The proteomic and isotopic analysis of parchment and their application to post-medieval sheep husbandry in Britain

Sean Doherty

PhD

University of York

Archaeology

April 2018

Abstract

The archives and libraries of Europe are full of dead animals. As the primary medium for writing for over 1000-years, tens of millions of durable parchment skins were manufactured, many of which survive to this day. While a few are illuminated Gospels or Royal Charters, the vast majority are mundane legal deeds concerning the ownership or right over property. When viewed simply as a textual resource, they are often considered to be of limited historic value and risk being deaccessioned or refused by archives. However, as a physical object they are an extraordinary high-resolution zooarchaeological and molecular archive, through which centuries of craft, trade and animal husbandry can be explored.

To explore the use of parchment as an isotopic resource, sheep, goat, pig and calfskin parchment was manufactured, identifying an impact from production on the measured values in fresh skin. The isotopic relationship between skin and bone was explored though paired samples to aid the integration of parchment results with bone collagen data; and a free-ranging diet study undertaken on a modern flock of sheep to examine the isotopic spacing between diet, bone, skin and parchment. These results were used to interpret data from 663 British legal deeds dating from the 12th to 20th century. Species identification via peptide mass fingerprinting indicted an almost exclusive use of sheepskin parchment throughout the medieval and post-medieval period. $\delta^{13}C$ and $\delta^{15}N$ isotope analysis revealed information on the use of domestic and potentially imported skins, as well as a likely preference for the skins of young lambs. Insight was also provided into the increasing geographical range livestock and skins moved across Britain facilitated by transport developments.

List of Contents

List of Tables

List of Figures

List of Equations

Acknowledgements

I am sincerely grateful to the following organisations for providing the funding which has made this research possible: The White Rose College of Arts & Humanities (WRoCAH, Arts & Humanities Research Council) (Award Ref: 1489527); The Natural Environment Research Council, Life Sciences Mass Spectrometry Facility, East Kilbride (NERC LSMSF) (Award Ref: EK259- 14/15); the Department of Archaeology, University of York; and The Society for Post-Medieval Archaeology.

For technical and administrative support at the BioArCh, University of York, I thank, Matt von Tersch, Keri Rowsell, Dr Sarah Fiddyment and Alizon Holland. For technical support at NERC LSMSF, I thank, Dr Jason Newton and Dr Rona McGill. For providing modern skins I thank, Dr Cluny Chapman, Jiří Vnouček, Jesse Meyer and Sue Hatton. For historic parchment samples, I thank, Tom Lord, Dave Lee, Ray Tye, and Richard and Pam Wills. I thank Christine McDonald (York Archaeological Trust), Guy Hunt and Matt Law (LP Archaeology) for granting destructive sampling of archaeological bone. I thank Jiří Vnouček (Royal Library of Copenhagen) for his invaluable assistance with parchment making, and aiding my understanding of skin processing.

Thanks also go to my friends and colleagues at BioArCh, for all the happiness they have brought to my life, and to my family for their unwavering support throughout. I am indebted to Prof. Matthew Collins, whose expertise, interdisciplinarity, and ideas have helped shape my research. I also thank the valuable comments and insight provided by my examiners Dr Richard Thomas and Dr Michelle Alexander.

Declaration

I declare that this is a presentation of original work and I am the sole author. This work has not been previously presented for an award at this, or any other, University. All sources are acknowledged as references. All chapters were written by myself, and the contribution of others is listed below. All chapters which are not explicitly mentioned were the sole work of myself.

Chapter 5: Examining the impact of parchment production on skin collagen stable $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ isotope values:

- Collagen extractions, sample preparation, data processing, interpretation and writing of the chapter were completed by SD.
- Experimental parchment production for Method 1 was undertaken by SD and Jiří Vnouček[1 2]. Parchment made using Method 2 was supplied by Jesse Meyer[3].
- Isotope analysis was carried out by SD and Dr Jason Newton[4].

Chapter 6: Examining the isotopic relationship between diet, bone, skin and parchment through a free-ranging diet study:

- Sample collection, collagen extractions, sample preparation, data processing, interpretation and writing of the chapter were completed by SD.
- Additional isotopic data of animal feed was supplied by Dr Isabella von Holstein[5].
- Bulk isotope analysis was carried out by SD, Dr Jason Newton and Matt von Tersch[1]

Chapter 7: $\delta^{13}C$ and $\delta^{15}N$ isotope analysis of post-medieval sheep bone collagen

- Sample collection, collagen extractions, sample preparation, data processing, interpretation and writing of the chapter were completed by SD.
- Additional collagen extractions were undertaken by Conor Jones (UG student), under SD's supervision.
- Bulk isotope analysis was carried out by Matt von Tersch.

Chapter 7: $\delta^{13}C$ and $\delta^{15}N$ isotope analysis of post-medieval sheepskin parchment deeds:

- Sample collection, collagen extractions, sample preparation, data processing, interpretation and writing of the chapter were completed by SD.
- Bulk isotope analysis was carried out by SD, Dr Jason Newton and Dr Rona McGill[4].

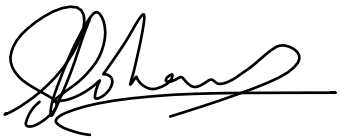
1 BioArCh, Department of Archaeology, University of York, York, UK

2 Department of Preservation, The Royal Library, Copenhagen, Denmark.

3 Pergamena, Montgomery, New York, USA

4 NERC Life Sciences Mass Spectrometry Facility, SUERC, East Kilbride, UK,

5 Department of Earth Sciences, Vrije Universiteit Amsterdam, Amsterdam, Netherlands



Sean Doherty 2018

Chapter 1

Research Framework and Aims

1.1 Research Context and Significance

Post-medieval parchment deeds are often considered to be of limited historic or scholarly value and risk being deaccessioned by archives and disposed of (National Archives 2015; Campagnolo *et al.* 2016; Giacometti *et al.* 2017). The same fate similarly befalls faunal material from this period (Thomas 2009; Broderick 2014; Rainsford *et al.* 2014). While digitisation may preserve the text, in a period of budgetary constraints for archival services, their physical value must be highlighted and the case made for their retention. Proteomic and stable isotope analysis offers the opportunity to highlight the value of this abundant but overlooked high-resolution zooarchaeological and molecular archive, providing archivists and conservators with greater information on their collections, and historians and archaeologists with the opportunity to explore the history of livestock husbandry, craft and trade.

Historians and archaeologists have often characterised post-medieval Britain (defined as post-1500 for the purposes of this thesis) as a country in 'revolution' (Deane 1965; Bagwell 1974; Overton 1996; Tarlow 2007). Through various new crops, field systems and farming techniques, the rural economy underwent significant transformations; changes which for some underpinned subsequent industrial and economic growth (Kerridge 1967). Historical enquiry into the pastoral economy has been hampered by the scarcity of livestock data, which is typically not of the chronological resolution required to explore this period of dramatic change. Substantial contributions have been made through zooarchaeological analysis, yet it too has been hindered by the often broad dating of post-medieval material (Thomas 2009; Morris 2014, 110). Analysis has indicated a size change during the 16th and 17th centuries, followed by a shape change in the 18th and 19th centuries as livestock became increasingly robust, with a lower bone-to-muscle ratio (O'Connor 1982; 1995; Albarella 1997; Davis & Beckett 1999; Thomas *et al.* 2013). However, a number of outstanding research questions remain, particularly the extent to which nutritional and land management changes influenced size and shape change? Stable isotope analysis of dated parchment deeds may therefore offer the possibility of addressing these questions which are not possible through traditional morphological analysis.

1.2 Proteomic and stable isotope analysis overview

Parchment, made from processed animal skin, is a collagenous material dominated by Type 1 collagen (Maxwell 2007, 22; Turner-Walker 2008, 5; Collins *et al.* 2002, 284; Covington 2009, 9, 23). Type 1 collagen, the protein focussed on here via peptide mass fingerprinting and stable isotope analysis, is constructed from two identical α 1 chains, and a third α 2 chain in a triple helix structure. This structure is facilitated by the repetitive amino acid sequence of Gly-X-Y, with glycine making up every third residue (Rich & Crick 1961). The X and Y position can be any other amino acid, although is often proline, hydroxyproline and lysine (Turner-Walker 2008). These macromolecules are organised into fibrils, which are then bound into fibres, and in turn into fibre bundles (Collins *et al.* 2002).

1.2.1 Stable isotope analysis

Stable isotope analysis is commonly applied to agricultural and archaeological material to reconstruct food webs, trophic relationships and farming practices (see Camin *et al.* 2007; Fisher & Thomas 2012; Madgwick *et al.* 2012). As the isotopic composition of tissue reflects that of the animal's diet (DeNiro & Epstein 1978; 1981), carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis of collagen from parchment offers the opportunity to examine change and continuity in animal diet, as well as important farming practices such as manuring, intensive folding, and the introduction of new crops (see Bogaard *et al.* 2007; Fraser *et al.* 2011; Szpak e*t al.* 2009; O'Leary 1988). Stable isotope analysis measures the relative abundance of 'light' and 'heavy' isotopes with material. Isotopes are atoms of an element which have the same number of protons but differ in the number of neutrons: $^{12}C/^{13}C$ and $^{14}N/^{15}N$ (Fry 2006, 4-11). For these elements, the lighter isotope is by far the most abundant (>98%) (de Hoffmann & Stroobant 2007, 252). Stable isotope ratios are expressed in δ-notation in units of per mille (‰) relative to international reference standards, of Vienna-Pee Dee Belemnite (VPDB) for carbon, and atmospheric nitrogen (AIR) for nitrogen. In the case of $\delta^{13}C$, for example, the following equation is used (Eq. 1):

$$\delta^{13}C = \left(\frac{^{12}C/^{13}C_{sample}}{^{12}C/^{13}C_{reference}} - 1\right) \times 10^3 \qquad \text{(Eq. 1)}$$

1.2.2 Peptide mass fingerprinting of COL1a1 using MALDI-ToF MS

Despite the potential of parchment deeds as an isotopic resource, uncertainty remains over the species used. Identification has traditionally been based upon the assessment of visual features, such as size, thickness, colour and follicle pattern (Saxl 1954; Ryder 1958; 1960a; 1960b; Poole & Reed 1962;

Ryan 1987; Clarkson 1992; Juchauld *et al.* 2010). Of these, identification using follicle patterning is the most common; however, not all parchment has an observable pattern (particularly post-medieval deeds), and requires considerable experience to make an accurate determination (see Ryder 1958; 1960a; 1983, 126-139; pers. comm. J. Vnouček[1]). Moreover, all of these features can be significantly altered during parchment manufacture and preparation, greatly affecting their reliability in being diagnostic. In the case of uterine vellum, for example, Fiddyment *et al.* (2015) demonstrated that visual identification can often be incorrect, with the majority of manuscripts classified as foetal calfskin in fact made from adult sheep and goatskin. Therefore, to ensure an accurate determination of species in order to better interpret the isotopic results from historic parchment, peptide mass fingerprinting will be used to identify the species used.

Like DNA-based method, proteomic analysis offers an absolute determination of the species used for parchment (Kirby *et al.* 2013; Teasdale *et al.* 2015; 2017; Fiddyment *et al.* 2015; *forthcoming*). Peptide mass fingerprinting uses enzymatic digestion to cleave the collagen into peptides, whose masses are measured by mass spectrometry. The amino acid sequence of collagen is genus-specific, and in some cases species-specific, and can therefore be used to determine the animal from which it derives (Buckley *et al.* 2009; 2010). While this has food authentication applications (Ortea *et al.* 2016), it is also useful for archaeological material where this is an absence of morphological markers (Welker *et al.* 2015a; 2015b; Fiddyment *et al.* 2015; Charlton e*t al.* 2016; Hendy *et al.* 2018). Identification via peptide mass fingerprinting is usually achieved by matching the mass spectrum from an unknown sample with mass spectrum from a known organism.

Using a protease such as trypsin, which cleaves after every lysine and arginine residue, a predictable pattern of peptides is produced, each with a variable mass (van Dorn 2012, 14-15). These peptides are analysed in this study using Matrix-assisted laser desorption ionisation – time of flight (MALDI-ToF) mass spectrometry. The digested samples are spotted onto a steel-plate and mixed with a matrix, before being ionised by a laser under vacuum. The matrix absorbs most of the energy and donates a positive charge in the form of a proton to the analyte. Once ionised, the peptides are guided towards

[1] Jiří Vnouček MA. is a renowned conservator and parchment maker, and a leading expert in the study of manuscripts. Jiří was the Head of Conservation at The National Library in Prague, CZ (1993-2005), and is currently a conservator at the Royal Library in Copenhagen, DK. He has worked as a consultant for many major libraries, including the Vatican Archives, and was part of an international team tasked with preserving archival material at the Iraq National Library and Archives in Bagdad, Iraq in 2004 and 2006. Jiří has been producing parchment for over 30 years, and has run courses at the Institut National du Patrimonine, Paris; The Bodleian Library, Oxford; and the J. Paul Getty Museum, Los Angeles. He is currently completing his PhD on the visual assessment of parchment at the Department of Archaeology, University of York, supervised by Prof. Matthew Collins and Dr Mary Garrison. An interview and profile of Jiří Vnouček's career can be found in Timecare Magazine (2013, 10-11).

a mass analyser, where they can be separated out by their mass-to-charge ratio (*m/z*) (van Dorn 2012, 8-18; 2014, 7998-8000). The mammalian species likely to be used for parchment (cattle, sheep, goat and deer; Ryder 1964) have highly similar collagen amino acid sequences, although a number of diagnostic peptides have been identified that make their separation possible (Buckley *et al.* 2009; 2010).

1.3 Aims

This PhD explores the potential for using parchment as an isotopic resource, and applied this to dated legal deeds (Figure 1.1) to provide a time sensitive analysis of sheep husbandry in a period of rapid agricultural development. This research has four primary aims:

1. To identify the species used for parchment legal deeds (Chapter 4)
2. To explore the use of parchment as an isotopic resource (Chapter 5)
3. To enable $\delta^{13}C$ and $\delta^{15}N$ isotope data from parchment to be integrated with those from bone collagen (Chapter 6)
4. To apply these methods to parchment and bone from post-medieval Britain to examine sheep husbandry and land management (Chapter 7 and 8).

These aims will be addressed through the analysis of 47 parchment skins manufactured by the author. The experimental manufacturing process used in this study was designed with, and supervised by master parchment maker Jiří Vnouček (Royal Library of Copenhagen), replicating late-medieval and post-medieval techniques detailed in historic recipes. A total of 41 sheepskins (*Ovis aries*) (Seaton Ross, E. Yorks., UK; Melton Mowbray, Leic., UK; Newark-on-Trent, Notts, UK; Prague, CZ Republic), 2 goatskins (*Capra aegagrus hircus*) (Prague, CZ), 2 calfskins (*Bos taurus*) (Vienna, AU) and 5 pigskins (*Sus scrofa domesticus*) (Beeston, Notts., UK) will be processed, and a further 5 finished parchment skins obtained from a commercial producer (Jesse Meyer, Pergamena, New York). These aims will also be addressed through the analysis of 26 paired bone and skin samples from modern livestock, and 663 historic legal deeds (Figure 1.2), obtained from the following private and public collections:

- Tye Collection (254 samples) – Collection of title deeds dating from AD 1650 to 1904, concerning the sale and lease of property in City of London. These documents were part of the Sun Fire Office insurance company archives, but were discarded and entered a private collection.

- Lee Collection (254 samples) – Artificial collections of various title deeds dating from 1499 to 1907, regarding the sale and lease of property across England and Wales.

- Lord Collection (50 samples) – Collection of title deeds dating from 1582 to 1893, concerning Lower Winskill Farm, Settle, North Yorkshire. This material has been continually held by the Foster-Lord family.

- Hull Collection (33 samples) – Artificial collection of various deeds dating from 1596 to 1969, discarded by Hull History Centre.

- Chester Collection (18 samples) – Artificial collection of various deeds dating from 1786 to 1813, held by Cheshire Records Office.

