

Please cite this article as: O'Sullivan R., Monahan F.J., Bahar B., Kirwan L., Pierce K., O'Shea A., McElroy S., Malone F., Hanafin B., Molloy S., Evans A.C.O. & Schmidt O., Stable isotope profile (C, N, O, S) of Irish raw milk: Baseline data for authentication., *Food Control* (2020), doi: https://doi.org/10.1016/j.foodcont.2020.107643.

Roisin O'Sullivan: Formal Analysis, Writing-Original Draft, Writing-Review & Editing, Visualization; **Frank J. Monahan**: Conceptualisation, Methodology, Data Curation, Writing-Original Draft, Writing-Review & Editing, Supervision, Project Administration, Funding Acquisition; **Bojlul Bahar**: Validation, Investigation, Data Curation, Visualization; **Laura Kirwan**: Software, Formal Analysis; **Karina Pierce**: Methodology, Writing-Review & Editing; **Audrey O'Shea**: Resources; **Shane McElroy**: Resources, Writing - Review & Editing; **Fionnuala Malone**: Resources, Writing - Review & Editing; **Brian Hanafin**: Resources; **Sean Molloy**: Resources; **Alexander C.O. Evans**: Conceptualisation, Resources, Supervision, Project Administration, Funding Acquisition; **Olaf Schmidt**: Conceptualisation, Methodology, Data Curation, Writing-Original Draft, Writing-Review & Editing, Supervision, Funding Acquisition

Stable isotope profile (C, N, O, S) of Irish raw milk: baseline data for authentication.

Roisin O'Sullivan[ab], Frank J. Monahan[ab*], Bojlul Bahar[ac], Laura Kirwan[a], Karina Pierce[ab], Audrey O'Shea[d], Shane McElroy[d], Fionnuala Malone[e], Brian Hanafin[e], Sean Molloy[e] , Alexander C.O. Evans[a], Olaf Schmidt[a]

[a] School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

[b] UCD Institute of Food and Health, University College Dublin, Belfield, Dublin 4, Ireland

[c] International Institute of Nutritional Sciences and Food Safety Studies
School of Sport and Health Sciences, University of Central Lancashire, Preston, Lancashire, PR1 2HE, United Kingdom

[d] Glanbia Ireland Plc, Glanbia House, Ring Road, Kilkenny, Co. Kilkenny, Ireland

[e] Glanbia Ingredients Ireland, Ballyragget, Co. Kilkenny, Ireland

*Corresponding author: frank.monahan@ucd.ie

ABSTRACT

Grass-based milk production is a major contributor to Irish agricultural output. The study characterized the Irish milk pool using stable isotope ratio analysis of carbon, nitrogen, oxygen and sulphur. Authentic raw milk samples were collected from 50 farms on five occasions over 13 months. Mean values of -27.11, 6.79, -3.27 and 6.16 ‰ were obtained for $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$, respectively. $\delta^{13}C$ values reflected a high level of grass input and values increased with increasing cereal concentrate feed input ($P<0.001$). $\delta^{18}O$ values were most negative in spring. There was a significant interaction between feed and season for $\delta^{13}C$ and $\delta^{15}N$ values ($P<0.05$), with the impact of concentrate feeding most evident in spring. $\delta^{34}S$ values were lowest at the highest level of concentrate input ($P<0.05$). The isotopic values reported here describe the Irish milk pool and may offer the potential to discriminate Irish milk and dairy products from similar commodities from other countries.

HIGHLIGHTS

- Stable isotope ratio analysis (C, N, O, S) was used to characterize Irish milk
- Isotopic values, particularly $\delta^{13}C$, reflected a high level of grass feeding
- Concentrate impact was clearest in spring, affecting $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values
- Isotopic values, particularly $\delta^{18}O$, were influenced by season
- Farm location (latitude and longitude) influenced $\delta^{18}O$ and $\delta^{34}S$ values

Keywords: food authentication; origin; milk production system; Isotope Ratio Mass Spectrometry; grass-fed dairy cattle

1. Introduction

Consumers have become more discerning about the background origin of the foods they consume as ethical concerns increasingly influence their food purchases (Sudbury-Riley & Kohlbacher, 2016). They often prefer to buy locally, or purchase foods that are native to a certain country, as they are more socially aware of the impact their choices have on the economy and the environment (Conner, Campbell-Arvai, & Hamm, 2008). In the case of foods derived from ruminant animals, positive connotations of phrases such as “pasture-raised” and “grass-fed” are often attractive to consumers who associate them with freshness, perceived health benefits, improved animal welfare and a more natural product overall (Shortall, 2019). Several studies have been conducted which support such claims. For example, it has been shown that when cows have access to pasture, aspects of animal welfare are improved (Hernandez-Mendo, von Keyserlingk, Veira, & Weary, 2007; Olmos et al., 2009). It has also been shown that pasture-fed dairying can have a lower environmental impact than a confinement system in terms of potential resource use and pollutants (Laca, Gomez, & Diaz, 2020) and that milk produced by animals raised on pasture has higher levels of desirable polyunsaturated fatty acids than milk produced from housed cows (Elgersma, 2015). Therefore, it is important for the industry to be able to guarantee that products claiming these advantages or origins are authentic. Presently, the quality, safety and provenance of Irish milk is subject to several electronic and paper-based traceability systems initiated at farm level and followed through processing to the finished product. With respect to provenance, the industry is increasingly interested in advanced independent scientific methods to verify product authenticity and support unique product values.

Ireland, as a dairy production location, is growing in importance. The country’s position on the edge of the Atlantic Ocean provides consistent, plentiful precipitation and the transatlantic Gulf Stream ensures moderate temperatures. This temperate climate permits dairy cows to graze outdoors for up to 300 days a year (O'Donovan, Lewis, & O'Kiely, 2011). As a result, Ireland’s dairy industry is centered on pasture-based milk production resulting in high quality milk (O'Brien et al., 1999). However, animals cannot be pasture-fed all year round and they receive non-grass feed inputs at various times of the year when grass growth is at a minimum (French, Driscoll, Horan, & Shalloo, 2015). Therefore, it is important to be able to characterize milk from a variety of feeding regimes throughout the year to understand how these factors influence the final milk composition and to enable differentiation based on level of grass feeding. This information is required to validate the grass-fed industry standards that are emerging to support grass-fed claims on dairy products (American Grass-fed Association, 2018; Bord Bia, 2020).

The application of stable isotope ratio analysis (SIRA) using isotopic ratio mass spectrometry (IRMS) to verify the authenticity of honey (Dong, Xiao, Xian, & Wu, 2018), wine (Wu et al., 2019), milk (Camin, Perini, Colombari, Bontempo, & Versini, 2008; Chung et al., 2020; Chung et al., 2019) and other dairy products (Bontempo et al., 2019; Camin et al., 2012) and of meat (Monahan et al., 2012; Monahan, Schmidt, & Moloney, 2018) is documented. Several studies have examined different milk pools in terms of extent of concentrate or grass-feeding across different production systems (Bontempo, Lombardi, Paoletti, Ziller, & Camin, 2012; Camin et al., 2008). Although SIRA has been used previously to identify specific regions within countries (Scampicchio et al., 2016) and to differentiate spot samples from a few countries (Luo et al., 2016), no study has characterized the isotopic profile from authentic samples representative of the entire milk pool of one country.

The objectives of this study were, firstly, to characterize, isotopically, a large sample of milk representative of the national milk pool in Ireland by generating isotopic profiles of light elements (C, N, O and S) of authentic, seasonally and on-farm sampled Irish raw milk, and, secondly, to analyse the effects of feeding regime (varying levels of cereal concentrate input), seasonality and farm location (latitude, longitude) within that pool.

2. Materials and Methods

2.1. Selection of dairy farms

A total of 50 farms were sampled in the mid-to-south east region of Ireland (Fig. S1). Farms were assigned to four different levels of cereal concentrate usage (feed intensity), based on questionnaires completed by each farmer and verified by concentrate purchasing records from Glanbia Plc (Kilkenny, Ireland). According to the classification used, cows were primarily grass-fed (receiving <500 kg concentrate per head annually) on 16 farms, partially concentrate-fed (receiving 500-750 kg concentrate per head annually) on 8 farms, moderately concentrate-fed (receiving 750-1000 kg concentrate per head annually) on 9 farms and highly concentrate-fed (receiving >1000 kg concentrate per head annually) on 17 farms. All farms supplied milk to Glanbia Plc and were chosen to be representative of the overall Irish milk pool. The co-ordinates (latitude and longitude) were recorded for each farm and authenticity was ensured by direct sampling from bulk tanks on the farms.

2.2. Sampling and sample processing

Milk samples (20-25 mL) were collected in May 2015, August 2015, November 2015, February 2016 and May 2016 on individual farms from the bulk milk tank and transported chilled to the Glanbia depot site together with the bulk milk. Samples were frozen (-20 °C) at the Glanbia laboratory prior to dispatch on dry ice to University College Dublin. In preparation for SIRA, samples (~20 mL) were thawed at room temperature (3 h) and centrifuged at 3334 g for 10 min (Hettich Rotofix 32A, Andreas Hettich GmbH & Co. Tuttlingen, Germany). The lower phase (skimmed milk) and the upper phase (milk fat) were carefully separated and collected. A sample of the skimmed milk was taken and stored at -20

°C for subsequent O stable isotope analysis. Casein was isolated from the lower phase (15 mL sample) by acidification to pH 4.3 with 525 μL of 2M HCl, followed by centrifugation (3334 g for 5 min). The casein was then washed with 30 mL deionized water, vortexed and centrifuged (3334 g for 2 min). The water was discarded and the residue (casein) lyophilized for 24 h before being used for C, N and S stable isotope analysis (Camin et al., 2008). Casein is frequently used for SIRA of milk and dairy products because it is a milk constituent common to many products, allowing inter-product isotopic comparisons.