- Glamorgan Collection (18 samples) – Collection of deeds paleographically dated to the 13th century, held by Glamorgan Archives.

- Wills Collection (16 samples) – Collection of title deeds dating from 1652 to 1790, concerning the sale and lease of land in Somerset, held by the Wills family.

- Lincoln Collection (9 samples) – Artificial collection of various deeds dating from 1742 to 1913, held by Lincoln Records Office.

- 1 title deed from the City of Westminster dating to 1707, discarded by Westminster City Archives.

- A further 8 samples were obtained privately from mortgages from 1913 to 1940.

Bone collagen isotope data from the following sites will also be used to address these aims:

- The Bedern, York (15th; 18th-19th century)
- Hungate, York (15th to 20th century)
- Walmgate, York (18th-19th century)
- Prescott Street, London (16th-19th century)

Figure 1.1: 15th to 20th century parchment. A) Title deed concerning the ownership of land in Enfield, Middlesex, signed and sealed 15th January 1499; B) Mortgage deed between Athelstan H. Highton Esq., Rev. Thomas Moulton and Sir Edmond Browne on property in Bolton, Lancashire, signed and sealed 3rd February 1927 (photos by S. Doherty)

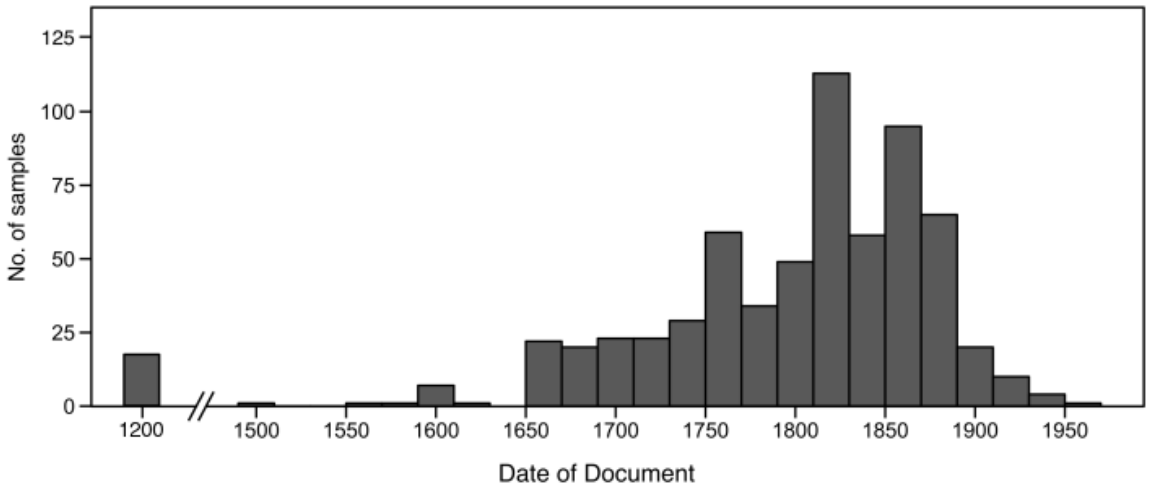


Figure 1.2: Distribution of historic parchment deeds analysed in this study

1.4 Thesis Structure

Chapter 2: Historical and zooarchaeological background to post-medieval sheep husbandry provides the context for the main proteomic and isotopic analysis, with particular focus on the nutritional and genetic 'improvement' of sheep. **Chapter 3: Background to sheepskin and parchment production** outlines the structure and biology of skin, and the methods of transforming the wet, perishable hide into a dry, durable sheet.

Chapter 4: Species identification of 13th to 20th century parchment deeds presents the analysis of 663 parchment deeds using peptide mas fingerprinting via MALDI-ToF analysis. Species identification of parchment is commonly made through visual assessment of features and follicles on the surface of the skin. Accurate determination can be challenging however, due to the high degree of similarity between species, and the removal or distortion of diagnostic features during manufacture. Consequently, the species used for parchment deeds is often unknown. In this chapter an absolute determination is made through proteomic analysis, which facilitates enquiry into the provisioning of skins through the analysis of 18th and 19th century stationer's accounts.

Chapter 5: Examining the impact of parchment production on skin collagen stable δ^{13}C and δ^{15}N isotope values explores the impact of manufacture on measured values. Despite previous published stable isotope analyses of parchment (23 manuscripts), the effect of structural and chemical modifications during production has not been assessed. To address this, this chapter presents the isotopic analysis of 52 paired skin and parchment samples to explore the use of parchment as an isotopic resource.

Chapter 6: Examining the isotopic relationship between diet, bone, skin and parchment through a free-ranging diet study explores isotopic spacing in a modern flock. Due to variations in tissue composition, routing of amino acids, and rate of growth, different tissues within the same individual often display different isotopic signatures. As ecological studies focus on non-invasive sampling and agricultural studies on muscle, much of our current knowledge on the isotopic relationship between bone and skin collagen δ^{13}C and δ^{15}N values comes from archaeological human remains. Due to the potential for unknown cultural factors to influence this spacing, such as abrupt changes in location or diet before death, a free-ranging diet study was undertaken on a modern flock grazing on a restricted diet and area. The results of this study aid the integration of parchment data with archaeological bone data.

Chapter 7: δ^{13}C and δ^{15}N isotope analysis of post-medieval sheep bone collagen details the isotopic analysis of faunal remains from 15th to 20th century York and London. These are synthesised

with other published results from the UK to explore temporal trends in $\delta^{13}C$ and $\delta^{15}N$ values. The results from York and London are integrated with zooarchaeological evidence to explore nutritional improvement as a driver behind conformational improvement.

Chapter 8: $\delta^{13}C$ and $\delta^{15}N$ isotope analysis of post-medieval sheepskin parchment deeds applies the information gained from the study of modern skins to 663 historic manuscripts. The results are integrated with historical records, and enable a discussion on the use of domestic and imported skin; the impact of transport improvements; and change in farming practice.

Chapter 10: Conclusion, summaries the outcomes of this study and discusses the major findings, before proposing future avenues of research.

1.5 Appendix

Table A.1 presents the full $\delta^{13}C$, $\delta^{15}N$, and elemental composition data for modern bone, skin and parchment samples for Chapter 5. **Table A.2** presents the proteomic identification of historic parchment samples for Chapter 4, and full $\delta^{13}C$, $\delta^{15}N$, and elemental composition data of all historic deeds for Chapter 8. **Table A.3** presents and full $\delta^{13}C$, $\delta^{15}N$, and elemental composition data for post-medieval sheep bones analysed in Chapter 7.

Chapter 2

Historical and zooarchaeological background to post-medieval sheep husbandry

This chapter provides a historical and zooarchaeological background to sheep husbandry in post-medieval Britain. The role of sheep in 16th to 19th century farming systems, and the impact of institutional and technological innovations will be detailed (2.2), before focussing on their nutritional (2.2.1) and genetic (2.2.2) improvement. Evidence for the 'improvement' of sheep using faunal remains will then be presented (2.4).

2.1 Introduction

Sheep have long played a central role in the British economy, first through the export of raw wool and finished textiles, which for centuries were the largest and most valuable exports (Bowden 1962; Carus-Wilson & Coleman 1963; Stephenson 1988) and later by the meat and manure that helped fuel an increasingly urbanised and industrialised population (Trow-Smith 1957; 1959; Clark 1993). At the turn of the 16th century, the country remained economically and socially "rooted in the land" (Campbell 2008, 3). Over 90% of the population lived in rural communities, earning a living from agricultural work, or engaged in proto-industrial activities utilising the skins, fibres, oil, fuel and food generated by this sector (Allen 1992; A'Hern 2004, 1). Although initially poorer than many of its continental neighbours, over the subsequent three centuries, the British economy displayed unprecedented growth, enabling it to catch and ultimately surpass all others to become the premier industrial, financial and imperial force of the 19th century (Broadberry *et al.* 2015, 371-383). The population transformed too, as urban centres grew and new industrial towns were established, and the islands inhabitants boomed from 2.8 million in 1550 to 5.2 million in 1700, before doubling to 10.6 million in 1800 and again to 20.6 million by 1850 (Wrigley 2004; Broadberry *et al.* 2015).

The demographic, industrial and agricultural developments of the period were intimately linked (Williamson 2013, 74-5). More reliable food supplies reduced the risk of famine and nutritional checks upon population growth, and diversification of the economy encouraged earlier marriage,

increasing fertility rates (2013, 75), resulting in a four-fold increase in population. Along with the bourgeoning industrial sector, this created greater demand for food and raw material, stimulating both agricultural and industrial development (Grigg 1980, 163-189; Williamson 2013, 75). The agricultural sector responded with increased productivity and output, more intensive cultivation, more productive livestock, and increased specialisation (Overton 1996, 8). Between 1550 and 1850, when the population grew from 3 to 20 million, cropped land increased by 150%; grain yields by 320%, sheep numbers by 170%, and mutton and wool yields all growing by more than 500% (Broadberry *et al.* 2015, 80-124), making British agriculture and its workers the most productive in Europe (Allen 2000, 21).

For some, this growth in output and productivity amounted to an 'Agricultural Revolution', often closely associated with contemporary 'industrial', 'transportation' and 'financial' revolutions (Toynbee 1884, Ernle 1912; Deane 1965; Kerridge 1967; Moykr 1985, 21; Overton 1996). More recently, however, the revolutionary nature of agricultural developments has been widely dismissed and the slow, gradual pace of improvements in practice has been highlighted through available historical records and zooarchaeological remains (i.e. Allen 1992; Clark 1993; Turner *et al.* 2001; Thomas 2005a; Thomas 2005b; Thomas *et al.* 2013). Most agree that the growth in output was the consequence of major institutional and technological innovations, particularly:

- Enclosure of open fields and commons, and the amalgamation of land into larger farms
- Adoption of new crops and field rotation systems
- New types of livestock
- Increased cultivation of forage and fodder crops, enabling a greater number of livestock to be supported
- Increased use of soil conditioner and fertilisers
- Increased mechanisation to support agriculture

Sheep were a central pillar of pastoral and arable farming systems, and closely associated with these changes. These will now be discussed with particular focus on their impact on the nutritional and genetic improvement of sheep.

2.2 Sheep in post-medieval farming

The landscape of open field agriculture and commons was not ubiquitous, but was wide-spread, and was the characteristic farming system of central England, from East Yorkshire to Dorset, and parts of Norfolk, Suffolk and Cambridgeshire (Rackham 1986, 2-5; Hall 2014, 72). Either side of this primary zone were areas where, often due to soil type and terrain, open fields had never existed, or

had disappeared by the 16th century (Smith 2012, 1); such as the uplands of the Welsh Marches, Pennines and Lakes, or the Essex Marshes where other farming systems prevailed (Thirsk 1967a). In its classic form, the parish land was divided into large fields designated as: arable for the cultivation of crops; meadow to grow hay for winter fodder; and pasture and wastes for permanent grazing. Villagers owned or rented dis-continuous strips, intermingled and undivided with those of their neighbours, requiring a high degree of cooperation in cultivation and management. The land was traditionally managed using a three- to five-field rotation system, with each field seasonally moving between a winter crop (wheat, rye or barley), spring crop (beans, peas, oats or barley) and a fallow, although they could be much more complex (Havinden 1961). Livestock communally grazed on common pastures, wastes and fallows, and were often turned out onto the stubble after harvest (Trow-Smith 1957, 158; Ault 1972; Overton 1996, 25), although access to this was often closely controlled (Clark 1993, 250).

The above description is an oversimplification of a system that existed in some areas for close to a thousand years, and one that developed to meet local needs and environmental conditions (Baker & Butlin 1973; Oosthuizen 2005; Hall 2014). What is clear, is the importance of livestock, particularly sheep, in maintaining soil fertility. In most agrarian systems, a paucity of nitrogen in the soil is the major limiting factor in plant growth due to the large amount removed at harvest (Touraine *et al.* 2001, 1). In the open field system, the main method of reducing nutrient depletion and returning nitrogen to the land was leaving a field fallow every third year and manuring. Fallowing performed three crucial restorative functions. First, it provided a 'cleaning' period where weeds could be removed by pasturing animals on bare land, or through repeated ploughing. Second, the earth was manured by the sheep folded onto it. To more effectively deposit and distribute the manure, folding, "a pillar of the farming system" (Thirsk 1984, 228), was used, where sheep grazed on pasture during the day and then driven onto fallows at night allowing their manure and urine to be spread and trodden into the earth before cultivation. Thirdly, mineralised nitrogen could accumulate and be available for subsequent crops (Allen 2008).

The open field system worked well in a period of a smaller population and limited levels of market specialisation, and it is clear that the rigidity and unproductiveness of the commons has previously been overstated (see Havinden 1961; Allen 1992, 133-7). However, communal rights were increasingly challenged from the 16th century as attitudes towards private property changed and as the permanent conversion of arable land to pasture became economically appealing as wool and meat prices continued to rise (Overton 1996; Blomley 2007, 2). Enclosure enacted by local lords, piecemeal accumulation or Act of Parliament (Mingay 1997, 7), removed communal rights and transferred control and ownership of the land into 'severalty' were owners had sole control over its use and access, marking a fundamental shift in an ancient way of life. Practically, it meant an end to

strip farming, common pasture and rights over wastes. Open fields were replaced with smaller fields bounded by hedgerow and stone walls.

The consensus had long been that the increase in agricultural output from the 16th century onwards was due to enclosure, as a sector dependent on "open field farmer and commoners, could never have fed a manufacturing population" (Ernle 1912, 351-2). Such views are no longer mainstream, but for many enclosure enabled greater experimentation, diversification and capital investment (Clark 1993; Overton 1996). Alternative field rotations were introduced which replaced bare fallows with ley grasses and root vegetables, dramatically increasing the crops available for livestock. These leys primarily featured small-seeded forage legumes such as clover, trefoils, sainfoin and lucerne (Turner *et al.* 2001). The actinorhizal plants increased the nutrient supply due to fixing their nitrogen from the atmosphere and not depleting the reserves in the soil. They provided a valuable livestock feed which would simultaneous manure the land, incorporating atmospheric nitrogen into the soil. The seeds of these plants were imported from the low countries by the 1620s, and were recommended to improving farmers in many mid-17th century farming manuals (Blith 1649; Weston 1650; Hartlib 1652). Turner *et al.* (2001) examined 979 farm accounts from England between 1700-1914, detailing cropping and husbandry patterns from across the country. Their analysis highlights the rapid spread of ley grasses, with 71% of sample farms growing clover by the early-18th century (Turner e*t al.* 2001, 72-3) (Figure 2.1).

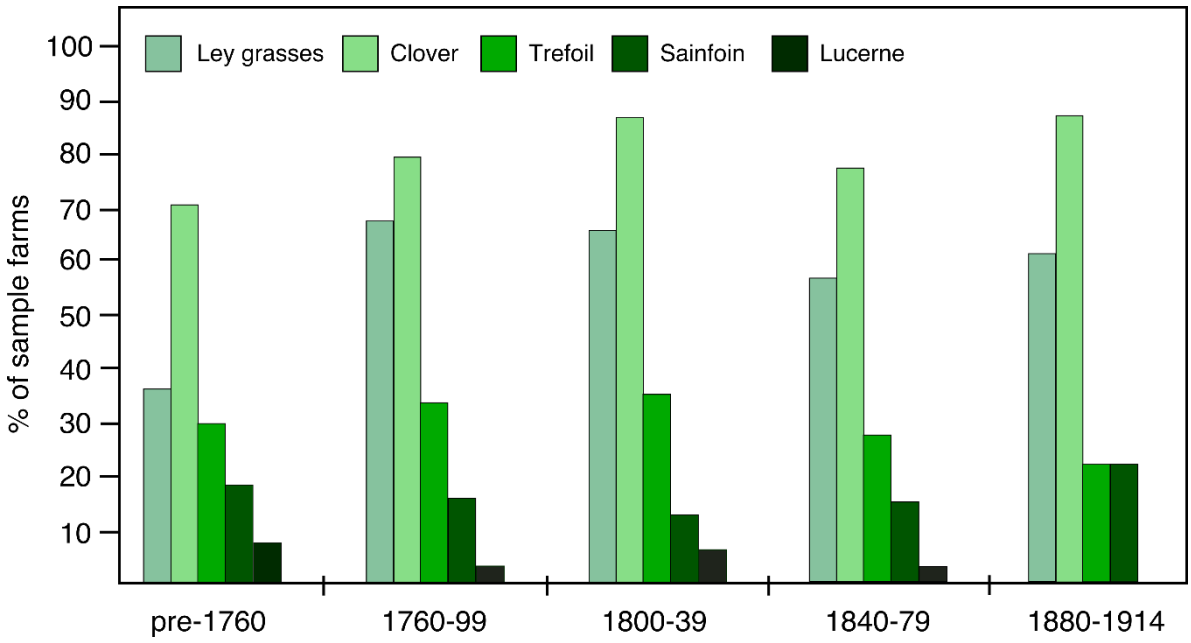


Figure 2.1: Temporary grasses and forage legumes grown in English farms, 1700-1914 (after Turner *et al.* 2001, 71) (Total of 979 farms)

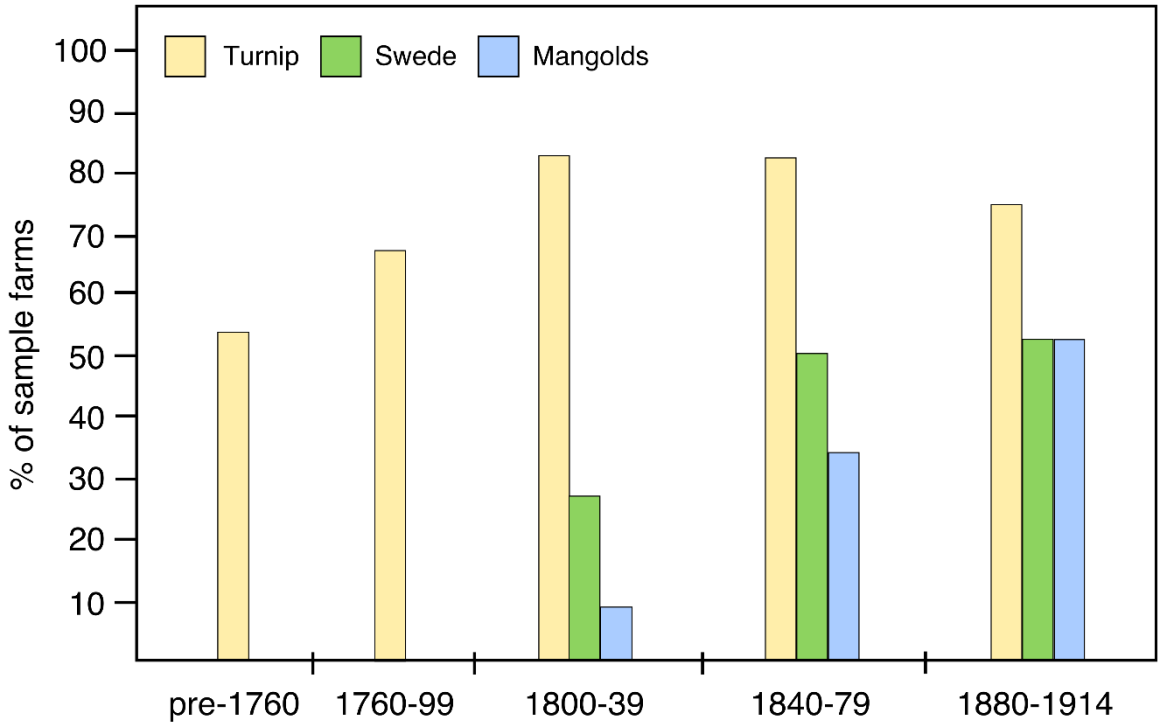


Figure 2.2: Root crops grown on English farms, 1700-1915 (after Turner *et al.* 2001, 73)
(Total of 979 farms)

The restorative function of land under grass had likely been known since the middle ages (Clark 1993, 250), but a practice that emerged between 1590 and 1660 (Kerridge 1967; Overton 1996, 117) was convertible husbandry, in which land which had been permanent pasture for decades was ploughed up and cropped with cereals for a few years, before again reverting to pasture. The store of nitrogen which had developed under pasture would result in a dramatic, if short-term increase in yields, but was likely to have returned to previous levels within a few years as nutrients were depleted. By the late-17th century, its popularity had declined (Overton 1996, 117), likely due to diminishing returns and the increased sowing of forage legumes (Allen 2008, 16-17).

Perhaps the most famous farming system of this period was the Norfolk four-course rotation of wheat ➞ root vegetables ➞ barley or oats ➞ clover and grass leys. Named after its likely place of origin, the major innovation of this system was the introduction of a 'cropped fallow', chiefly turnip, swede and mangolds. Following its introduction from the Low Countries in the late-16th century (Chambers and Mingay 1966, 56-60), many regard the turnip as having a revolutionary effect on British agriculture (Timmer 1969; Kerridge 1961; 1967, 28-29; Williamson 1998; Allen 2008) (Figure 2.2). Through the adoption of the Norfolk four-course, or a modified system more suited to local conditions (Kerridge 1967, 29), farmers could virtually eliminate uncropped land, which was both inefficient and unprofitable, and replace it with a cash or feed crop. By the mid-18th century, over half of all farms were cultivating turnips, which were later supplemented by swedes and mangolds

which provided vital feed later in winter and were more suitable than turnips to heavier soils (Turner *et al.* 2001, 72). Within a system such as this, the total acreage devoted to wheat declined, in favour of feed for livestock. However, by increasing the quantity of forage and fodder crops, more livestock could be maintained and overwintered, producing more manure, which in turn increased soil fertility and ultimately yields. The increase in fodder crops intensified the folding of sheep, as they could be driven directly onto a field of root vegetables and would uproot the plant themselves, with no need for harvest (Loudon 1831, 859). On the other hand, the turnips could be moved to a yard and the sheep stall fed to fatten them quickly (1835, 859).

To provide a dense and even covering, agriculturalists recommended that sheep were kept in temporary one-acre folds of at extreme densities of between 500 to 3,200 sheep per acre per night (Young 1770, 328; Home 1830, 251; Ellman 1831, 110). The density depended on the type of sheep, with the larger Lincoln and New Leicester often considered unsuited to folding (Billingsley 1795, 146; Youatt 1837; Tanner 1859, 63; Copus 1989, 38), and careful management required to ensure young or ill animals were not included or injured. These cramped and dirty conditions often downgraded the quality of the wool and the animal's rate of fattening, as well as increasing the risk of 'pelt rot', sheep scab and foot root (Lawrence 1809, 272). With the introduction of the turnip, and later oil cakes, the density of folds increased as more food allowed for more sheep, producing more manure (Philpotts 1863, 22; Ryder 1983, 675). Intensive folding had its critics, most notably Robert Bakewell who is reported to have considered keeping sheep in densities greater than 100 per acre 'a barbarous practice' (Lawrence 1809, 271). Folding could be detrimental to weaker sheep, and the daily driving between fields was not conducive to the development of fat (1809, 271). However, for Young (1809), the fact that folding kept the sheep lean was part of its continuing importance, especially considering the markets demands for leaner meat in the 19th century. Despite these recommendations in agricultural handbooks, folding sheep in such numbers was unrealistic for the majority of shepherds, with the average flock size post 16th century thought to be around 150 sheep (Ryder 1983, 477). It is clear, as Trow-Smith (1957, 247) acknowledged, that for much of this period that the sheep's wool, milk and "gift of fertility" were values more than its meat.

Soil fertility and nutrient availability was further improved through the increasing use of soil conditioners and fertilisers. A diverse range of organic fertilisers had been used for centuries, including human waste, seaweed and various animal products (Markham 1631; Blith 1649; Hartlib 1652), but their use significantly increased in the mid-19th century as agriculture entered a golden age of 'High Farming' (Collins 1995; Williamson 2002, 170-1) (Figure 2.3). Great effort was taken to conserve on farm fertilisers, and were supplemented with the highly nitrogenous Peruvian seabird guano (Nesbit 1852), and the use of artificial fertilisers such as superphosphate and nitrates of soda (Turner *et al.* 2001, 87) (Figure 2.4).

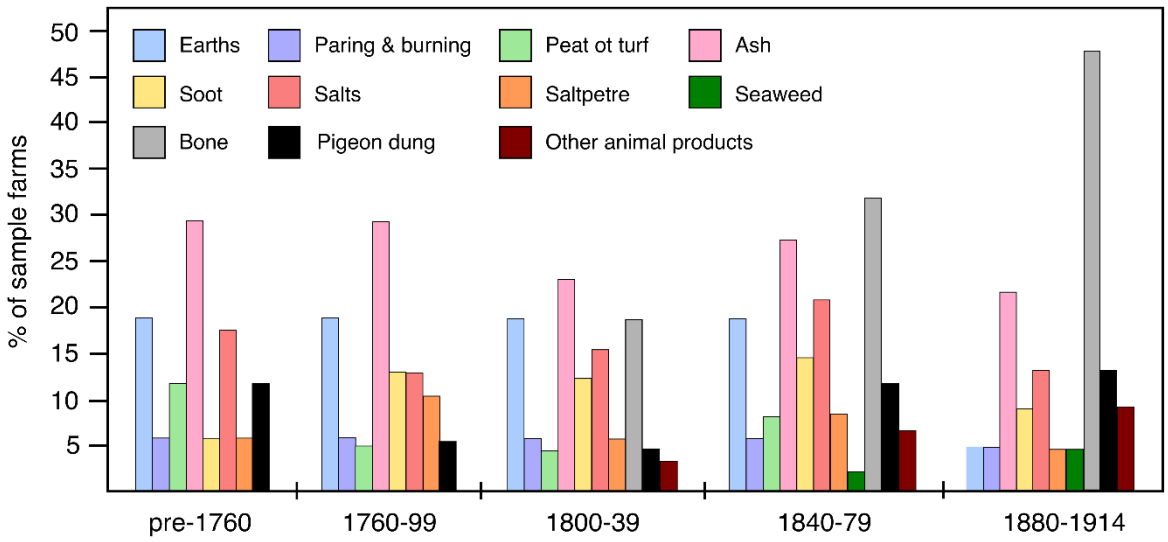


Figure 2.3: Traditional manures and fertilisers used on English farms, AD 1700-1914 (after Turner *et al.* 2001, 84) (Total of 979 farms)

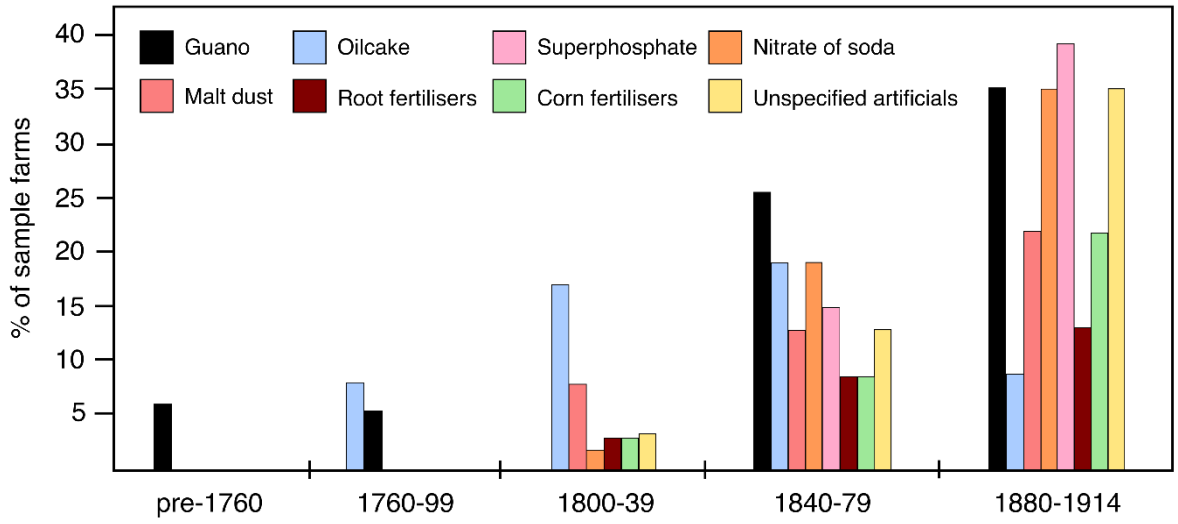


Figure 2.4: New manures and artificial fertilisers used on English farms, AD 1700-1914 (after Turner *et al.* 2001, 87) (Total of 979 farms)

2.2.1 Nutritional improvement

In his 1516 'Utopia', the later Lord Chancellor Sir Thomas More remarked that sheep

> "*once so meek and tame, and so small eaters …* [have] *become so great devourers … they consume, destroy and devour whole fields … and swallow down the very men themselves"* (More 1516 in Latin, translated in Craik 1916)

While More is referring to the depopulation wrought by enclosure, his writings draw upon the nutritional improvement brought by the adaption of field systems in the post-medieval period. Despite debates around the timing, nature and revolutionary qualities of agricultural developments, all agricultural historians agree on the central importance of increased variety and quantity of forage and fodder crops, and its role in increasing grain yields (see Kerridge 1967, 311; Ryder 1983, 495-6; Overton 1996, 111-21; Williamson 1998; Turner *et al.* 2001, 222). For Kerridge (1967, 311), *all* farming innovations from the 16th century onwards were designed first and foremost to increase the availability of crops for livestock. Their importance lay in the ability to support a greater number of animals, increasing the quantity of manure, thus raising arable yields. However, this increased nutritional intake also had a profound effect on the animal itself, improving aspects such as: carcass development, fertility rates, overall animal health, but also reducing the fineness of the fleece (Trow-Smith 1959, 36-41; Turner *et al.* 2001, 181-193).

By the 16th century, English shepherds were losing their pre-eminence as producers of fine wool, with cloth manufacturers turning to Merino wool from Spain for their finest items (Trow-Smith 1959, 9; Bowden 1962). At a higher plant of nutrition, the rate of wool growth increases, as does the length and diameter of the fibre, resulting in a heavier and coarser fleece (Henderson 1959, 74). Historical data on fleece weights is patchy, but indicates a trend for increasingly heavier and coarser fleeces through the post-medieval period (Fussell & Goodman 1930). In his review of wool exports, Chalmber (1782, 56) unquestionably links this increase in wool yields with enclosure, suggesting that a Hereford sheep would produce a fleece of ~2 lb in an open field, but ~9 lb when enclosed. While this must be viewed with some scepticism, it highlights the perception of the period that enclosure significantly increased wool yields, although potentially lowering quality.

Mutton yields also increased from the 16th century, with the recession in the wool trade and a growing population diverting breeder's attention towards meat (Broadberry *et al.* 2015, 105-9). Historical carcass weights are limited, restricted to post-1700, and not always comparable due to variations in age, sex and breed, but they suggest a gradual increase in the weight of 'sheep' at slaughter across the 18th and 19th centuries (Turner *et al.* 2001). More striking, is the increase in the weight of 'lambs'

across the same period, which almost doubled between 1800 and 1900, highlighting the increased rate of maturity (Figure 2.5). Some 'lambs' from the late-19th century are comparable in weight to 'sheep' from the mid-18th century. This suggests that the onset of early maturing sheep may have been quite late.