2.3. Stable isotope ratio analysis

For dual C and N analysis, freeze dried casein samples (1.0 ± 0.1 mg) were loaded into ultra-clean tin capsules (6 x 4 mm) and placed into a 96-multiwell plate. The isotopic ratios $^{13}C/^{12}C$ (expressed as $\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) in the milk casein samples were analysed by Elemental Analysis – Isotope Ratio Mass Spectrometry (EA-IRMS). Samples and references were loaded into an auto-sampler on a Europa Scientific elemental analyzer, then dropped in sequence into a furnace held at 1000 °C and combusted in the presence of oxygen. The tin capsules flash combusted, raising the temperature in the region of the sample to ~1700 °C. The combusted gases were swept in a helium stream over a combustion catalyst (Cr_2O_3), copper oxide wires (to oxidize hydrocarbons), and silver wool to remove sulphur and halides. The resultant gases, N_2, NO_x, H_2O, O_2, and CO_2 were swept through a reduction stage of pure copper wires held at 600 °C. This removed any oxygen and converted NO_x species to N_2. A magnesium perchlorate chemical trap was used to remove water.

Nitrogen and carbon dioxide were separated using a packed column gas chromatograph held at a constant temperature of 65 °C. The resultant nitrogen peak entered the ion source of the Europa Scientific 20-20 IRMS first, where it was ionized and accelerated. Nitrogen and carbon dioxide gas species of different masses were separated in a magnetic field and simultaneously measured using a Faraday cup collector array (Ishida et al., 2018) to measure the isotopomers of N_2 at *m/z* 28, 29, 30 and CO_2 at *m/z* 44, 45, 46. Both references and samples were converted to N_2 and CO_2 and analysed using this method. The analysis proceeded in a batch process whereby a reference was analysed followed by a number of samples and then another reference.

The reference material used for δ^{13}C and δ^{15}N analysis was IA-R042 (NBS-1577B bovine liver, $\delta^{13}C_{V\text{-}PDB}$ = -21.60 ‰, $\delta^{15}N_{AIR}$ = 7.65 ‰). Typical analytical precision (standard deviation) of IA-R042 (bovine liver) run with the sample batches was 0.08 ‰ (n = 8) for $\delta^{13}C_{V\text{-}PDB}$ and 0.04 ‰ (n = 8) for $\delta^{15}N_{AIR}$. Furthermore, IA-R042 as well as IA-R038 (L-alanine, $\delta^{13}C_{V\text{-}PDB}$ = -24.99 ‰, $\delta^{15}N_{AIR}$ = -0.65 ‰) and a mixture of IA-R006 (cane sugar, $\delta^{13}C_{V\text{-}PDB}$ = -11.64 ‰) and IA-R046 (ammonium sulfate, $\delta^{15}N_{AIR}$ = 22.04 ‰) were run as quality control check samples during analysis. Typical analytical precision of IA-R038 (L-alanine) run with the sample batches was 0.05 ‰ (n = 4) for $\delta^{13}C_{V\text{-}PDB}$ and 0.06 ‰ (n = 4) for $\delta^{15}N_{AIR}$ and for IA-R006/IA-R046 (mixture of cane sugar and ammonium sulfate) run with the sample batches was 0.09 ‰ (n = 4) for $\delta^{13}C_{V\text{-}PDB}$ and 0.03 ‰ (n = 4) for $\delta^{15}N_{AIR.}$ IA-R042 and IA-R038 were calibrated against and traceable to IAEA-CH-6 (sucrose, $\delta^{13}C_{V\text{-}PDB}$ = -10.43 ‰) and IAEA-N-1 (ammonium sulfate, $\delta^{15}N_{AIR}$ = 0.40 ‰). IA-R006 was calibrated against and traceable to IAEA-CH-6. IA-R046 was calibrated against and traceable to IAEA-N-1. IAEA-CH-6 and IAEA-N-1 are inter-laboratory comparison standards distributed by the International Atomic Energy Agency (IAEA).

For O analysis, two samples (1.8 mL) of skimmed milk were loaded into cryo-vials which were stored at -20 °C, packed in dry ice and transported as frozen. Oxygen-18 analysis was carried out in duplicate using the equilibration technique. A sample aliquot was pipetted into an Exetainer tube, sealed and then filled with pure carbon dioxide. Tubes were left overnight for complete equilibration of the water with the carbon dioxide. Reference waters, including quality control check samples, were prepared in the same manner. The samples and references were then analysed by continuous flow – isotope ratio mass spectrometry using a Europa Scientific ANCA-GSL and Hydra 20-20 IRMS.

The samples were measured against three reference standards (IA-R054 with $\delta^{18}O_{V\text{-}SMOW}$ = +0.56 ‰, IA-R052 with $\delta^{18}O_{V\text{-}SMOW}$ = -19.64 ‰ and IA-R053 with $\delta^{18}O_{V\text{-}SMOW}$ = -10.18 ‰). Typical analytical precision of IA-R053 run with the sample batches was 0.05 ‰ (n = 16) for $\delta^{18}O_{V\text{-}SMOW.}$ All three standards are traceable to the primary reference standards V-SMOW2 (Vienna-Standard Mean Ocean Water) and V-SLAP2 (Vienna-Standard Light Antarctic Precipitation) distributed by the IAEA. The IA-R054 standard was used as the reference to which the samples and other standards were measured. The IA-R052 standard was used for calibration of $\delta^{18}O$ and the IA-R053 standard was used as a check of this calibration.

For S analysis, freeze dried casein samples (5.0 ± 0.1 mg) were loaded into ultra-clean (8 x 5 mm) capsules along with vanadium pentoxide (8.0 mg) and placed into a 96-multiwell plate. Sulphur isotope analysis was also undertaken by EA-IRMS. Tin capsules containing reference or sample material plus vanadium pentoxide catalyst were loaded into an automatic sampler. From there they were dropped, in sequence, into a furnace held at 1080 °C and combusted in the presence of oxygen. Tin capsules flash combusted, raising the temperature in the region of the sample to ~ 1700 °C. The combusted gases were then swept in a helium stream over combustion catalysts (tungstic oxide/zirconium oxide) and through a reduction stage of high purity copper wires to produce SO_2, N_2, CO_2, and water. Water was removed using a Nafion™ membrane. Sulphur dioxide was resolved from N_2 and CO_2 on a packed GC column at a temperature of 32 °C. The resultant SO_2 peak entered the ion source of the IRMS where it was ionized and accelerated. Gas species of different masses were separated in a magnetic field and measured on a Faraday cup universal collector array. Analysis was based on monitoring of m/z 48, 49 and 50 of SO^+ produced from SO_2 in the ion source (Giesemann, Jager, Norman, Krouse, & Brand, 1994).

Both references and samples were converted to SO_2 and analysed using this method. The analysis proceeded in a batch process by which a reference was analysed followed by a number of samples and then another reference. The reference material used for sulphur isotope analysis of pre-weighed casein samples was IA-R061 (barium sulfate, $\delta^{34}S_{V\text{-}CDT}$ = +20.33 ‰). Furthermore, IA-R061, IA-R025 (barium sulfate, $\delta^{34}S_{V\text{-}CDT}$ = +8.53 ‰) and IA-R026 (silver sulfide, $\delta^{34}S_{V\text{-}CDT}$ = +3.96 ‰) were used for calibration and correction of the ^{18}O contribution to the SO^+ ion beam. IA-R061, IA-R025 and IA-R026 are in-house standards calibrated and traceable to NBS-127 (barium sulfate, $\delta^{34}S_{CDT}$ = +20.3 ‰) and IAEA-S-1 (silver sulfide, $\delta^{34}S_{V\text{-}CDT}$ = -0.3 ‰).

For quality control purposes test samples of IA-R061, IAEA-SO-5 (barium sulfate, $\delta^{34}S_{V\text{-}CDT}$ = +0.50 ‰) and NBS-1577B (bovine liver, $\delta^{34}S_{V\text{-}CDT}$ = +7.50 ‰) were measured as quality control checks during batch analysis of samples. NBS-127, IAEA-SO-5 and IAEA-S-1 are inter-laboratory comparison standards distributed by the International Atomic Energy Agency (IAEA) with internationally accepted $\delta^{34}S$ values. NBS-1577B is an inter-laboratory comparison standard with a generally agreed $\delta^{34}S$ value. Typical analytical precision of NBS-1577B (bovine liver) run with the sample batches was 0.19 ‰ (n = 8). All SIRA was conducted at Iso-Analytical [Marshfield Bank, Crewe, United Kingdom].

2.4. Statistical analysis

A multivariate repeated measures regression model was fitted to the isotope data (Table 1), including sampling time, feed intensity, feed intensity x sampling time and location (latitude and longitude) as fixed effects, and sampling time as a random effect (SAS v 9.4). Latitude and longitude were included as continuous variables and significant model slopes give predicted changes in isotopic values with increasing latitude and longitude. As there were no significant interactions of location with other factors, the effects of latitude and longitude are consistent across sampling time and feed intensity. Principal component analysis (PCA) was carried out using the mean δ^{13}C, δ^{15}N, δ^{18}O and δ^{34}S values for each farm (n=50) in order to visualize possible patterns in the data related to the production system (R Core Team, 2020).

3. Results

3.1 Isotopic profile of an authentic Irish milk pool

Based on the analysis of samples collected over the thirteen month period between May 2015 and May 2016, the Irish milk pool sampled in this study had mean (± standard deviation) values of -27.11 (± 1.79), 6.79 (± 0.85), -3.27 (± 1.03) and 6.16 (± 1.32) ‰ for δ^{13}C, δ^{15}N, δ^{18}O and δ^{34}S, respectively.