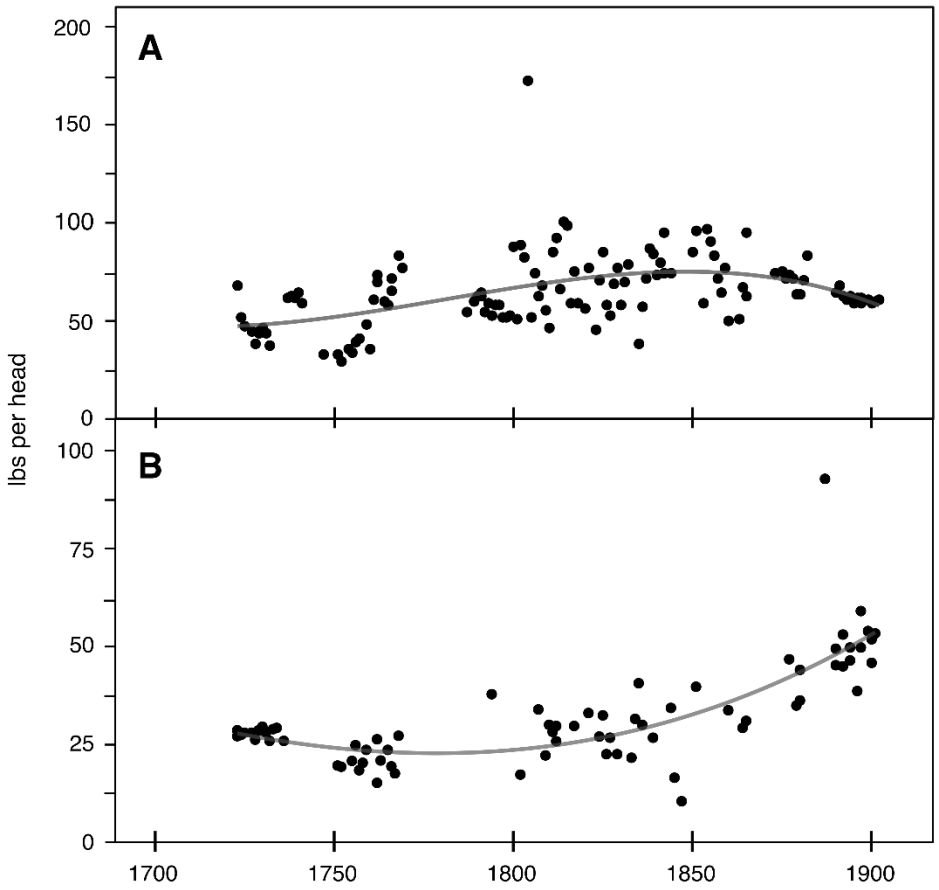


Figure 2.5: Annual average carcass weights for A) 'Sheep', and B) 'Lambs', 1700-1914 (after Turner *et al.* 2001, 191)

A further benefit from a higher plane of nutritional would have been an increase in prolificacy. A higher plane of nutrition results in an increase rate of ovulation, increasing the chances of twinning, and also providing greater nutrition for mother and offspring during pregnancy. Better nutrition also improves lactation meaning a greater number of offspring are likely to survive (National Academy of Science 1975, 3-5). In a practice known as 'flushing', nutritional intake is elevated during the breeding season to increase ovulation and conception rates (Robinson *et al.* 2002, 193-4). Although likely discovered accidentally at first, flushing became a common practice by the mid-18th century

(Ryder 1983, 498), no doubt facilitated by the greater availability of fodder, with ewes frequently fed on root vegetables, cabbages, hay and grains (Trow-Smith 1959, 200-1).

2.2.2 Genetic improvement

One of the major innovations facilitated by enclosure was the growth of selective breeding. While different 'types' had long been recognised, 'breeds' with homogenous appearances, characteristics or behavioural traits regulated by pedigrees and societies, only emerged in the early-19th century (Coates 1822). The development of the New Leicester and Southdown breeds provide a useful lens through which to examine the drive for greater profitability through selective breeding.

New Leicester

Robert Bakewell (1725-1795) and his New Leicester 'Dishley' sheep remains one of the most potent symbols of the age of improvement. For many contemporary agriculturalists, his Dishley Grange was the birthplace of selective breeding in livestock and scientifically informed farming (Tuke 1800, 90; Youatt 1837, 313-328). The late-18th and 19th century New Leicester was a white, hornless sheep; with a small head and neck; broad shouldered and a characteristic 'barrel-shaped' body; wide rumped and possessing proportionally smaller legs; light boned; bearing a fleece of around 7 lb (Marshall 1790; Price 1809, 182-188; Pitt 1809; Spooner 1844, 18-23). This conformation, fixed by Bakewellian principles of in-and-in breeding, contrasted with contemporary unimproved breeds such as the Welsh Mountain, which were typically of longer leg and neck; narrow chested with slender loin and rump; and coarse boned (Youatt 1837, 265-272; Spooner 1844, 19-21) (Figure 2.6).

The breed received international fame for three key characteristics. Firstly, through the repeated selection of fast-maturing animals, New Leicesters were ready for market at around two years of age, considerably quicker than other breeds (Youatt 1837, 265-9). Second, was their propensity to lay down large amounts of fat, with some reported to have developed as much as 6-7.5" of fat (Young 1788, 242) and backs "like the fattest bacon" (Culley 1788, 473). This rendered their meat inedible to all but the urban poor, but produced high amounts of valuable tallow (Price 1809; Trow-Smith 1959, 63; Copus 1989, 38). Thirdly, the refinement of the carcass and the high proportion of meat to bone maximised profitability (Parkinson 1810, xviii).

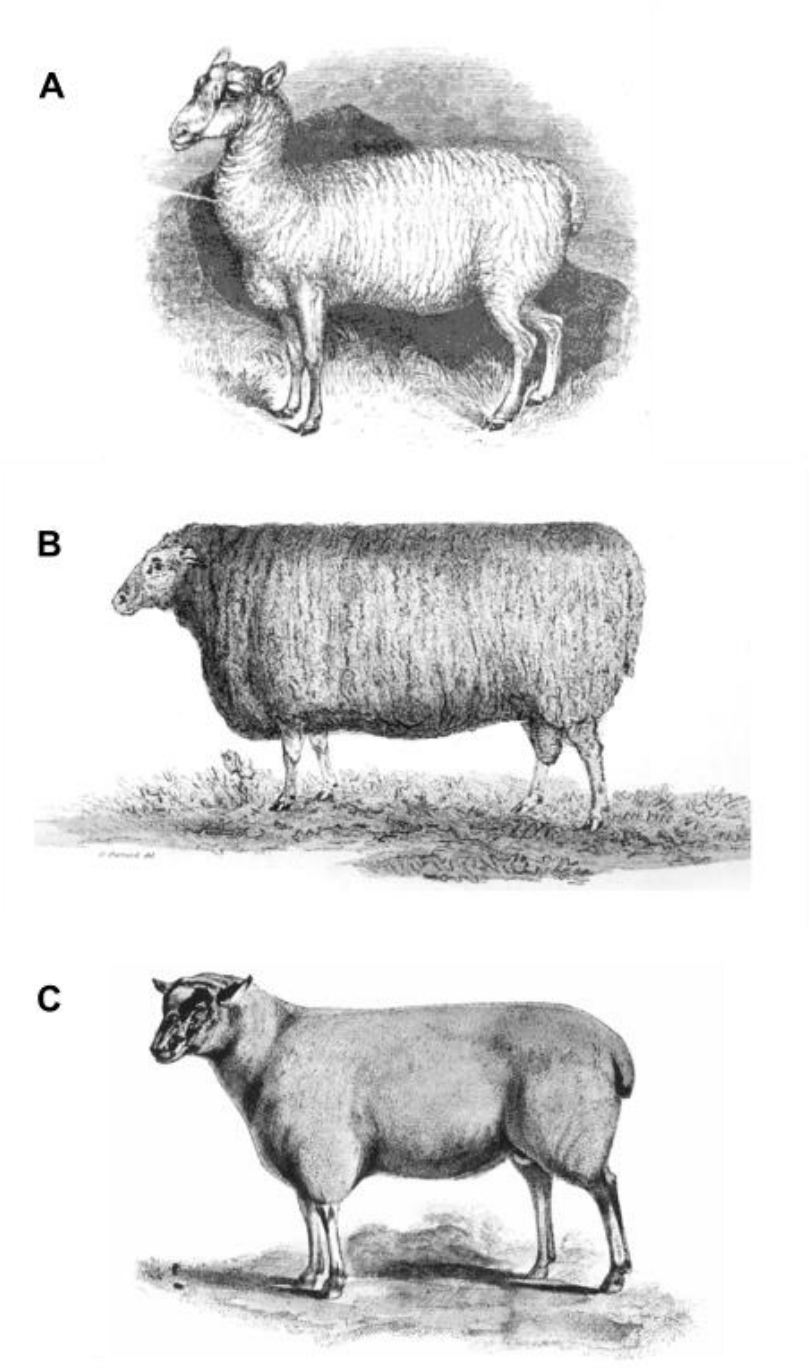


Figure 2.6: 19th century depictions of: A) an unimproved Welsh Mountain ewe (Youatt 1837, 272); B) New Leicester 'Dishley' ewe (Philips 1808); and C) a Southdown ram (Southdown Sheep Registry 2018)

Thanks to these characteristics, the spread of the New Leicester was rapid. In almost all of The Board of Agriculture's *General Views of Agriculture*, the necessary remedy for improving regional stock was to cross them with a New Leicester. By 1788, just 28 years after Bakewell began his breeding programme, George Culley remarked that few of the traditional native breeds did not contain some Dishley blood (1788, 472-4). Genetic analysis of sheepskin parchment from York has tentatively supported the rapid spread of this breed (Teasdale *et al.* 2015). Analysis of genetic-affinities of 17th century parchment to modern sheep breeds, showed strong affinity with northern Britain, specifically the black-faced breeds of the Swaledale, Rough Fell and Scottish Blackface (Teasdale *et al.* 2015, 5); types also identified by Ryder (1960a) in the fibre analysis of 17th-century parchment. The late-18th century material, however, showed closer affinity to the Midlands, and breeds such as the Border Leicester and Galway, the latter of which was developed out of the New Leicester. The parchment was not dated to the year of use, but dated paleographically. If this dating is correct, it supports the rapid expanse and crossing with the New Leicester only a few decades after Bakewell began breeding.

The development of the New Leicester marks a transition from the primary economic role of sheep as wool producers to that of producing meat and tallow (Ryder 1983, 147). Carcass conformation was put before fleece quality and folding ability (Copus 1989, 38-9). Bakewell had produced an animal ideal for the current market. The increasing coarseness of British wool brought about by nutritional improvement, left farmers struggling to compete with the high-quality Merino imports, resulting in a fall in the value of fleeces (Broadberry *et al.* 2015, 134). Moreover, during the 18th century, tallow was worth considerably more than mutton and Bakewell's "'beasts of tallow" (The Famers Magazine 1814) were in high demand. (Sinclair 1802, 49).

Southdown

Towards the end of the 18th century, large-scale imports from eastern Europe had depressed tallow prices, and by 1800 it had fallen below mutton (Copus 1989, 39). The once praised fat of the New Leicester was increasingly raised as a concern (Pitt 1810, 211) and breeder's attention quickly shifted towards high-quality mutton. The requirements of the market were best filled by the Southdown (more accurately the Southdown crosses; Trow Smith 1959, 131; Copus 1989, 39; Porter 1991, 20-22), a breed native to Kent, Sussex and Hampshire. The 19th-century Southdown was described as: polled with grey legs and face; a head clear of wool (unlike today); short but robust legs; light forequarters but full hindquarters; a 'barrel-shaped' deep chest with broad shoulder and a wide stance; and producing a short-wool fleece of c.2 lb with a 2-3" staple (Bingley 1809, 374; Lawson 1825, 431; Home 1830, 280; Youatt 1837, 235). Importantly for the changing market, they produced fine grained mutton of excellent flavour (Youatt 1837, 235; Home 1830, 469; Watson 1838, 469), and soon commanded the highest price at Smithfield's Market (Bingley (1809, 374). The Southdown

breed was 'improved' by John Ellman of Glynde, East Sussex (1753- 1832) and later by Jonas Webb, Suffolk (1796-1862), who like Bakewell sought to improve the native stock through in-and-in breeding. Their efforts, as well as those of less publicised breeders (Trow Smith 1959, 127) produced a faster maturing animal with an improved conformation, better bone-to-muscle ratio, and a propensity to lay down intramuscular fat. To the benefit of the farmer they were well suited to folding, and considered hardy and healthy (Youatt 1837, 235; Watson 1938, 468-9). The Southdown was highly praised in agricultural manuals, and soon became the 'must have' breed for the improving farmer.

2.3 Zooarchaeological evidence for improvement

The analysis of post-medieval faunal remains to explore livestock improvement is an area of considerable interest. Previous research has highlighted the value of synthesising historical and archaeological lines of evidence to provide a more nuanced understanding of animal husbandry and animal-based industry (Albarella 1997; 2003; Davis & Beckett 1999; Thomas 2005a; Thomas *et al.* 2013; Yeomans 2006). Despite the potential of this material, faunal remains from this period are frequently overlooked or discarded without analysis (Thomas 2009, 19-22; Broderick 2014). This neglect is perhaps understandable given the abundance of documentary records from this period (e.g. Fitzherbert 1534; Tusser 1580; Markham 1631; Brown 1793; Youatt 1837). However, a growing body of research has illustrated the considerable insight into past agricultural practices faunal remains can provide, including the health consequences of increasing industrialised farming (Fothergill *et al.* 2012; Gordon *et al.* 2015); changing human-animal relationships (Thomas 2005c; 2014; Sykes 2014, 46-9); and the timing and nature of livestock 'improvements' (O'Connor 1995; Albarella 1997; Davis & Beckett 1999; Thomas 2005a; Thomas *et al.* 2013). Unlike historical evidence, which has tended to focus on land management and cropping regimes, zooarchaeology can provide direct evidence from the animals themselves, and from a wider geographical and social range.

In general, the majority of zooarchaeological studies agree with the recent historical consensus that developments were never revolutionary in pace. They present biometrical evidence to suggest that livestock were increasing in size as early as the mid-late-14th century through better nutrition, and later through the introduction of new stock and selective breeding (Armitage 1980; Davis 1997; Albarella 1997; Davis & Beckett 1999; Thomas 2005a; 2005b; Thomas *et al.* 2013). Thomas (2005b, 73) notes that the two primary methods by which zooarchaeological data has been traditionally used to explore improvement are: 1) examining changes in the morphology and conformation of animals, conducted through biometrical analysis of skeletal elements; and 2) through the analysis of mortality profiles based on epiphyseal fusion and dental attrition.

Site	Period of size increase	Reference
Dominican Friary, Beverley	no change	Gilchrist 1996
Hall Garth, Beverley	no change	Dobney *et al.* 1994
Chester	late-17th-19th	Gordon 2015
Okehampton Castle, Devon	late-16th-17th	Maltby 1982
Launceston Castle, Devon	15th-mid-19th	Albarella & Davis 1996
Exeter	16th	Maltby 1979
Wigmore Castle, Herefordshire	20th	Thomas & Vann 2015
Beverley Gate, Hull	no change	Scott 1989
Baynard's Castle, London	no change	Armitage 1977
London	14th, 15th, 16th, 17th, 19th	Thomas *et al.* 2013
Blackgate, Newcastle	17th	Rackham 1983
Blackfriars, Newcastle	no change	Rackham n.d.
Closegate, Newcastle	17th-18th	Davis 1991
Prudhoe Castle, Northumberland	no change	Davis 1987
Castle Mall, Norwich	late-16th-early-18th	Albarella e*t al.* 1997
Dudley Castle, West Midlands	mid-14th, 14th-15th, 17th	Thomas 2005a
Pontefract Castle, West Yorkshire	by 17th	Richardson 2002
Aldwark, York	no change	O'Connor 1995
Walmgate, York	no change	O'Connor 1995
The Bedern, York	19th	O'Connor 1995

Table 2.1: Evidence for size increases in sheep bones at various English sites

2.3.1 Morphology and conformation

The primary factors that control animal growth and size are its genetic background, nutritional intake, and the environment it is exposed to (Hossner 2005, 4-10). Optimal growth and productivity relies on the complex interplay of all these factors. The genetic background provides the capacity for the rate of growth, and heritable traits such as bone density, muscling and fat deposition (Purchas *et al.* 1991; Campbell *et al.* 2004; Wood *et al.* 2008). For an animal to achieve its genetic potential, it is essential that it receives adequate nutritional intake as deficiency either *in utero* or post-weaning can permanently stunt animal size (Pálsson & Vergés 1952a; 1952b; National Academy of Sciences 1975, 2; Hossner 2005, 6). Environmental factors such as temperature, nutrient availability and disease can also influence animal size (Atkinson & Sibly 1997; Davis 1981).

Biometrical data indicates that the majority of early-modern sheep were not dissimilar in size to those of the Iron Age, and are frequently compared in conformation to modern island breeds such as the Shetland and Soay (Armitage 1977, 65-8; O'Connor 1982; Bond & O'Connor 1999; Albarella & Davis 1994; Dobney *et al.* 1995, 41). Metrical data shows some regional variation in size, and a range greater than the homogeneity suggested in historical sources (Trow-Smith 1957) (Table 2.1). In general, available evidence suggests an increase in the size of sheep from the 16th and 17th centuries onwards. However, at some sites, particularly London and Dudley Castle (Thomas 2005a; Thomas *et al.* 2013), sheep have an increasingly longer, broader and deeper conformation from the 14th century onwards, a development Thomas associates with changes in agricultural and tenurial organisation in the post-Black Death period.

Bone size is significantly affected by nutritional intake, with modern sheep receiving a higher plane of nutrition shown to grow taller and broader (Pálsson & Vergés 1952a; 1952b; Popkin *et al.* 2012). The size increase seen in early-modern sheep may therefore reflect nutritional 'improvement', brought about the increased availability of forage and fodder crops. It is perhaps not surprising then that the earliest evidence for a size increase comes from elite castle assemblages. The disparity between elite and lower status sites was also observed by Armitage (1977) who demonstrated a significant difference in cattle size between contemporary 14th to 16th century dumps in London and those from Baynard's Castle, with the latter's occupants consuming substantially larger animals. This may reflect different financial resources and the ability of demesne farms to provide greater provisions for their livestock, particularly during winter, which was unaffordable to most farmers (Ryder 1983). This increased nutritional intake may have allowed sheep to achieve their genetic potential. Increasingly larger sheep are seen entering London from the 14th century onwards, which may suggest a preferential selection of larger animals for the capital's markets, or better fed animals in the surrounding counties.

By the later post-medieval period, evidence for larger taller is more widespread, with the average size continuing to increase into the 19th century. The increase is small and gradual, but suggests that contemporary with wider changes in agriculture, particularly the growth of enclosure and the cultivation of new forage and fodder crops, sheep were increasing in size. Regional variation becomes more pronounced, with areas such as Lincoln, East Anglia, Sussex and London presenting evidence for a size increase from the 16th century onwards (Dobney *et al.* 1995; Albarella *et al.* 1997; Connell *et al.* 1997; Thomas *et al.* 2013). In contrast, in the north of England there is no discernible increase in many sites from York (O'Connor 1984a; 1984b), Hull (Scott 1989; Saunders and Phillips 1993; Carrott *et al.* 1995), Beverley (Scott 1988; 1989; 1991; 1993; Dobney *et al.* 1994), Newcastle (Rackham 1977; 1978; 1988; Gidney 1989; Davis 1991a), or Carlisle (Rackham n.d) until well into the 19th century. This may reflect a retention of existing husbandry strategies and role of wool

production in these areas, which gave little financial incentive to improving the inherently suitable native stock, as the production of wool presupposes nothing about the size of the animal. While tooth size can be influenced by nutrition and environment, as much as 90% of observed variation is due to genetic influence (Larsen 2015, 26). Changes in size may therefore be the consequence of selective breeding or the introduction of new stock (Thomas 2005b, 74). Unfortunately, there is a paucity of published dental measurement data, however, changes in tooth size have been observed at Castle Mall, Norwich and Dudley Castle in the 15th and 16th centuries (Albarella *et al.* 1997; Thomas 2005a), suggesting that biometrical variation in long bones was at least partly the result of increasing genetic variation.

2.3.1.1 Analysis of nutritional and conformation improvement using metacarpals

Somewhat naively, the above assumes that 'large = improved'. This may well have been the case pre-1750, in which the 'biggest-boned' (Fitzherbert 1534; Markham 1648) was the ideal, but does not fully appreciate the nature of post-medieval improvement which was the development of early-maturing, fat and mutton bearing sheep, which were of a deeper and broader conformation, but not necessarily taller. A 'leggy' carcass was often considered inferior (Young 1793, 47-9; Robertson 1799, 540), as the deeper 'barrel-shaped' breeds were more profitable due to increased muscling and the increased surface area for wool (D'Arcy 1990, 13). Evidence for refinement of the carcass, and increasing the ratio of muscle to bone has been seen in 19th-century London, where the size of sheep long-bones decreases relative to previous centuries (Thomas *et al.* 2013, 17). These animals were not raised in London, so again may suggest an element of selection for the London market.

Some of the most significant contributions to identifying post-medieval improvement have been made by O'Connor (1982; 1995). By drawing upon modern agricultural research and analysis of extant breeds, O'Connor illustrated that the morphology of sheep metacarpals can be used to assess the extent of nutritional and conformational improvement, and the rate of skeletal maturation. His conclusions have been supported by subsequent studies which have illustrated that this abundant element is highly reflective of environmental and phenotypic factors, and provides a valuable tool for assessing the extent of 'improvement' (Guintard & Lallemand 2003; Davis 2008; Vann & Grimm 2010; Popkin *et al.* 2012).

Nutritional Improvement

In long bone development, longitudinal growth is predominantly dictated by *in utero* nutrition and circumferential growth by post-weaning diet (Hammond 1932; Pálsson & Vergés 1952a; 1952b). In the metacarpal, O'Connor (1982, 25) suggested that the relationship between the greatest length (GL) and minimum shaft diameter (SD)[2] could therefore be used as an indicator of the plane of nutrition, by calculating a slenderness index:

$$(100 \times SD) / GL \qquad \text{(Eq. 2)}$$

In breeds considered primitive (Shetland, Soay and Orkney), O'Connor (1982, 60-1) noted that their metacarpals were short and proportionally slender, indicative of both poor *in utero* nutrition and post-weaning diet, a trait observed in other unimproved flocks and wild Mouflon (Noddle 1978, 133-4; O'Connor 1982; Dobney *et al.* 1994; Davis 1997; Salvagno & Albarella 2017). In contrast, the metacarpals of more 'improved' breeds (Clun Forest and SouthdownXRomney) were more robust, with a greater shaft diameter (Figure 2.7). Despite similar slenderness indices, the two breeds differ in absolute length, a feature O'Connor (1982, 62) attributes to the rate of skeletal maturation, as improved commercial Southdowns mature early, leading to a proportional shortening of the limb bone (Reitz & Wing 2008, 64-5), while the slower maturing Clun Forests grow taller. Metapodia shape is, however, also affected by sex, with ewes typically having short and slender bones, rams longer and more robust, and wethers long and slender (Davis 2000, 389; Popkin *et al.* 2012), a factor which must be caution the examination of nutritional and conformational improvement.

This 'primitiveness' index has been applied at a number of sites and demonstrates a gradual increase in the robustness of sheep metacarpals from the late-medieval through to the post-medieval period (Noddle 1978; O'Connor 1982; Dobney *et al.* 1995; Vann & Grimm 2010; Thomas *et al.* 2013; Gordon 2015, 202-3) (Figure 2.8). Despite overlap between periods, and considerable range within a single site, the post-medieval remains from major cities including Hull, Lincoln, Chester, Coventry, Norwich, Newcastle, York and Hereford, present very little evidence for 'improved' individuals (Rackham 1978; Noddle 1978; 2002; O'Connor 1982; 1995; Davis 1991a; Dobney *et al.* 1995; Gordon 2015). Statistical analysis shows no significant difference in the slenderness indices between Coventry, Hull or Lincoln (Kruskal Wallis test, $p=0.12$)[3]. In Lincoln, O'Connor remarked that sheep

[2] O'Connor (1982) used the abbreviations MaxL, instead of the more commonly used GL, and MinShB instead of SD. These are the same measurements as defined by von den Driesch (1976).
[3] Non-parametric test used due to the non-normal distribution of data from Coventry (Shapiro-Wilks test, $p=0.03$). Sample size: Hull (n=11), Coventry (n=11), Lincoln (n=5).

contemporary with the celebrated Lincoln Longwool were largely indistinguishable from those from the Iron Age (1982, 215).

The variation in size and shape was even greater in London, and while many retained a morphology similar to those of primitive breeds, increasingly robust metacarpals were recovered from the 17th century onwards (Thomas *et al.* 2013, 3316-7) (Figure 2.9). However, there is no statistically significant difference in the slenderness indices of metacarpals across the mid-15th to late-19th century (Table 2.2). The earlier presence of these more improved morphotypes in London, may again suggest the preferential driving of larger, broader and more improved sheep into the capital for slaughter. Vann & Grimm (2010) applied this criteria to a discrete cache of late-18th and 19th century metapodia from Tiverton, Devon, and noted the presence of a significant number of short and robust metacarpals. While acknowledging the impact of sex, the data suggests both nutritionally and early-maturing individuals, although perhaps more contemporary with the traditional post-1750 period of improvement.

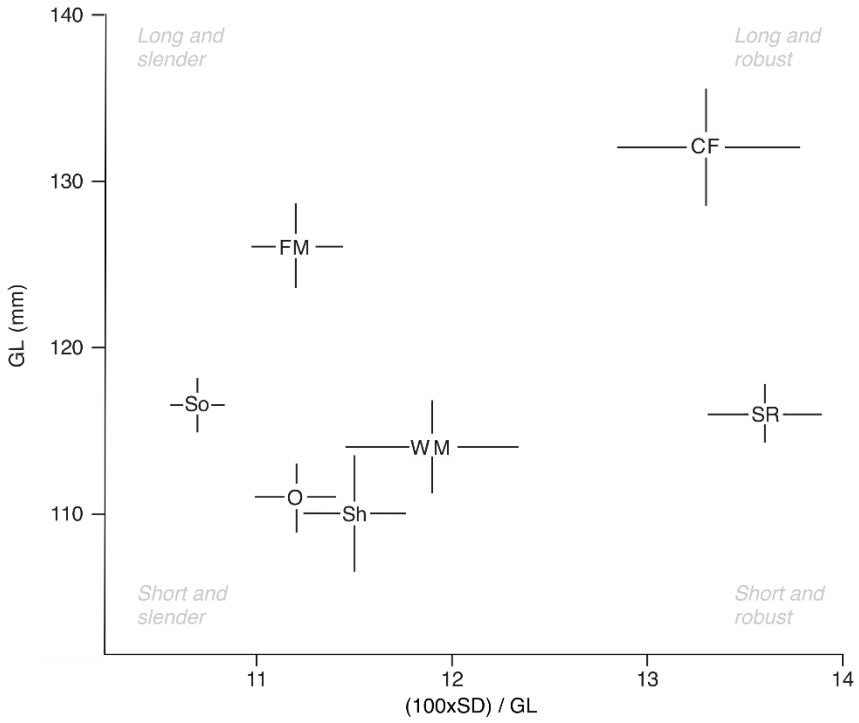


Figure 2.7: Metacarpal slenderness in a range of modern breeds:
Sh – Shetland, O – Orkney, So – Soay, WM – Welsh Mountain, FM – FinnXMerino,
CF – Clun Forest, SF – SouthdownXRomney. Breed data is mean and standard deviation (after O'Connor 1982, 62)

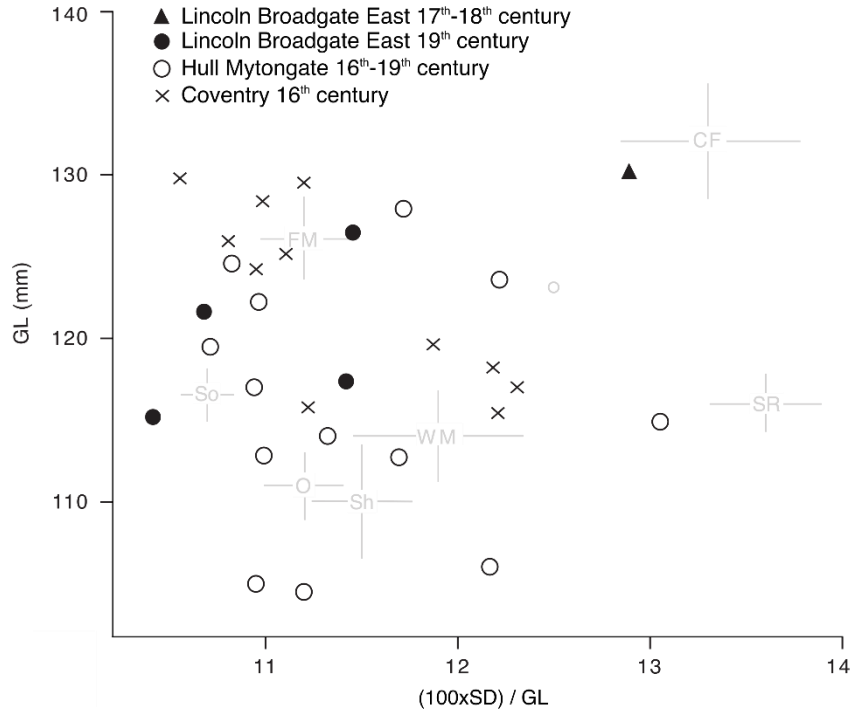


Figure 2.8: Metacarpal slenderness from a range of 16th to 19th century assemblages. Breed data is mean and standard deviation (after O'Connor 1982, Appendix I)

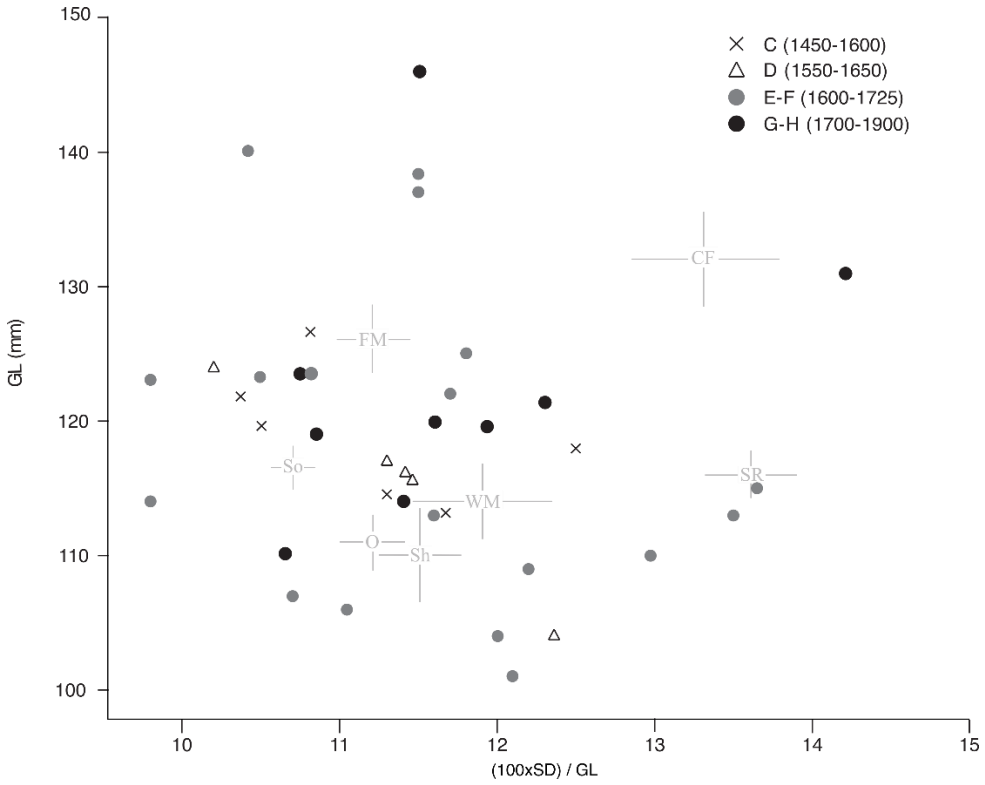


Figure 2.9: Metacarpal slenderness from 15th to 19th century London. Breed data is mean and standard deviation (after Thomas *et al.* 2013)