3.2 δ^{13}C values

δ^{13}C values ranged from -20.33 to -30.49 ‰ across all feeding regimes (Fig. 1) but there was a significant effect of feed intensity (Table 1). δ^{13}C values decreased in the order: highly concentrate-fed > moderately concentrate-fed > partially concentrate-fed > primarily grass-fed at each sampling time. Thus, δ^{13}C values were less negative for milk samples collected from highly concentrate-fed animals (range, -20.67 to -28.65 ‰; mean, -25.83 ‰) (Fig. 1D) compared to those collected from primarily grass-fed animals (range, -25.11 to -30.49 ‰; mean, -28.09 ‰) (Fig.1A). The range in δ^{13}C values was -25.27 to -29.59 ‰ (mean, -27.9 ‰) and -20.33 to -28.80 ‰ (mean, -27.05 ‰) for milk from animals that were partially (Fig. 1B) and moderately (Fig. 1C) concentrate-fed, respectively.

There was a significant effect of sampling time on $\delta^{13}C$ values (Table 1). $\delta^{13}C$ values were least negative across all feed intensities in February 2016, i.e. in winter (range, -20.33 to -28.74 ‰; mean, -25.78 ‰), whereas the most negative $\delta^{13}C$ values ranged from -22.59 to -30.49‰ (mean, -27.84 ‰) and -24.67 to -29.14 ‰ (mean, -27.79 ‰) in May 2015 and May 2016, respectively (Fig. S2A). The range of $\delta^{13}C$ values was narrowest for milk samples collected from primarily grass-fed cows (range, -27.37 to -28.71 ‰) (Fig. 1A) across all collection dates and widest for samples collected from highly concentrate-fed cows (range, -23.84 to -26.91 ‰) (Fig. 1D).

There was a significant feed intensity x sampling time interaction whereby differences due to feed intensity were greater in November 2015 and February 2016 than at other sampling times (Fig. S2A).

3.3 $\delta^{15}N$ values

$\delta^{15}N$ values ranged from 4.7 to 8.93 ‰ across all feeding regimes (Fig. 2). $\delta^{15}N$ values were more positive for samples collected from primarily grass-fed cows (range, 4.88 to 8.7 ‰; mean, 7.13 ‰) (Fig. 2A) compared to those collected from highly concentrate-fed cows (range, 4.75 to 8.54 ‰; mean 6.64 ‰) (Fig. 2D) and samples from partially (range, 5.51 to 8.93 ‰; mean, 6.9 ‰) (Fig. 2B) and moderately (range, 4.7 to 8.28 ‰; mean, 6.4 ‰) (Fig. 2C) concentrate-fed cows.

Across all feed intensities, $\delta^{15}N$ values were lowest in samples collected in May 2016 (range, 4.7 to 8.03 ‰; mean, 6.6 ‰) (Fig. 2). Also, $\delta^{15}N$ values were more variable across all feed intensities in February 2016 (range, 4.75 to 8.7 ‰; mean, 6.8 ‰) compared to values in August 2015 (range, 5 to 8.37 ‰; mean, 6.87 ‰) and November 2015 (range, 5.02 to 8.51 ‰; mean, 6.83 ‰) (Fig. 2).

There was a significant feed intensity x sampling time interaction for δ^{15}N values (Table 1). Thus, there was a clear separation in δ^{15}N values between the more highly concentrate-fed groups and the other feed intensities in February 2016 (winter) but not at the other sampling times. (Fig. S2B).

3.4 δ^{18}O values

δ^{18}O values across all feeding regimes ranged from -0.85 to -5.34 ‰ (Fig. 3). There was a significant effect of sampling time on δ^{18}O values whereby values were most negative across all feed intensities in February 2016, i.e. in winter (range, -5.34 to -3.22 ‰; mean, -4.40 ‰) and least negative in May 2016 (range, -3.09 to -0.85 ‰; mean, -1.91‰) followed by May 2015 (range, -4.98 to -1.9‰; mean, -3.25 ‰) (Fig. S2C).

3.5 δ^{34}S values

δ^{34}S values ranged from 3.12 to 9.58 ‰ across all feeding regimes (Fig. 4). There was a significant effect of feed intensity on δ^{34}S values (Table 1) with milk samples from animals with the highest level of concentrate input having the lowest δ^{34}S values (range 3.12 to 7.62 ‰; mean, 5.47 ‰) (Fig. 4D) compared to the moderately (range 3.43 to 8.94 ‰; mean, 6.53 ‰) (Fig. 4C), partially (range, 3.7 to 8.23 ‰; mean, 6.28 ‰) (Fig. 4B) concentrate-fed groups and the primarily grass-fed group (range, 3.86 to 9.58 ‰; mean, 6.62 ‰) (Fig. 4A).

There was a significant effect of sampling time on δ^{34}S values with lowest values recorded in August 2015, i.e. in late summer (range, 3.12 to 8.09 ‰; mean, 5.82‰) compared to the other sampling times (Fig. S2D).

3.6 Principal Component Analysis

Principal component analysis (PCA) based on the mean $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$ values for each farm (n=50) is shown in Fig. 5. Primarily grass-fed (<500 kg concentrate per head annually) and highly concentrate-fed samples (>1000 kg concentrate per head annually) were clearly separated mainly by PC1 which explained 60.2% of the data variation.

3.7 Influence of farm location (latitude and longitude)

Latitude and longitude had statistically detectable effects on the $\delta^{34}S$ and $\delta^{18}O$ values of the milk samples ($P<0.05$) (Table 1). When calculated using the data collected over the full year, there was a decrease in $\delta^{18}O$ values (± standard error) of 0.55 ± 0.07 ‰ per degree increase in latitude and an increase in $\delta^{18}O$ values of 0.17 ± 0.07 ‰ per degree increase in longitude. There was a decrease in $\delta^{34}S$ values of 0.82 ± 0.24 ‰ per degree increase in latitude and a decrease in $\delta^{34}S$ values of 0.49 ± 0.23 ‰ per degree increase in longitude.

4. Discussion

4.1 $\delta^{13}C$ values

The mean $\delta^{13}C$ values for Irish milk from the four feed intensity ranges were more negative than those reported in studies undertaken in some other countries (Table 2), most likely reflecting the highly grass-based nature of Irish milk production. The values were in agreement with those temperate regions of the world where grass-based (C3 grasses) milk production is possible for some, or all, of the year (e.g. New Zealand). The less negative mean $\delta^{13}C$ values for countries such as the USA, China, Malaysia, Germany and France may be attributed to the unavailability of fresh pasture, or a lack of pasture at certain times of the year, which would result in the requirement for high levels of cereal concentrate input to support milk production.

The results are in good agreement with data for Irish beef reported by (Osorio, Moloney, Schmidt, & Monahan, 2011) where $\delta^{13}C$ values for beef samples from grass-fed animals had significantly lower $\delta^{13}C$ values than concentrate-fed animals, with the differences being attributed to the more negative $\delta^{13}C$ values of pasture compared to the cereal concentrates consumed by the animals. In the current study, the less negative values for casein in the highly and moderately concentrate-fed groups compared to the other groups may also reflect some level of maize input, particularly in winter. The clear separation between the primarily grass-fed and highly concentrate fed is mainly due to difference in $\delta^{13}C$ values (see Table 2 and Figure 5).

Of importance for comparisons with international samples is the fact that animals consuming C3 plant materials, e.g. temperate grasses, grass silage or barley, which have low $\delta^{13}C$ values (between -35 and -21 ‰), have lower tissue $\delta^{13}C$ values than those consuming C4 plants, e.g. maize ($\delta^{13}C$ between -14 and -10 ‰) (Bahar et al., 2005; Heaton, Kelly, Hoogewerff, & Woolfe, 2008; Schmidt et al., 2005). One special case is organic milk production which may be highly grass-based across different countries, leading to similarity in $\delta^{13}C$ values in milk from temperate regions particularly. However, the analysis of multiple elements, some of which are influenced by geographic factors (notably $\delta^{18}O$ and $\delta^{34}S$) is likely to permit discrimination of Irish samples among international organic grass-fed milk (Chung et al., 2018).

The findings relating to seasonal differences in $\delta^{13}C$ values are also in agreement with (Kornexl, Werner, Rossmann, & Schmidt, 1997) who reported more positive $\delta^{13}C$ values in Bavarian milk casein samples taken during winter months. Similarly, in Irish beef, (Bahar et al., 2008) found in a seasonal survey that $\delta^{13}C$ values became more positive between December and early summer, most likely reflecting a higher level on concentrate feeding, possibly including maize, over the winter/spring period when the grass supply is limited (French et al., 2015).

4.2 δ^{15}N values

Mean δ^{15}N values were more positive compared to values reported in other countries (e.g. Germany, Italy, the US, Brazil, and China) (Table 2). This may be related to high synthetic nitrogen fertilizer application in Ireland and high levels of precipitation on fresh pasture leading to higher δ^{15}N values (Cook, 2001). The data agrees with previous studies in which meat from countries in north-western Europe, such as Ireland and the UK, had relatively more positive δ^{15}N values than other countries (Camin et al., 2007; Osorio et al., 2011). The more pronounced differences in mean δ^{15}N values between feeding intensities in springtime (February 2016) probably reflects a higher level on cereal concentrate input across all feeding regimes at this time of the year when grass is limiting.

4.3 δ^{18}O values

δ^{18}O values of Irish skimmed milk reported here were considerably more positive when compared to other countries (Australia, New Zealand, France, Germany, the US and China) analysed in previous studies (Table 2). Precipitation δ^{18}O values are known to decrease with increasing latitude, elevation and continentality (Bowen, 2010). In accordance with this, Ireland, as a small island country would be expected to have more positive δ^{18}O values than land-locked countries and, in our current study, δ^{18}O values for skimmed milk increased with increasing longitude. The findings in our study also agree with (Bowen & Revenaugh, 2003) where a decrease in δ^{18}O values was experienced as latitude (distance from the equator) increased. Although latitude and longitude had a significant influence on δ^{18}O values of skimmed milk, (van der Veer, Voerkelius, Lorentz, Heiss, & Hoogewerff, 2009) suggested that surface temperature, which will vary with time of sampling, is a better explanatory factor for global δ^{18}O isotopic variation. The δ^{18}O values we report are in agreement with results collected by (Kornexl et al., 1997) where milk water was observed to be enriched in δ^{18}O during the summer. In terms of seasonal effects, the low δ^{18}O values of skimmed milk in February and to a lesser extent, November, most likely reflects the seasonal effect of temperature on rainfall with higher δ^{18}O values in the summer months when surface temperature is warmest compared to winter when surface temperature is coldest (van der Veer et al., 2009).