Phase	MinShB index	
	U	*P*
C (1450-1600) (n=6) – D (1550-1650) (n=6)	16.0	0.82
D (1550-1650) (n=6) – E-F (1600-1725) (n=16)	47.0	0.97
E-F (1600-1725) (n=16) – G-H (1700-1900) (n=9)	61.0	0.56

Table 2.2: Mann-Whitney U tests of 'slenderness indices' from London (Thomas *et al.* 2013). Significance level (p=<0.05)

Conformational improvement

A further suggestion of O'Connor (1982) was that the morphology of the metacarpal could be used to examine conformational 'improvement', and the development of proportionally deeper carcasses. Most primitive breeds are characterised by long, slender legs, proportional to the body, with a long neck and light forequarters. Consequently, the most profitable parts of the carcass, i.e. the proximal part of the hind leg and the shoulder regions, are under-developed. Conformational improvement was concerned with the expansion of these most desirable joints at the expense of less profitable parts, particularly the neck, feet and head. The results of this were most pronounced in the New Leicester, and more refined Southdown, famed for their short-legged, 'barrel-shaped' body. O'Connor examined the antero-posterior depth (DFp) and depth (DFd)[4] of the metacarpal using the equation:

$$(100 \times \text{DFd}) / \text{DFp} \qquad \text{(Eq. 3)}$$

and concluded that the ratio should be lower in modern breeds reflecting the deeper and more robust conformation of their carcass, with the proximal part of the bone better developed in proportion to the distal. This was confirmed when tested upon a range of modern breeds (Table 2.3). While the range from primitive to modern is small, the coefficient of variables was low in most cases (mean 3.23), and suggests that in more 'improved' breeds the proximal part of the bone is better developed. Depth and diameter is also less affected by sex (O'Connor 1982, 100-2; Davis 1996; Popkin *et al.* 2012). By examining both metacarpal slenderness and depth conformation, this provides a useful assessment of 'improvement'. Unfortunately, this assessment relies on the presence of complete specimens and for a measurement of proximal depth to be taken, which is quite uncommon. Therefore, an assessment of conformational improvement of specimens from Launceston Castle or Dudley Castle is not possible. These measurements are available from post-medieval Lincoln (Figure

[4] O'Connor (1982) used the abbreviations ProxD and DistD, more commonly termed DFp (greatest depth of proximal fusion point) and DFd (greatest depth of distal fusion point)

2.10). As with the slenderness index, they indicate that between the 16th and 18th century there was considerable variation but no statistically significant difference (Student's t test, p=0.51)[5].

Sample	*n*	Index	SD	SE
Soay (Whipsnade)	8	100.3	3.3	0.4
Soay (Hirta)	70	99.3	3.6	1.3
Orkney	39	99.0	4.2	0.7
Welsh Mountain	15	96.9	3.7	0.9
Hebridean	23	96.3	2.7	0.5
FinnXMerino	30	94.7	2.5	0.4
Shetland	13	93.9	3.4	1.0
Clun Forest	22	92.1	2.3	0.4
Southdown XRomney	30	89.6	2.5	0.5
Cheviot Suffolk	17	88.2	3.8	0.9

Table 2.3: Conformational improvement indices for a range of modern breeds (after O'Connor 1982)

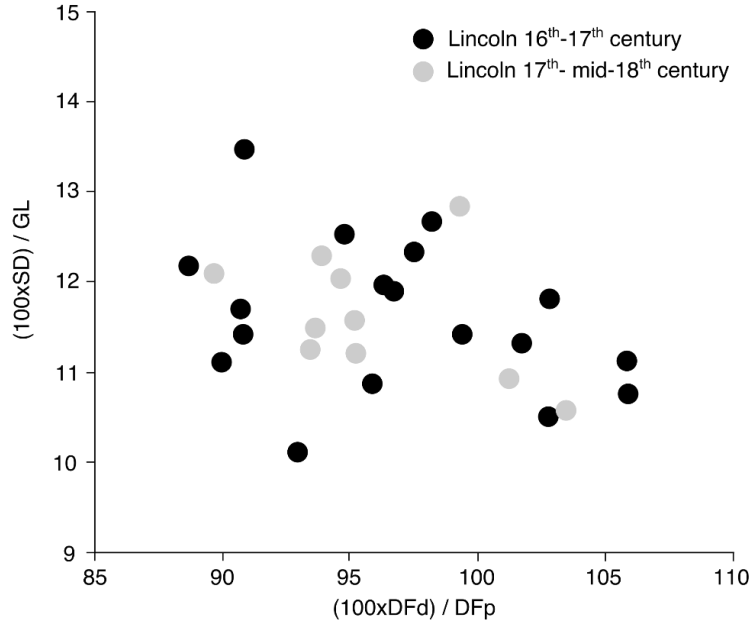


Figure 2.10: Nutritional and conformational improvement of sheep metacarpals from post-medieval Lincoln, using O'Connor's (1982) criteria. Data from Dobney *et al.* (1995)

[5] Parametric test used due to the normal distribution of both 16th-17th century samples (Shapiro-Wilks test, p=0.46, n=16), and 17th to mid-18th century samples (p=0.36, n=10).

2.3.2 Liveweight

Biometrical data permits the estimation of an animal's liveweight, and the opportunity to explore the documented increase in mutton yields during this period. As bone breadth is more correlated with carcass weight than length (Pálsson & Vergés 1952a; 1952b), O'Connor (1988) developed a regression formula using the distal breadth (Bd) of fused radii from a range of modern breeds of varying 'primitiveness', to create an average relationship between bone size and body weight:

$$(1.79 \times Bd) - 13.3 \qquad \text{(Eq. 4)}$$

This formula was applied to fused sheep/goat radii from late-medieval and post-medieval assemblages, and again suggests the dominance of animals comparable in weight with those of the Soay breed (Table 2.4), and only a modest increase in weight as attested in the historical data.

	n	Date (century)	Liveweight (kg)	
			Mean	Range
Modern:				
Soay Ram	-	-	38	-
Soay Ewe	-	-	24	-
Welsh Mountain Ram	-	-	75	-
Welsh Mountain Ewe	-	-	50	-
Border Leicester Ram	-	-	125	102 – 147
Border Leicester Ewe	-	-	96	70 – 122
Southdown Ram	-	-	155	130 – 180
Southdown Ewe	-	-	95	86 – 104
Archaeological:				
Beverley	7	16th	32.8	17.8 – 39.3
Chester	5	17th - 19th	34.5	31.9 – 38.1
Exeter	35	12th - 15th	32.8	-
	34	16th - 17th	32.3	-
	21	17th - 18th	35.6	-
	9	18th - 19th	37.7	-
Hereford	4	14th - 15th	32.3	27.8 – 35.0
	15	post-16th	35.0	31.4 – 38.6
London, Baynard's Castle	28	14th	33.5	28.7 – 37.7
	50	16th	36.5	30.7 – 40.4
London, Aldwark	4	17th - 18th	36.5	32.1 – 38.7
Winchester	16	11th - 15th	33.1	-
	23	16th - 19th	33.7	-
York, Aldwark	26	14th - 16th	36.5	-
	39	16th - 19th	37.9	-

Table 2.4: Liveweight estimates for various sheep breeds, and late-medieval to post-medieval remains using O'Connor's (1988) formula. - = no data (St Kilda Soay Project; Oklahoma State Department of Animal Science; O'Connor 1988; Sykes *et al.* n.d.; Armitage 1977; 1983; Maltby 1979; Gilchrist 1996; Noddle 2002)

2.3.3 Age profiles

Analysis of mortality profiles may have the potential to identify animals which were ready for slaughter at an earlier age. The hastening of skeletal maturation, muscle growth and fat deposition was a key achievement of the improvement period and has been closely associated with Robert Bakewell whose New Leicesters were finished as early as 2 years of age (Youatt 1837; Chambers and Mingay 1996, 67). Historical evidence indicates that this separation of skeletal development and soft-tissue mass only occurred after the mid-18th century (Thomas 2005b, 74). This is supported by the analysis of metacarpal morphology, which suggests that prior to this period, few specimens presented an 'improved' morphology and were maturing at a rate which may have proportionally shortened bone length, due to early epiphyseal closure, as seen in the Southdown breed.

The ageing data from most post-medieval sites reflects a mixed farming economy, with the presence of 'prime meat' lambs, yearling and older mutton sheep. At sites such as Launceston Castle (Albarella & Davis 1994) and Castle Mall, Norwich (Albarella *et al.* 2009), the presence of considerably older individuals in the 16th to 18th centuries, some in excess of 6 years old, highlights the continuing importance of wool to many famers (Ryder 1983, 147). In northern cities such as York (O'Connor 1984a; 1984b), Hull (Scott 1989; Carrot *et al.* 1995), Beverley (Scott 1988; 1991; Dobney *et al.* 1994) and Newcastle (Rackham 1977; 1978; 1988), older sheep dominate, likely slaughter after producing many wool clippings. The mixed pastoral economy can be clearly seen in the contemporary material from Castle Mall and Heigham Street, Norwich (Figure 2.11). At Castle Mall, the majority of sheep across the 14th to 18th century survived beyond 3 years, while at Heigham Street, few survived beyond full maturity (Weinstock 2002). Statistical analysis of the distribution of ages between 16th-18th century Castle Mall (n=75) and 17th-19th century Heigham Street (n=12) shows a significant difference between the mortality profiles at these sites (Levene's test, p=<0.01). The latter suggests a production model likely geared towards meat production (Marom & Bar-Oz 2002), although the small sample size must be noted. While changes in mortality profiles are identifiable, there is limited evidence for any change in the rate of skeletal maturity prior to the 19th century, and the clearest evidence still comes from historical slaughter weights.

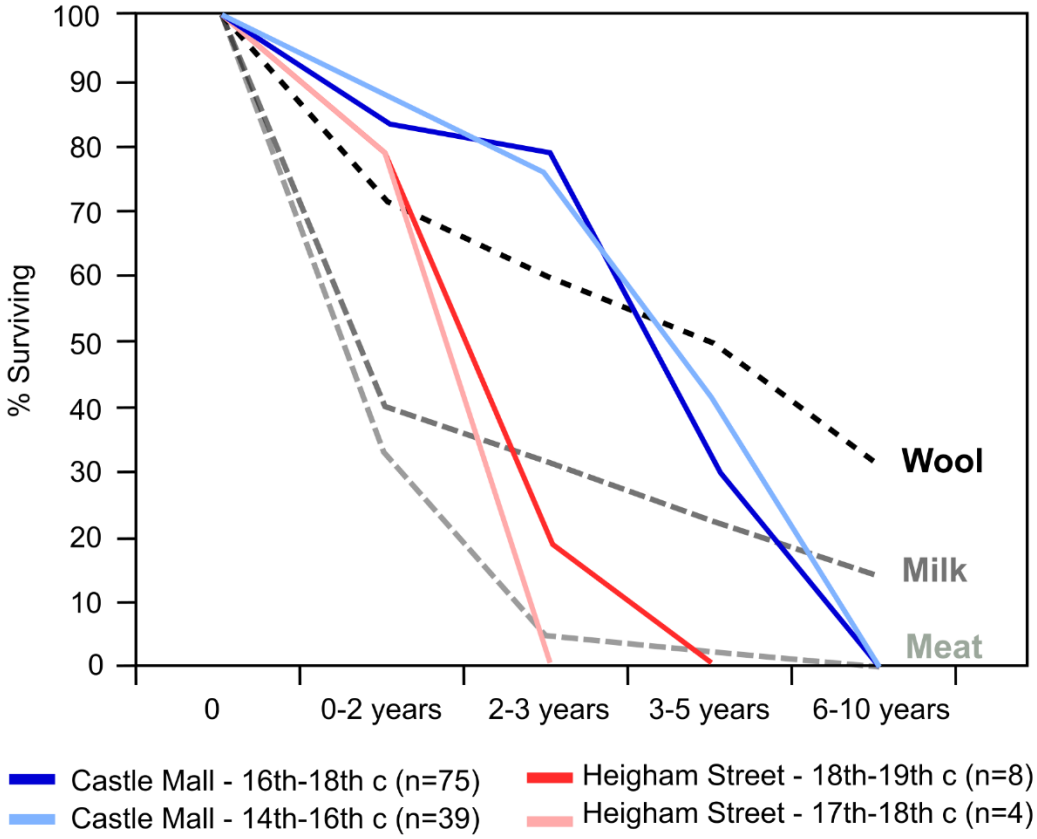


Figure 2.11: Survivorship curves for post-medieval Norwich using dental eruption and attrition data (Castle Mall – Albaralla *et al.* 1997; Heigham Street – Weinstock 2002). Sample numbers in parentheses. Modem theoretical survivorship curves from Marom & Bar-Oz 2009

3.4 Summary

The rural economy of Britain underwent considerable change between the 16th and 19th centuries. New field systems were adopted, new rotations practiced, new crops sown, new fertilisers sourced. However, historical and zooarchaeological data indicates that these developments were never revolutionary in pace, and instead, 'improvement' and the adoption of new practices slowly diffused across the country and down to smaller farms. The impact that these wider agricultural developments had on sheep is still unclear, with the more 'natural' sheep considered less suited to 'High Farming' (Trow-Smith 1959, 319-321). Biometrical evidence indicates an increase in sheep size from the 16th century onwards, although it is temporally and geographically variable. This size increase likely reflects the nutritional improvement that was facilitated by the increased availability of forage and fodder crops in these later centuries. This may have enabled livestock to achieve their genetic potential, which was further enhanced with selective breeding which was facilitated by enclosure.

Chapter 3

Background to sheepskin and parchment production

This chapter provides an overview of sheepskin, and the methods of parchment production. Skin biology and structure will be outlined (3.1), with specific reference to the impact of environmental and physiological factors on the tissue and resulting parchment (3.2). Each step of the production process will be detailed (3.3), exploring both medieval and post-medieval methods, before outlining the manufacturing process followed in this study (3.4).