δ^{18}O values of milk samples differ from precipitation values since fractionation and enrichment occurs during milk production (Abeni et al., 2015). Oxygen in an animal's diet has many inputs (e.g. atmospheric oxygen, drinking water, plant water) and outputs (e.g. CO_2 production, sweat water, urine water), all of which have an overall influence on the δ^{18}O values of milk produced (Chen, Schnyder, & Auerswald, 2017). Therefore, geographical and fractionation factors must be considered and, in this context, the δ^{18}O values for Irish skimmed milk reported probably reflect some enrichment relative to water ingested by the animals; in an earlier study we reported values of -5.0 to -6.7 ‰ for Irish water (Harrison et al., 2011).

4.4 δ^{34}S values

Few other studies have examined the δ^{34}S values of milk casein (Table 2), however, the δ^{34}S values of Irish milk casein in this study were lower than those of samples collected from coastal regions of Australia and New Zealand (Crittenden et al., 2007). Coastal regions are known to have more positive δ^{34}S values as ocean water and sea spray will enrich soil with sulphur which in turn increases the δ^{34}S values of locally grown feedstuffs and, ultimately, animal derived foods (Rossmann, 2001). Therefore, Ireland, as an island country would be expected to have higher δ^{34}S values than land-locked countries. Although no study of milk samples comparing coastal and land-locked countries is available, defatted lamb protein samples taken from coastal and land-locked countries were examined by (Camin et al., 2007) with Irish samples having an average δ^{34}S value of 9.2 ‰, which was among the highest values reported. This value is in agreement with the high δ^{34}S values observed in the current study.

The values reported here are also similar to those reported for sheep's wool in parts of the midlands and east of Ireland; furthermore, our finding of decreasing δ^{34}S values with increasing latitude is in agreement with the data on sheep's wool, reflecting the influence of sea spray and prevailing winds from the south and west (Zazzo, Monahan, Moloney, Green, & Schmidt, 2011). This study provides evidence of a promising δ^{34}S isotopic signature for Irish milk samples and future work should compare these values with other international signatures.

5. CONCLUSIONS

The highly grass-fed nature of the Irish milk pool compared to milk from some other countries is evident, particularly from analysis of C and N stable isotope ratios. The study shows that the level of concentrate feeding is most clearly reflected in milk in the winter when animals are temporarily housed indoors. Ireland's island geography and the prevailing climatic factors, combined with the highly grass-fed nature of its milk production, may have the potential to generate a signature for the Irish milk pool. This may offer an opportunity to scientifically validate the provenance, origin and claims, such as grass-fed, of milk and dairy products.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENTS

The authors acknowledge funding from Glanbia Ingredients Ireland (GII) to support the research.

REFERENCES

Abeni, F., Petrera, F., Capelletti, M., Dal Pra, A., Bontempo, L., Tonon, A., & Camin, F. (2015). Hydrogen and Oxygen Stable Isotope Fractionation in Body Fluid Compartments of Dairy Cattle According to Season, Farm, Breed, and Reproductive Stage. *Plos One, 10*(5), 18. doi:10.1371/journal.pone.0127391

American Grass-fed Association (2018). Grass-fed dairy standard. Retrieved from https://www.americangrassfed.org/wp-content/uploads/2018/11/aga-grassfed-dairy-standards.pdf

Bahar, B., Monahan, F. J., Moloney, A. P., O'Kiely, P., Scrimgeour, C. M., & Schmidt, O. (2005). Alteration of the carbon and nitrogen stable isotope composition of beef by substitution of grass silage with maize silage. *Rapid Communications in Mass Spectrometry, 19*(14), 1937-1942. doi:10.1002/rcm.2007

Bahar, B., Schmidt, O., Moloney, A. P., Scrimgeour, C. M., Begley, I. S., & Monahan, F. J. (2008). Seasonal variation in the C, N and S stable isotope composition of retail organic and conventional Irish beef. *Food Chemistry, 106*(3), 1299-1305. doi:10.1016/j.foodchem.2007.07.053

Behkami, S., Gholami, R., Gholami, M., & Roohparvar, R. (2020). Precipitation isotopic information: A tool for building the data base to verify milk geographical origin traceability. *Food Control, 107*. doi:10.1016/j.foodcont.2019.106780

Bontempo, L., Barbero, A., Bertoldi, D., Camin, F., Larcher, R., Perini, M., Sepulcri, A., Zicarelli, L., & Piasentier, E. (2019). Isotopic and elemental profiles of Mediterranean buffalo milk and cheese and authentication of Mozzarella di Bufala Campana PDO: An initial exploratory study. *Food Chemistry, 285*, 316-323. doi:10.1016/j.foodchem.2019.01.160

Bontempo, L., Lombardi, G., Paoletti, R., Ziller, L., & Camin, F. (2012). H, C, N and O stable isotope characteristics of alpine forage, milk and cheese. *International Dairy Journal, 23*(2), 99-104. doi:10.1016/j.idairyj.2011.10.005

Bord Bia (2020). Grass-fed dairy standard. Retrieved from https://www.bordbia.ie/farmers-growers/get-involved/become-quality-assured/sustainable-dairy-assurance-scheme-sdas/

Bostic, J. N., Hagopian, W. M., & Jahren, A. H. (2018). Carbon and nitrogen stable isotopes in US milk: Insight into production process. *Rapid Communications in Mass Spectrometry, 32*(7), 561-566. doi:10.1002/rcm.8069

Bowen, G. J. (2010). Isoscapes: Spatial Pattern in Isotopic Biogeochemistry. *Annual Review of Earth and Planetary Sciences, Vol 38, 38*, 161-187. doi:10.1146/annurev-earth-040809-152429

Bowen, G. J., & Revenaugh, J. (2003). Interpolating the isotopic composition of modern meteoric precipitation. *Water Resources Research, 39*(10). doi:10.1029/2003wr002086

Camin, F., Bontempo, L., Heinrich, K., Horacek, M., Kelly, S. D., Schlicht, C., Thomas, F., Monahan, F. J., Hoogewerff, J., & Rossmann, A. (2007). Multi-element (H,C,N,S) stable isotope characteristics of lamb meat from different European regions. *Analytical and Bioanalytical Chemistry, 389*(1), 309-320. doi:10.1007/s00216-007-1302-3

Camin, F., Perini, M., Colombari, G., Bontempo, L., & Versini, G. (2008). Influence of dietary composition on the carbon, nitrogen, oxygen and hydrogen stable isotope ratios of milk. *Rapid Communications in Mass Spectrometry, 22*(11), 1690-1696. doi:10.1002/rcm.3506

Camin, F., Wehrens, R., Bertoldi, D., Bontempo, L., Ziller, L., Perini, M., Nicolini, G., Nocetti, M., & Larcher, R. (2012). H, C, N and S stable isotopes and mineral profiles to objectively guarantee the authenticity of grated hard cheeses. *Analytica Chimica Acta, 711*, 54-59. doi:10.1016/j.aca.2011.10.047

Chen, G., Schnyder, H., & Auerswald, K. (2017). Model explanation of the seasonal variation of δ18O in cow (Bos taurus) hair under temperate conditions. *Scientific Reports, 7*(1), 1-15. doi:10.1038/s41598-017-00361-y

Chesson, L. A., Valenzuela, L. O., O'Grady, S. P., Cerling, T. E., & Ehleringer, J. R. (2010). Hydrogen and Oxygen Stable Isotope Ratios of Milk in the United States. *Journal of Agricultural and Food Chemistry, 58*(4), 2358-2363. doi:10.1021/jf904151c

Chung, I.-M., Kim, J.-K., Yarnes, C. T., An, Y.-J., Kwon, C., Kim, S.-Y., Yang, Y.-J., Chi, H.-Y., & Kim, S.-H. (2019). Fatty Acid- and Amino Acid-Specific Isotope Analysis for Accurate Authentication and Traceability in Organic Milk. *Journal of Agricultural and Food Chemistry, 67*(2), 711-722. doi:10.1021/acs.jafc.8b05063

Chung, I. M., Kim, J. K., Lee, K. J., Son, N. Y., An, M. J., Lee, J. H., An, Y.J., Kim, S. H. (2018). Discrimination of organic milk by stable isotope ratio, vitamin E, and fatty acid profiling combined with multivariate analysis: A case study of monthly and seasonal variation in Korea for 2016-2017. *Food Chemistry, 261*, 112-123. doi:10.1016/j.foodchem.2018.04.017

Chung, I. M., Kim, J. K., Yang, Y. J., An, Y. J., Kim, S. Y., Kwon, C., & Kim, S. H. (2020). A case study for geographical indication of organic milk in Korea using stable isotope ratios-based chemometric analysis. *Food Control, 107*. doi:10.1016/j.foodcont.2019.106755

Conner, D. S., Campbell-Arvai, V., & Hamm, M. W. (2008). Value in the values: pasture-raised livestock products offer opportunities for reconnecting producers and consumers. *Renewable Agriculture and Food Systems, 23*(1), 62-69. doi:10.1017/S1742170507002086

Cook, G. D. (2001). Effects of frequent fires and grazing on stable nitrogen isotope ratios of vegetation in northern Australia. *Austral Ecology, 26*(6), 630-636. doi:10.1046/j.1442-9993.2001.01150.x

Crittenden, R. G., Andrew, A. S., LeFournour, M., Young, M. D., Middleton, H., & Stockmann, R. (2007). Determining the geographic origin of milk in Australasia using multi-element stable isotope ratio analysis. *International Dairy Journal, 17*(5), 421-428. doi:10.1016/j.idairyj.2006.05.012

Dong, H., Xiao, K. J., Xian, Y. P., & Wu, Y. L. (2018). Authenticity determination of honeys with non-extractable proteins by means of elemental analyzer (EA) and liquid chromatography (LC) coupled to isotope ratio mass spectroscopy (IRMS). *Food Chemistry, 240*, 717-724. doi:10.1016/j.foodchem.2017.08.008

Ehtesham, E., Baisden, W. T., Keller, E. D., Hayman, A. R., Van Hale, R., & Frew, R. D. (2013). Correlation between precipitation and geographical location of the δ2H values of the fatty acids in milk and bulk milk powder. *Geochimica et Cosmochimica Acta, 111*, 105-116. doi:10.1016/j.gca.2012.10.026

Elgersma, A. (2015). Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: A review of the contributing factors, challenges and future perspectives. *European Journal of Lipid Science and Technology, 117*(9), 1345-1369. doi:10.1002/ejlt.201400469

French, P., Driscoll, K. O., Horan, B., & Shalloo, L. (2015). The economic, environmental and welfare implications of alternative systems of accommodating dairy cows during the winter months. *Animal Production Science, 55*(7), 838-842. doi:10.1071/an14895

Garbaras, A., Skipityte, R., Meliaschenia, A., Senchenko, T., Smoliak, T., Ivanko, M., Sapolaite, J., Ezerinskis, Z., Remeikis, V. (2018). REGION DEPENDENT C-13,N-15,O-18 ISOTOPE RATIOS IN THE COW MILK. *Lithuanian Journal of Physics, 58*(3), 277-282

Garbaras, A., Skipityte, R., Sapolaite, J., Ezerinskis, Z., & Remeikis, V. (2019). Seasonal Variation in Stable Isotope Ratios of Cow Milk in Vilnius Region, Lithuania. *Animals, 9*(3). doi:10.3390/ani9030069

Giesemann, A., Jager, H. J., Norman, A. L., Krouse, H. P., & Brand, W. A. (1994). ONLINE SULFUR-ISOTOPE DETERMINATION USING AN ELEMENTAL ANALYZER COUPLED TO A MASS-SPECTROMETER. *Analytical Chemistry, 66*(18), 2816-2819. doi:10.1021/ac00090a005

Harrison, S. M., Schmidt, O., Moloney, A. P., Kelly, S. D., Rossmann, A., Schellenberg, A., Camin, F., Perini, M., Hoogewerff, J., Monahan, F. J. (2011). Tissue turnover in ovine muscles and lipids as recorded by multiple (H, C, O, S) stable isotope ratios. *Food Chemistry, 124*(1), 291-297. doi:10.1016/j.foodchem.2010.06.035

Heaton, K., Kelly, S. D., Hoogewerff, J., & Woolfe, M. (2008). Verifying the geographical origin of beef: The application of multi-element isotope and trace element analysis. *Food Chemistry, 107*(1), 506-515. doi:10.1016/j.foodchem.2007.08.010

Hernandez-Mendo, O., von Keyserlingk, M. A. G., Veira, D. M., & Weary, D. M. (2007). Effects of Pasture on Lameness in Dairy Cows. *Journal of Dairy Science, 90*(3), 1209-1214. doi:10.3168/jds.S0022-0302(07)71608-9

Ishida, A., Kitajima, K., Williford, K. H., Tuite, M. L., Kakegawa, T., & Valley, J. W. (2018). Simultaneous In Situ Analysis of Carbon and Nitrogen Isotope Ratios in Organic Matter by Secondary Ion Mass Spectrometry. *Geostandards and Geoanalytical Research, 42*(2), 189-203. doi:10.1111/ggr.12209

Knobbe, N., Vogl, J., Pritzkow, W., Panne, U., Fry, H., Lochotzke, H. M., & Preiss-Weigert, A. (2006). C and N stable isotope variation in urine and milk of cattle depending on the diet. *Analytical and Bioanalytical Chemistry, 386*(1), 104-108. doi:10.1007/s00216-006-0644-6

Kornexl, B. E., Werner, T., Rossmann, A., & Schmidt, H. L. (1997). Measurement of stable isotope abundances in milk and milk ingredients - A possible tool for origin assignment and quality control. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung a-Food Research and Technology, 205*(1), 19-24. doi:10.1007/s002170050117

Laca, A., Gomez, N., & Diaz, M. (2020). Overview on GHG emissions of raw milk production and a comparison of milk and cheese carbon footprints of two different systems from northern Spain. *Environmental Science and Pollution Research*, 17. doi:10.1007/s11356-019-06857-6

Luo, D. H., Dong, H., Luo, H. Y., Xian, Y. P., Guo, X. D., & Wu, Y. L. (2016). Multi-Element (C, N, H, O) Stable Isotope Ratio Analysis for Determining the Geographical Origin of Pure Milk from Different Regions. *Food Analytical Methods, 9*(2), 437-442. doi:10.1007/s12161-015-0204-9

Martinelli, L. A., Nardoto, G. B., Perez, M. A. Z., Arruda, G., Fracassi, F. C., Oliveira, J. G. G., Ottani, I. S., Lima, S. H., Mazzi, E. A., Gomes, T. F., Soltangheisi, A., Abdulla, A. L., Mariano, E., Costa, F. J. V., Duarte-Neto, P. J., Moreira, M. Z., Camargo, P. B. (2020). Carbon and Nitrogen Isotope Ratios of Food and Beverage in Brazil. *Molecules, 25*(6). doi:10.3390/molecules25061457

Monahan, F. J., Moloney, A. P., Osorio, M. T., Röhrle, F. T., Schmidt, O., & Brennan, L. (2012). Authentication of grass-fed beef using bovine muscle, hair or urine. *Trends in Food Science & Technology, 28*(2), 69-76. doi:10.1016/j.tifs.2012.05.005

Monahan, F. J., Schmidt, O., & Moloney, A. P. (2018). Meat provenance: Authentication of geographical origin and dietary background of meat. *Meat Science, 144*, 2-14. doi:https://doi.org/10.1016/j.meatsci.2018.05.008

O'Brien, B., Dillon, P., Murphy, J. J., Mehra, R. K., Guinee, T. P., Connolly, J. F., Kelly, A., Joyce, P. (1999). Effects of stocking density and concentrate supplementation of grazing dairy cows on milk production, composition and processing characteristics. *Journal of Dairy Research, 66*(2), 165-176. doi:10.1017/S0022029999003544

O'Donovan, M., Lewis, E., & O'Kiely, P. (2011). Requirements of future grass-based ruminant production systems in Ireland. *Irish Journal of Agricultural and Food Research, 50*(1), 1-21.

Olmos, G., Boyle, L., Hanlon, A., Patton, J., Murphy, J. J., & Mee, J. F. (2009). Hoof disorders, locomotion ability and lying times of cubicle-housed compared to pasture-based dairy cows. *Livestock Science, 125*(2), 199-207. doi:10.1016/j.livsci.2009.04.009

Osorio, M. T., Moloney, A. P., Schmidt, O., & Monahan, F. J. (2011). Beef Authentication and Retrospective Dietary Verification Using Stable Isotope Ratio Analysis of Bovine Muscle and Tail Hair. *Journal of Agricultural and Food Chemistry, 59*(7), 3295-3305. doi:10.1021/jf1040959

Potocnik, D., Necemer, M., Perisic, I., Jagodic, M., Mazej, D., Camin, F., Eftimov, T., Strojnik, L., Ogrinc, N. (2020). Geographical verification of Slovenian milk using stable isotope ratio, multi-element and multivariate modelling approaches. *Food Chemistry, 326*. doi:10.1016/j.foodchem.2020.126958

R Core Team. (2020). R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. https://doi.org/http://www.r-project.org/

Rossmann, A. (2001). Determination of stable isotope ratios in food analysis. *Food Reviews International, 17*(3), 347-381. doi:10.1081/fri-100104704

Scampicchio, M., Eisenstecken, D., De Benedictis, L., Capici, C., Ballabio, D., Mimmo, T., Robatscher, P., Kerschbaumer, L., Oberhuber, M., Kaser, A., Huck, C.W., Cesco, S. (2016). Multi-method Approach to Trace the Geographical Origin of Alpine Milk: a Case Study of Tyrol Region. *Food Analytical Methods, 9*(5), 1262-1273. doi:10.1007/s12161-015-0308-2

Scampicchio, M., Mimmo, T., Capici, C., Huck, C., Innocente, N., Drusch, S., & Cesco, S. (2012). Identification of Milk Origin and Process-Induced Changes in Milk by Stable Isotope Ratio Mass Spectrometry. *Journal of Agricultural and Food Chemistry, 60*(45), 11268-11273. doi:10.1021/jf302846j

Schmidt, O., Quilter, J. M., Bahar, B., Moloney, A. P., Scrimgeour, C. M., Begley, I. S., & Monahan, F. J. (2005). Inferring the origin and dietary history of beef from C, N and S stable isotope ratio analysis. *Food Chemistry, 91*(3), 545-549. doi:10.1016/j.foodchem.2004.08.036

Shortall, O. (2019). Cows eat grass, don't they? Contrasting sociotechnical imaginaries of the role of grazing in the UK and Irish dairy sectors. *Journal of Rural Studies, 72*, 45-57. doi:10.1016/j.jrurstud.2019.10.004

Sudbury-Riley, L., & Kohlbacher, F. (2016). Ethically minded consumer behavior: Scale review, development, and validation. *Journal of Business Research, 69*(8), 2697-2710. doi:https://doi.org/10.1016/j.jbusres.2015.11.005

van der Veer, G., Voerkelius, S., Lorentz, G., Heiss, G., & Hoogewerff, J. A. (2009). Spatial interpolation of the deuterium and oxygen-18 composition of global precipitation using temperature as ancillary variable. *Journal of Geochemical Exploration, 101*(2), 175-184. doi:10.1016/j.gexplo.2008.06.008

Wu, H., Tian, L., Chen, B., Jin, B. H., Tian, B., Xie, L. Q., Rogers, K.M., Lin, G. H. (2019). Verification of imported red wine origin into China using multi isotope and elemental analyses. *Food Chemistry, 301*. doi:10.1016/j.foodchem.2019.125137

Zazzo, A., Monahan, F. J., Moloney, A. P., Green, S., & Schmidt, O. (2011). Sulphur isotopes in animal hair track distance to sea. *Rapid Communications in Mass Spectrometry, 25*(17), 2371-2378. doi:10.1002/rcm.5131

TABLES CAPTIONS

Table 1 Summary outputs of multivariate repeated measures regression models for the C, N, O and S stable isotope composition of raw milk from Irish dairy farms. Values in bold are significant.