3.1 Introduction to sheepskin

Skin, and the wider integumentary system, is one of the largest and most complex organs in the body. It provides a vast physical barrier between the organism and its environment, and is integral to: thermal regulation; the storage of water and lipids; the excretion of waste products (as perspiration); the secretion of sebum (primarily as lanolin in sheep); the synthesis of vitamin D_3; as well as detecting and responding to a range of environmental information (Lyne 1964; Tregear 1966; Millington & Wilkinson 1983; Odland 1991; Brodal 2010; Montagna & Parakkal 2012; Handle *et al.* 2016). Sheepskin is characterised by a loose dermal fibre network; the presence of wool fibres and a high follicle density; significant levels of cutaneous fat; and a weak *papillary-reticular* junction. In biological literature, skin is typically divided into the epidermis, dermis and subcutaneous layer. In leather and parchment technology nomenclature, these layers are typically referred to as the grain, corium and flesh (Figure 3.1)

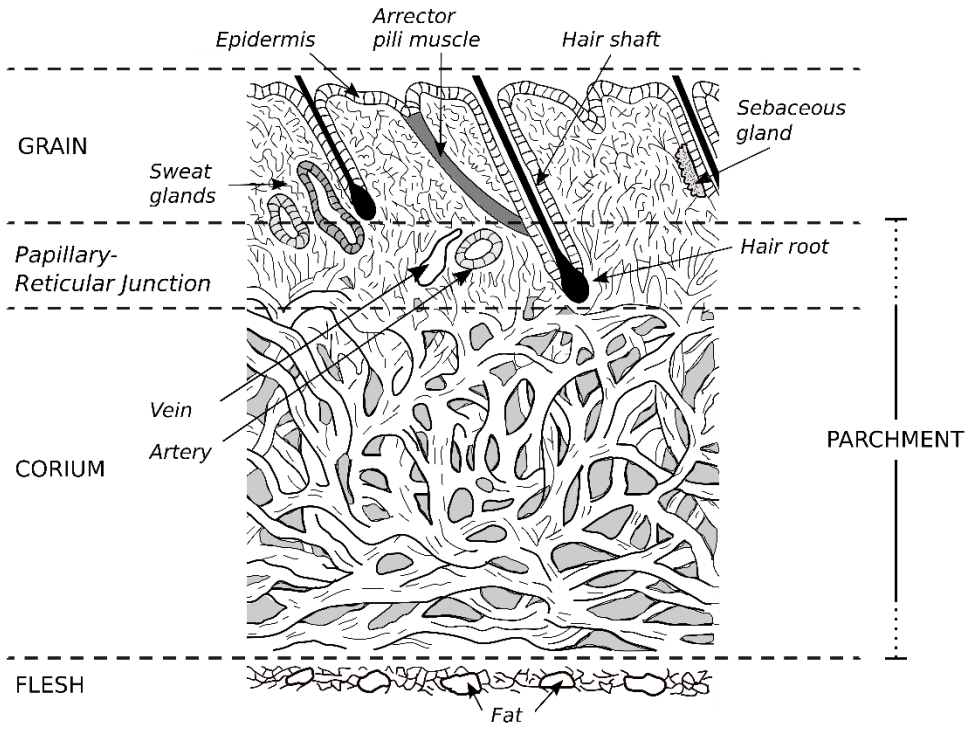


Figure 3.1: Structure of mammalian skin and parchment, with key features labelled (Drawn by S. Doherty)

3.1.1 The epidermis

The epidermis is the outermost layer of the skin, and constitutes four discrete layers: the *stratum basale*, *stratum spinosum*, *stratum granulosum* and the *stratum corneum*. It is in a state of constant renewal, with the *basale* keratinocytes and melanin granules dividing and displacing outwards through the overlying layers to enter the *corneum*, forming a flat, densely keratinised layer (Ryder 1973; 1; Dover & White 1991). The rate of epidermal turnover is variable, taking between 26-56 days in humans (Halprin 1972; Koster 2009), but less time in smaller mammals (Potten *et al.* 1987). The epidermis forms between 2-20% of the total thickness of sheepskin, varying between 30 µ in wool- and hair-bearing regions, to 500 µ in spare or naked regions (Lyne 1957; Lyne & Hollis 1968; Brown *et al.* 2010). It is composed almost entirely of 'soft' epithelial keratin, which has a lower cysteine content, but higher methionine and glycine levels than 'hard' hair or nail keratins (Bowes & Elliot 1957; Resing & Dale 1991). During parchment production, the epidermis is completely removed.

3.1.2 The dermis

The dermis is a collagen rich layer which form the majority of the skins thickness, and is the portion remaining after parchment and leather production. Sheepskin dermis is typically 1-5 mm thick (Lyne

& Hollis 1968; Brown *et al.* 2010), and commonly divided into the upper *papillary dermis* and lower *reticular dermis.* The *papillary dermis* is the thinner of these and is moulded against the overlying epidermis, producing distinct ridges and grooves seen in unsplit leather.

The intersection of these two layers is known as the *papillary-reticular* junction, or the grain-corium junction (Covington 2009, 33; Michel 2014, 35), and marks the transition from the fine dermal fibres of the *papillary* to the much larger fibres of the *reticular dermis*. Sheepskin is characteristically weak at this junction due to the abrupt change in structure (Reed 1973, 33-5; Covington 2009, 67; Michel 2014, 35). The separation of these two layers is known as 'delamination', and is facilitated by the presence and removal of cutaneous fat cells which form within the junction (Figure 3.2). If large amounts of these are removed during processing, particularly through the saponification of triglycerides during liming, this can produce voids in the structure, allowing the two layers to detach.

Water accounts for ~80% of the dermal content, with the remaining ~20% predominantly collagen, elastin and non-structural proteins (Maxwell 2007, 25). The dense collagen fibres are arranged as interwoven strands, largely in parallel with the surface of the skin, and are responsible for providing its strength and flexibility (Odland 1991, 14). This layer also contains hair shafts and roots, along with a range of mechanoreceptors and nerves.

3.1.3 Subcutaneous tissue

Subcutaneous tissue, or hypodermis, is the innermost layer of the integumentary system and separates the skin from the underlying muscle. It is composed of loose areolar and adipose connective tissue, and displays substantial regional, species, breed, age and sex related variation in thickness (Odland 1991, 3; Wood *et al.* 2008). Adipose tissue provides insulation and shock-absorbance for the underlying muscle structure and organs. Excessive quantities of subcutaneous adipose tissue are uncommon in most sheep breeds, as fat cells are instead distributed throughout the skin, particularly at the *papillary-reticular* junction (Ryder 1958, 310); however, fat deposits around the rear and shoulders can be common in older animals.

3.1.4 Lipids

Sheepskin has an inherently high lipid content. As much as 22% of the epidermis dry weight, and 30-50% of the dermis is composed of lipids, even within a skin that would not be classified as 'greasy' (Koppenhoefer 1938; 1939, 207; Ockerman & Hansen 2000, 140). In comparison, cattle dermis varies between 2-3%, horsehide between 0.5-4%, and goat between 3-10% (Bieńkiewicz 1983; Palop 2016). Unsurprising, the lipid content is most pronounced in the *papillary-reticular*

junction. The amount of cutaneous fat varies by breed and season, with levels highest in sheep bred for wool, and at their greatest in autumn and early winter, and lowest in late winter (Dempsey 1955, 108). Epithelial lipids are composed primarily of waxes (particularly lanolin), cholesterol and its esters, phospholipids and free fatty acids, while the dermis and subcutaneous tissue are composed predominantly of triglycerides (Koppenhoefer 1939).

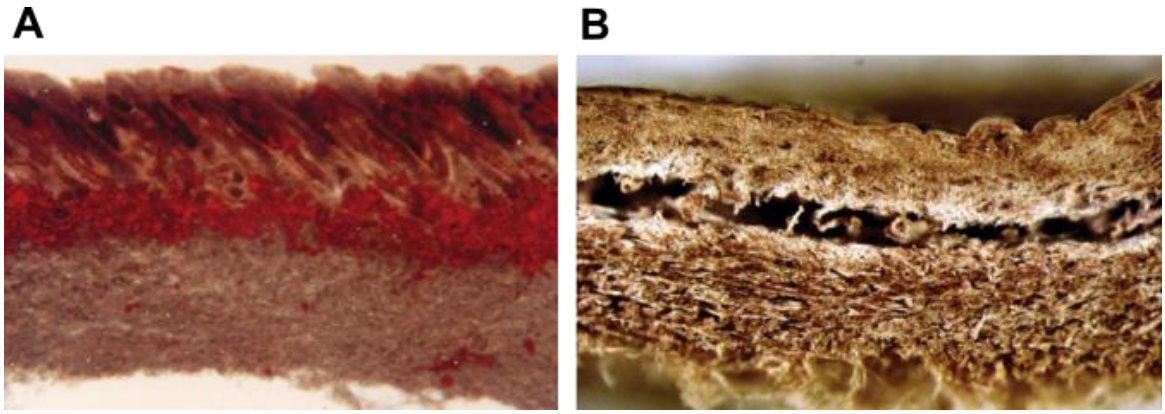


Figure 3.2: A) Histology of fresh sheepskin with fat lipocytes (stained red) within the *papillary-reticular* junction (after Covington 2009, 34); B) Cross section of sheepskin leather showing delamination of the layers, x25 magnification (after Michel 2014, 35)

3.2 The impact of physiological and environmental factors on sheepskin and parchment

Skin responds continuously to physiological and environmental changes, thus reflecting many characteristics unique to the individual animal (Saxl 1954; Reed 1973; Bieńkiewicz 1983; Clarkson 1992). Both the fresh skin and resulting parchment are chemically and structurally heterogeneous, with variations in: the thickness of layers, lipid content and composition; tissues and structures present; follicle density; and tightness of the dermal fibre network, all dependent on species, breed, age, sex, diet, health, season and location on the body (Koppenhoefer 1938; 1939; McLaughlin & Theis 1945; Dowling 1955; Wodzicka 1958; Rushmer *et al.* 1966; Ryder 1968; Haines 1999; Maxwell 2007; Palop 2016).

3.2.1 Breed

Breed can have a significant impact upon the quality of the sheepskin and its suitability for parchment, as the preferred qualities of the fibre and fleece relate inversely to the preferred qualities of the skin (Figure 3.3) (Covington 2009, 67). Skin thickness and dermal fibre tightness are linked

to follicle density (Wodzicka 1958); therefore, wool-bearing breeds with a high-follicle density have a weaker skin, and make poorer parchment. The Merino breed, for example, produces exceptionally fine wool, but their skin has a high fat content and a loose fibre network, and can be wrinkled due to the weight of the wool, resulting in a skin of limited value (Leach 1995, 27; Covington 2009, 67). In contrast, goats or hair-sheep breeds such as the Wiltshire Horn have a fibre of low value but their skin has a tight structure with minimal fat, producing a hard-wearing material used for the finest gloving leather (Covington 2009, 67). Breeds reared primarily for their meat also have their issues, as in fast-maturing animals the dermal collagen is often poorly cross-linked, also resulting in a weaker skin (Henrickson *et al.* 1984, 168).

Cutaneous and subcutaneous fat is one of the most significant impediments to successful parchment making, not only due to its effect on the integrity and strength of skin, but that it inhibits the penetration of aqueous solutions, making the skin difficult and lengthier to process (Leach 1995, 81; Ockerman & Hansen 2000, 162).

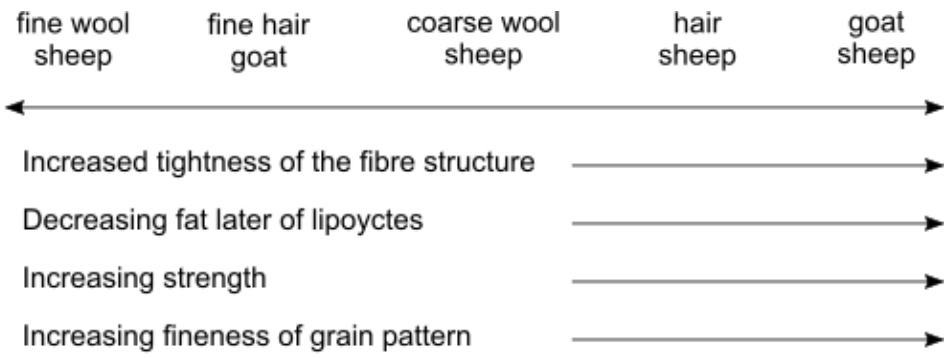


Figure 3.3: Properties of sheep and goatskin (after Covington 2009, 67)

3.2.2 Age and Sex

The most significant change to skin with age is increasing size and thickness with maturity. Thickness is also seasonally variable; influenced by temperature; varying planes of nutrition; and reproduction (Ryder 1958; Wodzicka 1958; Lyne 1961; Dowling 1964). While calfskin >6-8 weeks of age is typically too thick for anything other than leather (pers. comm. J. Vnouček), sheepskin of any age can be used for parchment. Older animals, however, are more likely to display visual imperfections such as scars, warts, parasites and ringworm (Ryder 1983), with parasitic attack more likely in older sheep with longer and more soiled wool (D'Arcy 1990, 313-4). These dermatological issues are visible in parchment (Reed 1973; Ryan 1987), so the finest manuscripts were made from

young lamb or calfskins (Ryder 1964, 391; 1983, 732; Reed 1973, 35-6). There are limited data on the influence of sex upon sheepskin, although rams often have thicker skins than ewes and wethers (Henrickson *et al.* 1984; Schummer *et al.* 1981, 506), particularly during the mating season (Adam & Findlay 1997, 123).

3.2.3 Diet

Adequate nutrition is required for the healthy growth, maintenance and repair of skin, with poor nutrition leading to the development of smaller collagen fibres and impaired cross-linking, resulting in a weaker skin (Clark 1986). In more severe cases of malnutrition, the skin can be extremely thin and exhibit 'ribness/skeleton-marked' patterns over bony structures such as the ribs and pelvis (Dempsey 1955, 103; Reed 1973, 37). An early-20th century tanner remarked that *"everyone knows the feel of a starved skin …* [from those of] *good feeding and care"* (Thomas 1912, 29). Conversely, overfeeding and the consumption of supplementary feeds (such as root vegetables and protein concentrates) can lead to the deposition of excessive cutaneous fat, weakening the fibre structure, reducing the skins tensile strength and resistance to abrasion (Anon. 1947, 750; Reed 1973, 37; Henrickson e*t al.* 1984).

3.2.4 Environment

Stall-fed, indoor reared sheep are said to have a thinner skin than those reared outdoors; similarly, hill sheep have thicker and stronger skins than those reared in lowland areas (Reed 1973, 36-7). This may be due to thermal regulation, as the thickness of the skin is highly temperature dependent, increasing in thickness in cooler environments (Wodzicka 1958, 601-606; Wodzicka-Tomaszewska 1960; Jolly & Lyne 1970). Perhaps due to these variations, some regard the leather produced from animals reared in cool and moist climates to be of a higher quality than those from hot, dry countries (Reed 1973, 42; NIIR 2011, 17).

From the points raised above, it can be considered that the parchment makers 'ideal' skin comes from a <12-month-old, slow maturing hair-sheep or coarse-woolled lamb, which has been raised in upland pastures, receiving adequate nutrition and good care. Such ideals are not always possible, and the skill of the parchment maker lies in their ability to 'read' the skin and adapt the method of manufacture to suit its unique characteristics.

3.3 Parchment manufacture

Gullick (1991) has convincingly argued that the transformation of the wet sheepskin described above, into a dry durable sheet of parchment, should be divided into two separate processes: *manufacture* – from the skin's removal from the animal to its removal from the parchment makers frame; and *preparation* – during which the parchment is ruled and made ready to be written on. In post-medieval Britain, manufacture was undertaken by the skinner, fellmonger and parchment maker; and preparation by a stationer and scribe, although separation of these roles may not have always been clearly defined.

3.3.1 Flaying

Flaying is usually performed by a skinner or butcher shortly after the animal's death, prior to disarticulation of the carcass. It is essential that as little damage as possible is made during removal of the skin, as small cuts can become large holes during stretching. Some skinners, would therefore 'punch' the skin off by hand, which would result not only in a higher quality skin, but also more attractive cuts of meat (Price 1939, 18; Sharphouse 1971, 8-9). In early-modern France, many parchment makers would only procure skins from those who flayed by hand (Hagadorn 2012, 169).

Throughout the 18th century, skin processors petition parliament for legislation to prevent *"the gnashing and cutting of"* hides by negligent skinner, which rendered them unusable for either leather of parchment (Journal of the House of Commons 1801, 270; 1803, 294). In 1800 the 'Flaying Acts' (1800, 39 Geo. 3 c.66) imposed severe fines on those who damaged skins, but were considered oppressive by butchers (Anon. 1806), although favoured by most manufacturers (House of Commons 1837, 100-8). A report into the effectiveness of the legislation by the House of Commons, indicates that parchment makers were far more concerned with the flaws that arise from parasites than from flaying (1837, 106).

3.3.2 Curing

Once removed from the carcass, the skin is highly susceptible to putrefaction and bacterial attack. In as little as 24 hrs, the skin can be significantly damaged and discoloured, leading to a loss of tensile strength and prominent vein marking (Covington 2009, 72). If the skin cannot be processed immediately, the most common method of retarding proteolytic enzyme degradation historically has been curing with salt (Thomson 2006, 68). Sodium chloride is applied to the flesh side, dehydrating the skin, reducing the viability of bacterial activity. If salting is undertaken correctly, the skin can be left in this state for several months and is easily reversed through rehydration.

3.3.3 Rehydration and washing

The first job of the parchment maker or fellmonger is to rehydrate and wash the skin, removing detritus such as curing salts, dung, blood and plant material (Figure 3.4). This is achieved by immersion in clean water, and often accelerated with rotating paddle drums, pounding them with hammers, or treading them underfoot (Thomson 2006, 68). The skin must be completely rehydrated to ensure an even penetration of lime.