Table 2 Stable isotope composition (‰, mean or range) of milk or its constituents from different countries.

FIGURE CAPTIONS

Fig. 1 Carbon isotope ratios (δ^{13}C, ‰) in raw milk from 50 Irish farms with levels of cereal concentrate input ranging from <500 kg/head/year to >1000 kg/head/year: (A), <500 kg/head/year; (B) 500-750 kg/head/year; (C) 750-1000 kg/head/year; (D) >1000 kg/head/year. Milk sampling took place at intervals of 3 months between May 2015 and May 2016 inclusive, with 5 sampling points in total: May 2015, August 2015, November 2015, February 2016 and May 2016.

Fig. 2 Nitrogen isotope ratios (δ^{15}N, ‰) in raw milk from 50 Irish farms with levels of cereal concentrate input ranging from <500 kg/head/year to >1000 kg/head/year: (A), <500 kg/head/year; (B) 500-750 kg/head/year; (C) 750-1000 kg/head/year; (D) >1000 kg/head/year. Milk sampling took place at intervals of 3 months between May 2015 and May 2016 inclusive, with 5 sampling points in total: May 2015, August 2015, November 2015, February 2016 and May 2016.

Fig. 3 Oxygen isotope ratios (δ^{18}O, ‰) in raw milk from 50 Irish farms with levels of cereal concentrate input ranging from <500 kg/head/year to >1000 kg/head/year: (A), <500 kg/head/year; (B) 500-750 kg/head/year; (C) 750-1000 kg/head/year; (D) >1000 kg/head/year. Milk sampling took place at intervals of 3 months between May 2015 and May 2016 inclusive, with 5 sampling points in total: May 2015, August 2015, November 2015, February 2016 and May 2016.

Fig. 4 Sulphur isotope ratios (δ^{34}S, ‰) in raw milk from 50 Irish farms with levels of cereal concentrate input ranging from <500 kg/head/year to >1000 kg/head/year: (A), <500 kg/head/year; (B) 500-750 kg/head/year; (C) 750-1000 kg/head/year; (D) >1000 kg/head/year. Milk sampling took place at intervals of 3 months between May 2015 and May 2016 inclusive, with 5 sampling points in total: May 2015, August 2015, November 2015, February 2016 and May 2016.

Fig. 5 Plot of the first and second principal component using the mean values of all four stable isotope ratios collected over 13 months.

Table 2

Country	Sample	δ^{13}C (‰)	δ^{15}N (‰)	δ^{18}O (‰)	δ^{34}S (‰)	Time of sampling	Feed type	Reference
Ireland	Casein	-20.33 to -30.49	4.7 to 8.93	-	3.12 to 9.58	Over full year	Varying levels of concentrate input	Current study
	Milk Water	-	-	-0.85 to -5.34	-	Over full year	Varying levels of concentrate input	Current study
Italy	Casein	-24 to -17.2	3 to 5.9	-	-	-	Alfalfa and maize silages	Scampicchio et al. (2012)
	Casein	-20.6 to -17.5	4.5 to 5.8	10.5 to 15.9	-	Spring	Varying levels of maize	Camin, Perini, Colombari, Bontempo, & Versini (2008)
Germany	Whole milk	-26.3 to -22	3.7 to 4.1	-	-	Sept - May	44-100% C4 plants	Knobbe et al. (2006)
	Casein	-26.5 to -29.4	3.5 to 5	-	-	-	Grass, grain, corn	Kornexl, Werner, Rossmann, & Schmidt (1997)
Germany and France	Milk Protein	-21.85 ± 0.56	5.2 ± 0.16	-	-	-	Assumed C-4 pastures	Luo et al. (2016)
	Milk Protein	-	-	-10.46 ± 0.38	-	-	Assumed C-4 pastures	
Vilnius Region, Lithuania	Bulk Milk Powder	-31.2 to -27.6	2.9 to 6	-	-	2014 to 2016	Grass and hay	Garbaras, Skipityte, Sapolaite, Ezerinskis, & Remeikis (2019)

	Milk Water	-	-	-9.8 to -2.2	-	2014 to 2016	Grass and hay	
Belarus	Whole milk	-30.2 to -20	3.63 to 5.66	-	-	Summer and 2 Winter samples	Varying levels of C-4 plants	Garbaras et al. (2018)
	Milk water	-	-	-8.67 to -3.87	-	Summer and 2 Winter samples	Varying levels of C-4 plants	
Slovenia	Casein	-28.2 to -17.8	2.5 to 9.6	9 to 14.6	0.5 to 7.7	Summer and Winter 2012 - 2014	-	Potocnik et al. (2020)
	Raw milk	-	-	-8 to 0.3	-	Summer and Winter 2012 - 2014	-	
China	Milk Protein	-15.99 ± 0.50	4.55 ± 0.11	-	-	-	Assumed C-4 pastures	Luo et al. (2016)
	Milk Water	-	-	-13.06 ± 0.60	-	-	Assumed C-4 pastures	
Australia and New Zealand	Casein	-25.94 to -10.22	5.2 to 7.26	-	7.7 to 14.83	Mid-late Autumn	Varying levels (18-92%) of C_4 grasses	Crittenden et al. (2007)
	Milk Protein	-28.46 ± 0.70	5.8 ± 0.16	-	-	-	Assumed C-4 pastures	Luo et al. (2016)
	Milk Water	-	-	-11.1 ± 0.36	-	-	Assumed C-4 pastures	
New Zealand	Bulk Milk Powder	-30.14 ± 0.58	5.93 ± 0.53	-	-	Representing Spring milk	Predominantly pasture	Ehtesham et al. (2013)

Malaysia	Whole milk	-19.81 to -9.09	2.17 to 6.61	-	-	-	Assumed C-4 pastures	Behkami, Gholami, Gholami, & Roohparvar (2020)
United States of America	Milk Protein	-21.16 ± 0.11	4.65 ± 0.07	-	-	-	Assumed C-4 pastures	Luo et al. (2016)
	Milk water	-	-	-22.4 ± 2.3	-	-	Assumed C-4 pastures	
	Whole Milk	-19.19 ± 1.1	5.09 ± 0.41	-	-	-	-	Bostic, Hagopian, & Jahren (2018)
	Milk Water	-	-	-13 to -3.6	-	-	-	Chesson, Valenzuela, O'Grady, Cerling, & Ehleringer (2010)
Brazil	Whole milk	-15.9	5.4	-	-	2015-2019	-	Martinelli et al. (2020)
	Milk Powder	-16.5	5.7	-	-	2015-2019	-	

Table 1 Summary outputs of multivariate repeated measures regression models for the C, N, O and S stable isotope composition of raw milk. Values in bold are significant.

Type III Tests of Fixed Effects for
Carbon

Effect	Num DF	Den DF	F Value	Pr > F
Feed intensity	3	44	19.56	**<.0001**
Sampling Time	4	183	19.88	**<.0001**
(Feed intensity)x(Sampling Time)	12	183	2.94	**0.0009**
Latitude	1	44	0.01	0.9237
Longitude	1	44	0.01	0.9256

Type III Tests of Fixed Effects for
Nitrogen

Effect	Num DF	Den DF	F Value	Pr > F
Feed intensity	3	44	3.5	**0.0231**
Sampling Time	4	44	2.75	**0.0399**
(Feed intensity)x(Sampling Time)	12	44	3.07	**0.0033**
Latitude	1	44	0.03	0.8624
Longitude	1	44	0.06	0.8149

Type III Tests of Fixed Effects for
Oxygen

Effect	Num DF	Den DF	F Value	Pr > F
Feed intensity	3	44	2.32	0.088
Sampling Time	4	44	249.92	**<.0001**
(Feed intensity)x(Sampling Time)	12	44	1.69	0.1011
Latitude	1	44	54.16	**<.0001**
Longitude	1	44	5.56	**0.0229**

Type III Tests of Fixed Effects for
Sulphur

Effect	Num DF	Den DF	F Value	Pr > F
Feed intensity	3	44	4.2	**0.0107**
Sampling Time	4	44	7.45	**0.0001**
(Feed intensity)x(Sampling Time)	12	44	0.92	0.5344
Latitude	1	44	11.58	**0.0014**
Longitude	1	44	4.44	**0.0407**

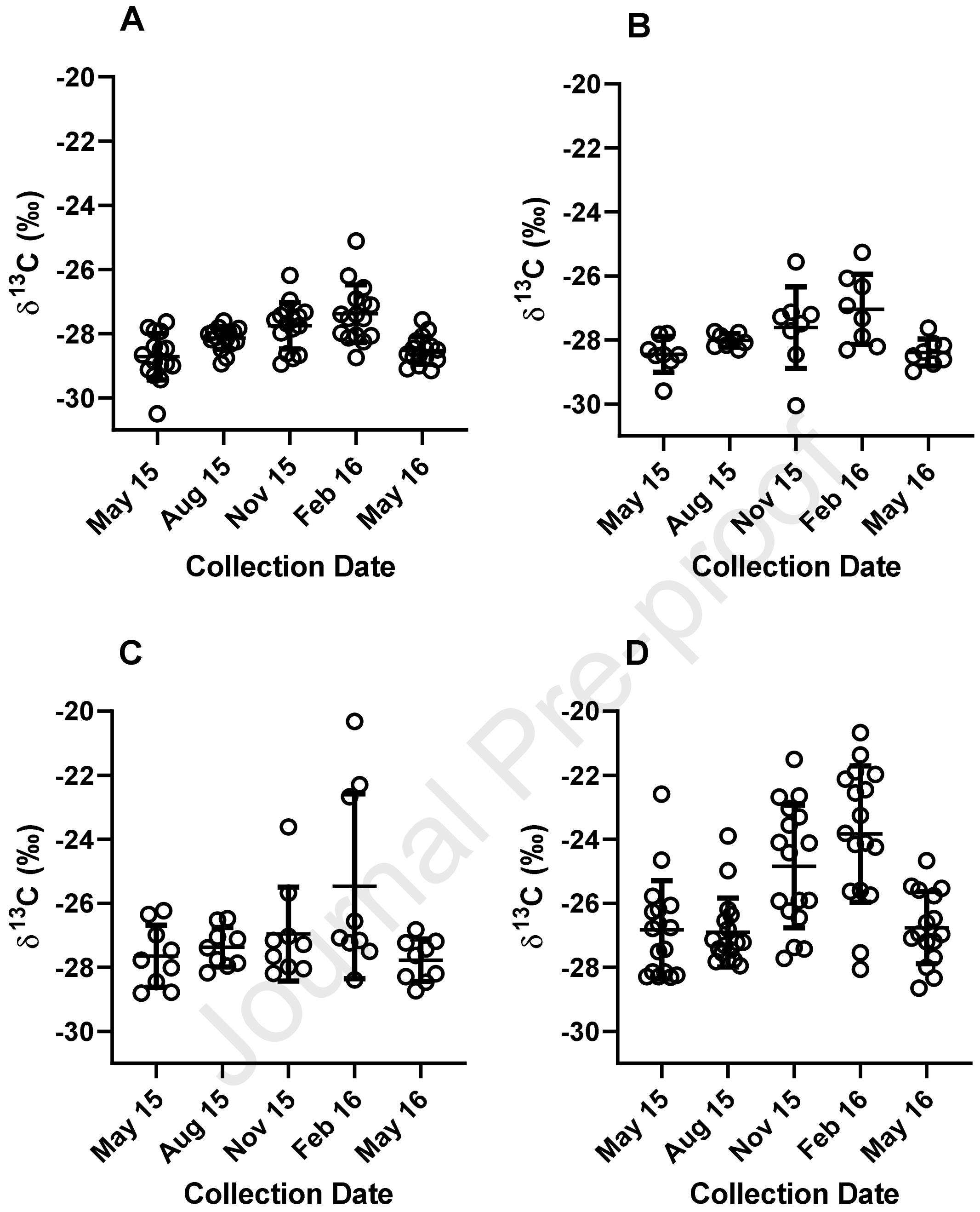

A
B
C
D
-20
-22
-24
-26
-28
-30
$\delta^{13}C$ (‰)
May 15
Aug 15
Nov 15
Feb 16
May 16
Collection Date

Fig. 1

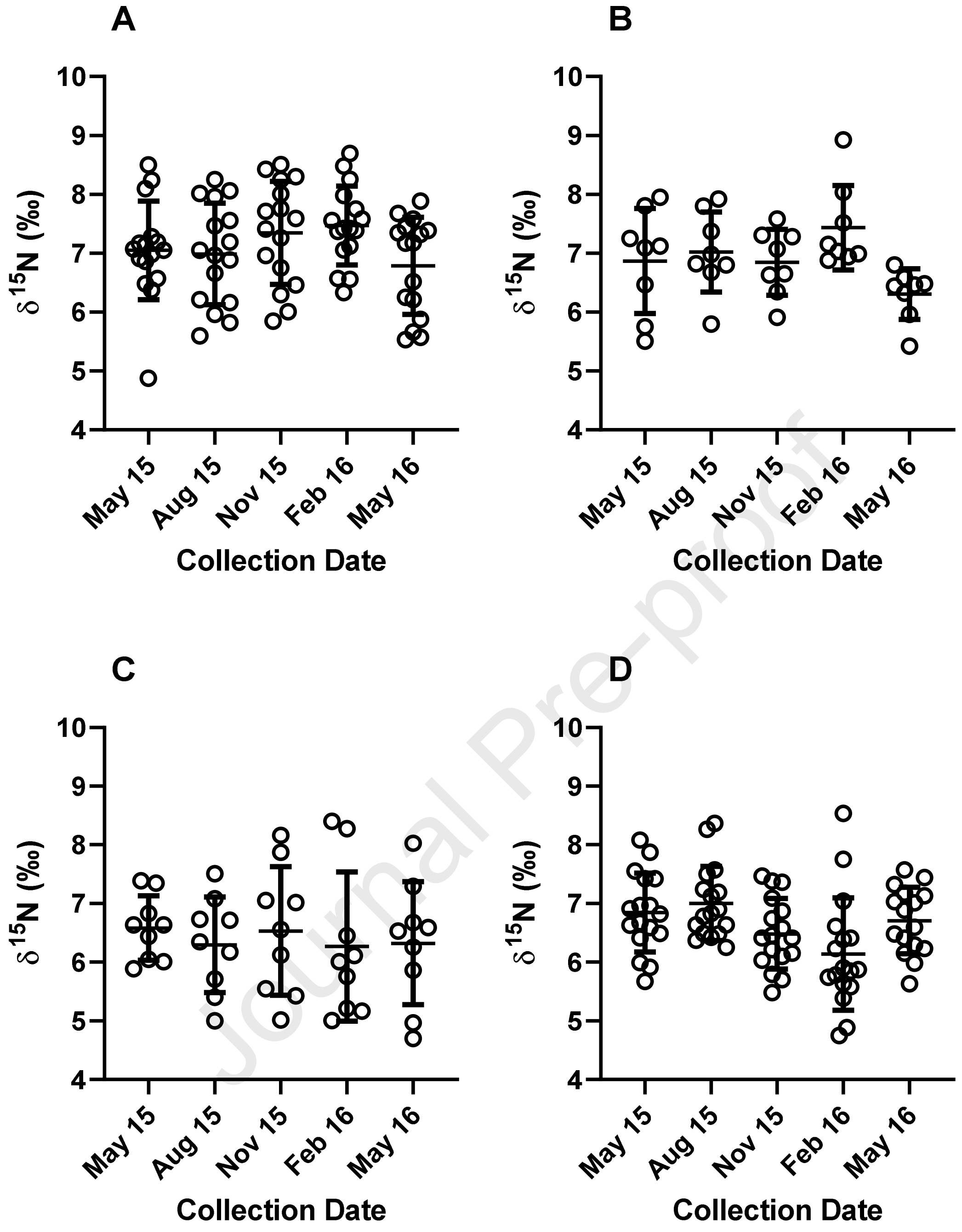

A
B
C
D
10
9
8
7
6
5
4
δ 15N (‰)
May 15
Aug 15
Nov 15
Feb 16
May 16
Collection Date

Fig. 2

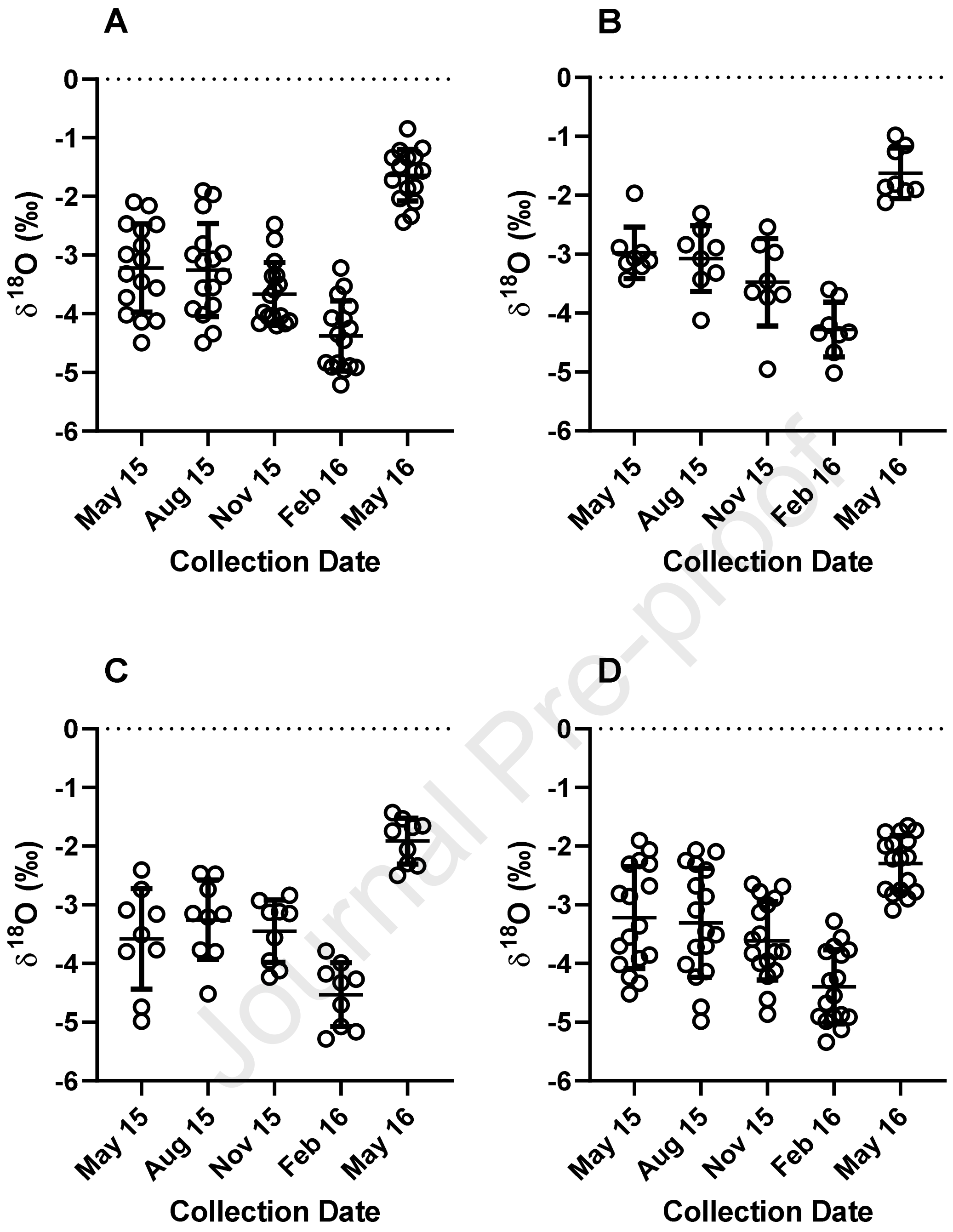

A
0
-1
-2
-3
-4
-5
-6
δ18O (‰)
May 15
Aug 15
Nov 15
Feb 16
May 16
Collection Date
B
0
-1
-2
-3
-4
-5
-6
δ18O (‰)
May 15
Aug 15
Nov 15
Feb 16
May 16
Collection Date
C
0
-1
-2
-3
-4
-5
-6
δ18O (‰)
May 15
Aug 15
Nov 15
Feb 16
May 16
Collection Date
D
0
-1
-2
-3
-4
-5
-6
δ18O (‰)
May 15
Aug 15
Nov 15
Feb 16
May 16
Collection Date

Fig. 3

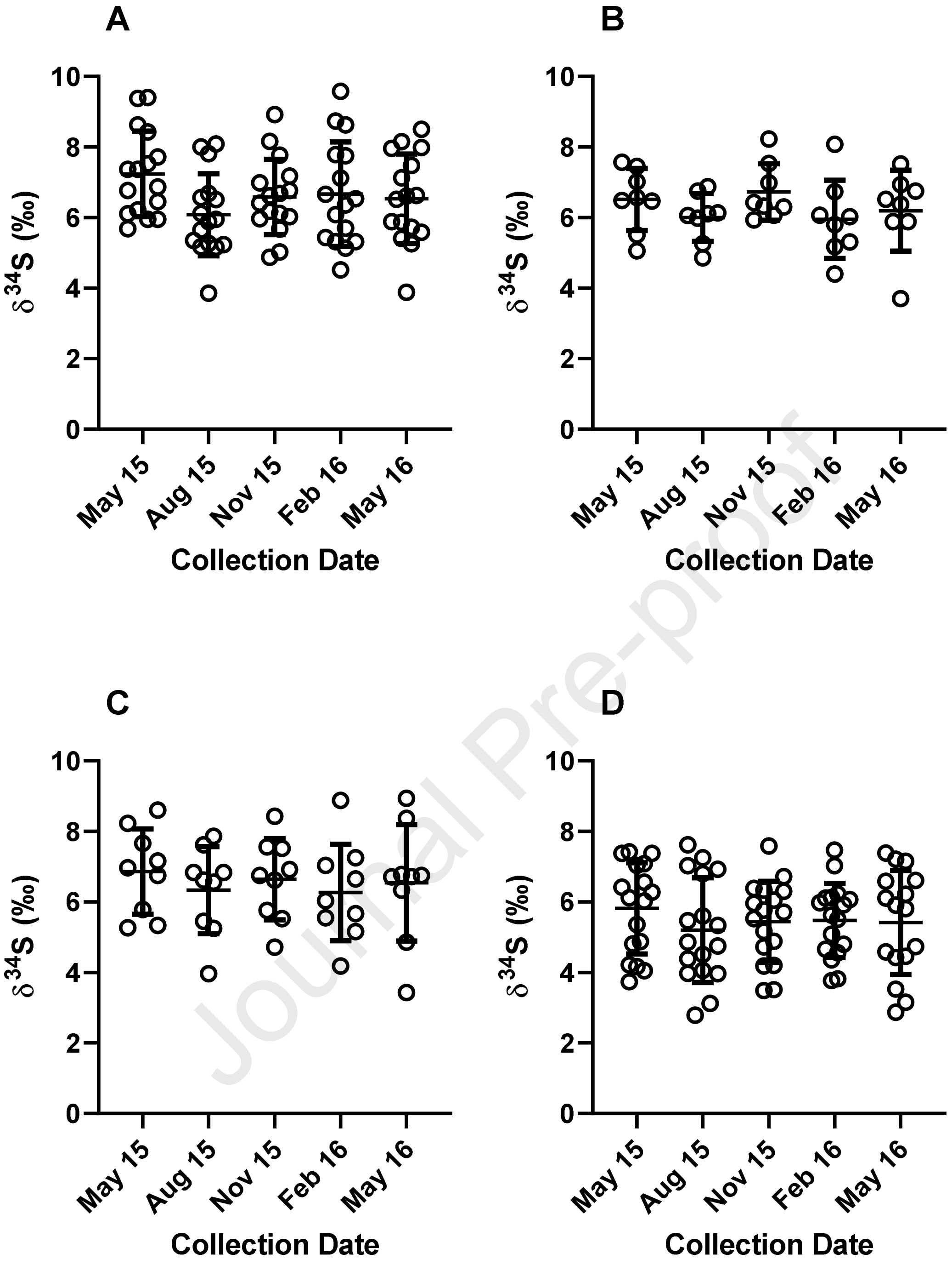
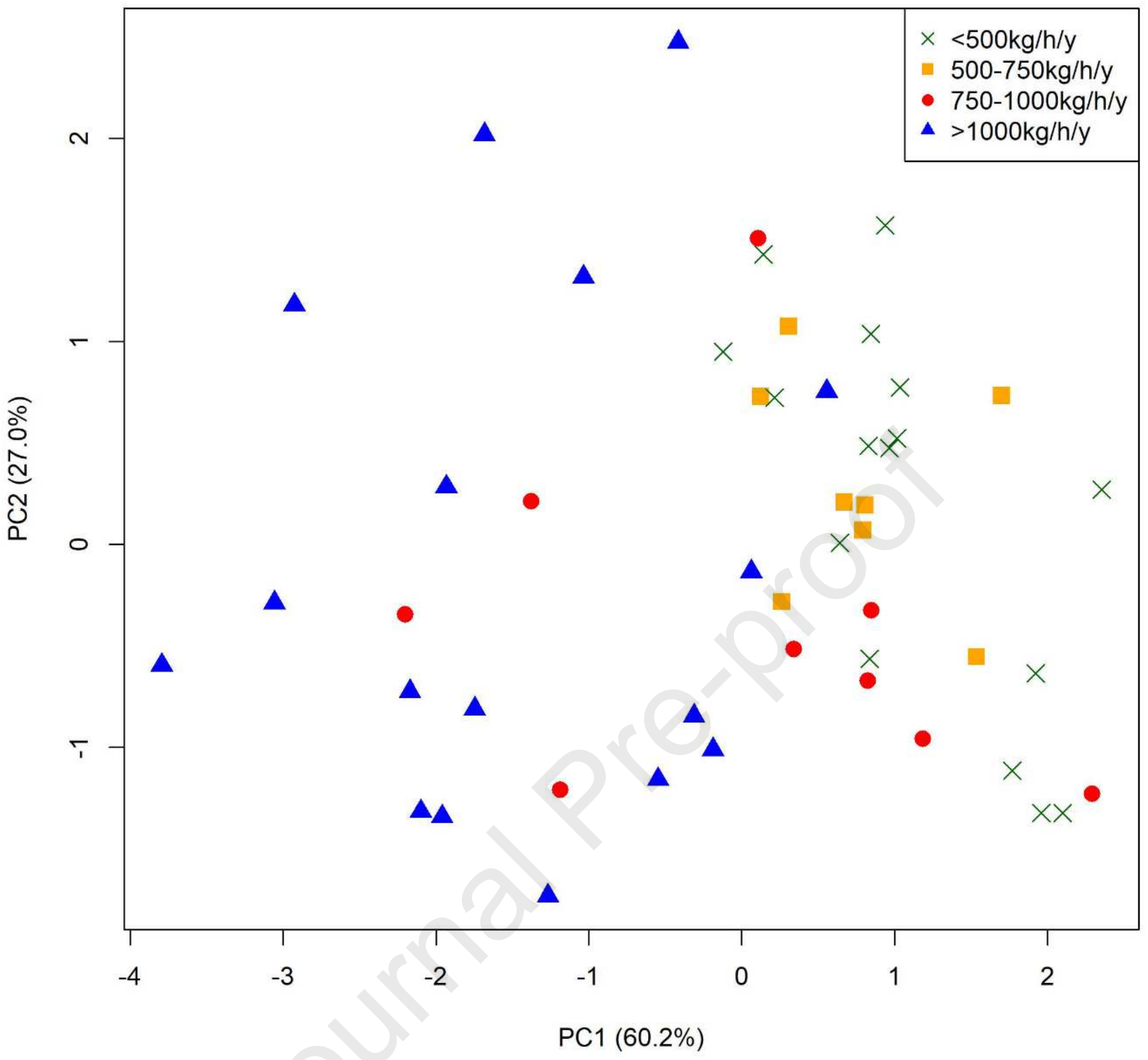

A
B
C
D
δ^{34}S (‰)
0
2
4
6
8
10
May 15
Aug 15
Nov 15
Feb 16
May 16
Collection Date

Fig. 4



× <500kg/h/y
■ 500-750kg/h/y
● 750-1000kg/h/y
▲ >1000kg/h/y
PC2 (27.0%)
2
1
0
-1
-4
-3
-2
-1
0
1
2
PC1 (60.2%)

Fig. 5

HIGHLIGHTS

- Stable isotope ratio analysis (C, N, O, S) was used to characterize Irish milk
- Isotopic values, particularly $\delta^{13}C$, reflected a high level of grass input
- Concentrate impact was clearest in spring, affecting $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values
- Isotopic values, particularly $\delta^{18}O$, were influenced by season
- Farm location (latitude and longitude) influenced $\delta^{18}O$ and $\delta^{34}S$ values

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.