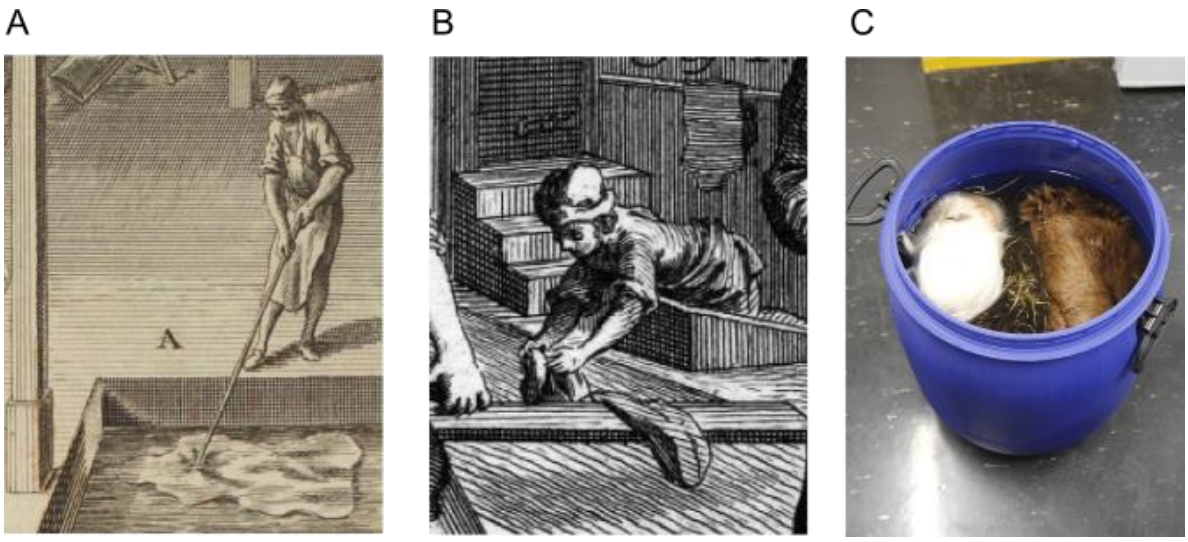


Figure 3.4: Rehydration and washing: A) Detail from Plate I *'Que représente le travail du Mégisserie'*, Lalande 1762, with skins washed in a pit of water; B) Detail from plate *'Chamoiseur et Mégisserie'*, Diderot & d'Alembert 1776, showing skins being washed in the river; C) Skins from this study rehydrating in a barrel of fresh water (by S. Doherty)

3.3.4 Liming and unhairing

One of the central processes of parchment making is the application of lime (Figure 3.5); derived from roasting limestone (calcium carbonate) to form quicklime (calcium oxide), which reacts with water (slaking) to produce calcium hydroxide. Liming serves a range of important functions, in particular: ceasing putrefaction; swelling the skin and opening up the collagen fibres; degrading the keratinous wool and epidermis; saponification of lipids; and the hydrolysis of peptide bonds and amide side chains in the controlled damage of the collagen (Covington & Alexander 1993; Menderes *et al.* 1999; Maxwell *et al.* 2006; Covington 2009).

The major chemical modification of collagen during liming is the hydrolysis of some amide groups attached to aspartic and glutamic acid residues (Menderes *et al.* 1999). The deamidation reaction converts amide function groups to carboxyl groups, liberating ammonia, lowering the isoelectric point of the collagen (Jones 2004, 31-32). The repelling effect of the negatively charged centres forces the collagen fibres apart, opening up the fibre network allowing the water and lime solution in (Menderes 2002, 53; Covington 2009, 140). This swelling, followed by manual squeezing on the beam during fleshing, aids the removal of non-collagenous proteins (such as albumins and globulins) and substrates (such as hyaluronic acid), resulting in a parchment made up almost entirely of collagen (Haines 1999, 22; Kennedy & Wess 2003, 70; Maxwell 2007, 40).

In the high alkalinity of the lime solution, the disulphide bonds in the keratinous fibre and epidermis undergo hydrolysis, facilitating unhairing. This reaction decomposes the young keratin at the base of the hair follicle, so that it is held in place by friction alone (Covington 2009, 119). The ability to remove the hair is traditionally appraised by the thumbnail test, to see if the pressure of the nail against the surface of the skin is sufficient to dislodge the hair. In a relatively weak solution this is achieved within 7 days, although the reaction can be accelerated by increased temperature and alkalinity. From the Victorian period onwards, additional depilatory agents ('sharpeners') such as sodium cyanide and arsenic sulfides, or now more commonly sodium sulfide or sodium hydroxide, have been added to accelerate keratin degradation, and for unhairing to be achieved in <48 hrs (Covington 2009, 122).

These processes irreversibly degrade the keratin of the wool, limiting the fibre's future use. Prior to the 18th century, when wool was of greater value than meat, fat or skin (Clark 2004), wool-saving methods of unhairing were used by fellmongers who removed the fibre for resale to the textile industry, before selling the skin directly to parchment makers (Houghton 1694 in Bradley 1727, 325-327; Lalande 1762, 2-3; Thomas 1983, 72; Thomson 2006, 71-2). The most basic of these is 'sweating', allowing the epidermis to decompose to the extent that the fibre detaches at the root and can be removed by hand. While the wool is undamaged by harsh chemicals, this uncontrolled method risks significant deformation to the surface of the skin and to the structural integrity of the collagen and other proteins (Covington 2009, 75-76), and will ultimately require the application of lime to cease putrefaction.

The more common and controlled method is the use of 'lime paint', where unhairing is driven from the flesh-side, without exposing the wool to the alkalies (Petrie 1995, 53-55; Covington 2009, 150). In this process, a thick lime paste is applied to the full extent of the flesh-side. The strength must be sufficiently high to penetrate the skin by diffusion alone, and is usually made with a concentration of >75% calcium hydroxide, although commercially available paints often contain sodium sulfide

and sodium hydroxide (Petrie 1995, 52-53; Covington 2009, 130). The skin can usually be unhaired in 24 hours, considerably faster than when immersed in a lime solution, although it can cause lime-burns to the skin due its high concentration. The additional benefit of this technique is that once clear of wool, the condition of the skin can be more accurately assessed and the appropriate end product selected. At Messrs. Russell Leather & Parchment Works, Hitchin, Herefordshire, in the early-20th century, a 'puller' removed and graded the wool, after which a 'sorter' inspected the skins and selected those with the least imperfections for parchment making, and the remaining for different types of leather goods (Hampshire County Council 2008).

A further alternative to unhairing with lime and sharpeners is enzymatic dehairing, with the use of proteolytic enzymes obtained from bacterial fermentation to degrade the keratin and epidermis (Covington 2009, 128-130). Like sweating, this is a high-risk process as it can result in the degradation of collagen and non-structural proteins (such as elastin) too. Enzymatic unhairing has been tried by the author, and proved successful in digesting the wool, but then ultimately the entire skin.

Figure 3.5: Liming and unhairing: A) Detail from Plate I, Lalande 1762, showing the skin being placed in a lime pit with the aid of long-handled tongs; B) Photo from Havant Parchment Makers 1928, removing skins from the lime pits after treatment (Hampshire County Council 2008); C) Lime barrels from this study, of both new and 'old' lime (by S. Doherty)

3.3.5 Fleshing

Cutaneous and subcutaneous triglyceride are removed during manufacture through chemical and mechanical processes (Figure 3.6). Chemically, triglycerides esters undergo alkaline hydrolysis during liming, releasing fatty acid salts and glycerol (saponification), which are leached out into the lime solution. As much as 50% of the total lipid content of the skin is removed during liming (Koppenhoefer 1938; 1939; McLaughlin and Theis 1945), with more likely removed during washing,

deliming, shaving and degreasing. Despite this, sheepskin parchment can have in excess of 7% lipid content (Ghioni *et al.* 2005).

Mechanically, the skin is placed over the beam at an angle of around 45º, and the fat and flesh manually removed with a sharp, double-handled knife. Effort must be taken not to cut the skin, as shallow cuts can tear during stretching. Fleshing is usually conducted after unhairing, as the skin needs to lay flat on the beam; however, as large subcutaneous fat deposits impede the penetration of the lime, excess fat can be removed before liming, known as 'green fleshing' (pers. comm. J. Vnouček). In 1809, a leather-splitting machine was invented that could split the skin into any desired thickness. The skin was split with a band-knife moving between two rollers, producing an upper 'grain split' and a lower 'flesh split'. This technique is predominantly used on thicker cattle hides, but was used for sheepskins, when the upper 'grain split' was known as a 'skiver' (The Society for the Diffusion of Useful Knowledge 1842, 214).

Figure 3.6: Unhairing and fleshing on the beam: A) Detail from Plate I, Lalande 1762, showing the unhairing of the skin; B) Still from '*The Romance of Leather'*, capturing workers from Richard Hodgson & Sons Tannery, Beverley, E. Yorkshire, fleshing skins on the beam; (Debenhams 1932) C) A skin from this study being unhaired and fleshing in the same manner using the traditional double-handed curved knife (by S. Doherty)

3.3.6 Deliming

Once unhaired and fleshed, the pH must be lowered to neutralise the alkalinity from the liming process. This has traditionally been achieved by washing the skin in clean water for around 72 hrs (*Schedula diversarum atrium*, MS Harley 3915. Fol 1282, c.12th century; Houghton 1694 in Bradley 1727, 326-7; Lalande 1762; Saxl 1954; Reed 1973). The process can be accelerated through the addition of acid, which in the early-modern period may have included acetic acid (vinegar) or acidic salts (such as sodium bicarbonate), while stronger agents such as formic acid are used today. The

deliming stage is where parchment and leather manufacture diverge, where the skin would now be subjected to vegetable or mineral tanning agents.

3.3.7 Tensioning and drying

The neutralised skin is stretched and dried under tension (Figure 3.7). Traditionally, the skin is attached to a rectangular or circular frame by ropes, but can be stretched by a variety of means such as weights or pinned to a board. Effort is taken to preserve symmetry and apply a uniform tension, although the same level of stretching cannot be achieved across the entire skin. Of those measured in this study, the skin decreased in thickness on average by 50%, while neck to tail lengths increased 24%, and shoulder width increased by 14%. This considerable pressure forces out moisture, and has the effect of altering the orientation of the collagen fibres in the skin to become more parallel to the surface (Kennedy & Wess 2003, 70).

3.3.8 Shaving and pumicing

Once dry on the frame, effort is made to further reduce the parchments thickness via shaving. Using a sharp lunar-shaped knife (lunellum), the skin is shaved in one direction slowly reducing the thickness (Figure 3.8). Shaving is usually conducted on both the flesh- and hair-side, with the result of producing a dermis-rich final product (Kennedy and Wess 2003, 70). Shaving can leave scratches on the surface due to small nicks in the lunellum. Therefore, shaving is often followed with pumicing, which helps to smooth out the parchment surface.

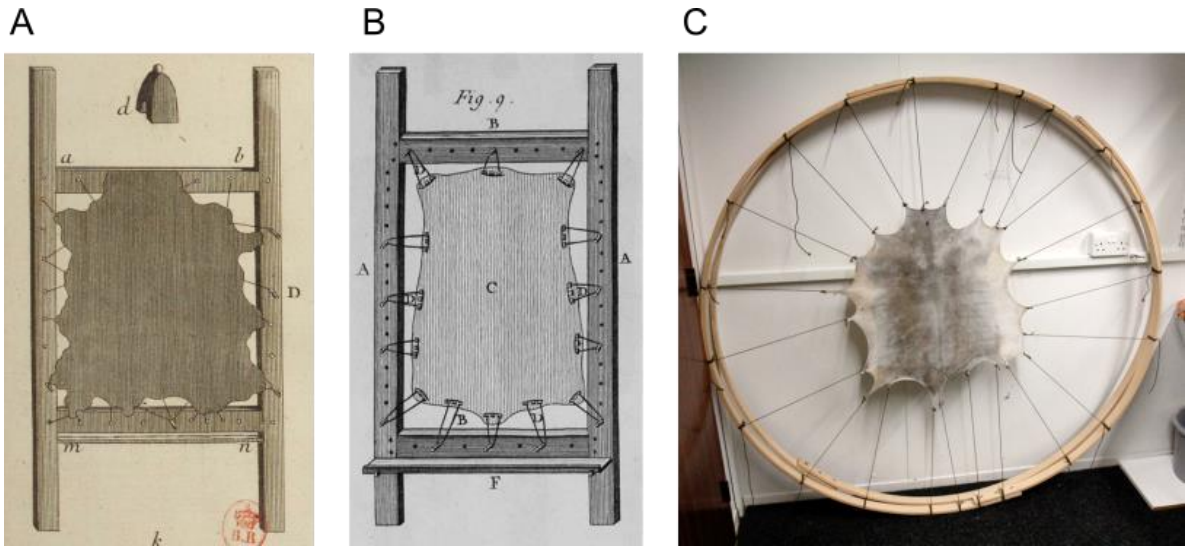


Figure 3.7: Tensioning and drying: A) Detail from Plate II *'Que représente le travail du Parcheminier'*, Lalande 1762, showing a rectangular frame or 'herse', and the skin attached by individual anchors on the skin; B) Detail from Plate 'Parcheminier', Diderot & d'Alembert 1776, depicting a rectangular frame, with the skin anchored via skewers threaded through the skin; C) A sheepskin from this study drying upon a circular frame in the classical Italian style, which distributes an even tension. Here the skin is anchored via individual stones in the skin (by S. Doherty)

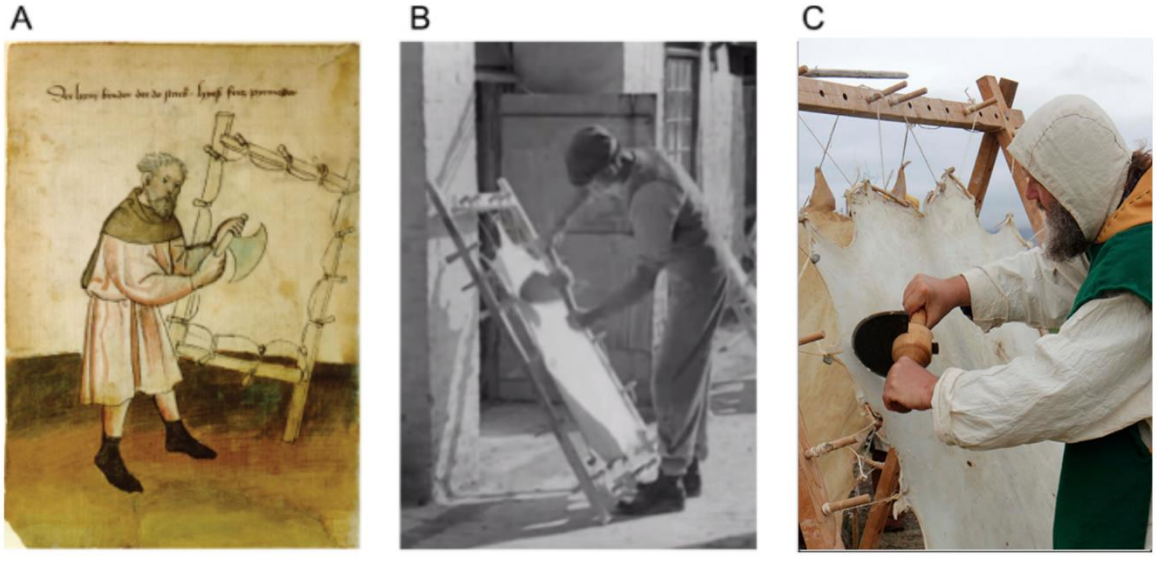


Figure 3.8: Shaving the parchment on the frame: A) Depiction of a parchment maker shaving the skin with a lunellum, *Folio 34 Mendel Housebook* c.1425, *Amb. 317.2*; B) Still from *'The Very Idea'*, 1939 (British Pathé) from a parchment makers in Brentford, Middlesex; C) J. Vnouček embodying Mendel's parchment maker nearly 600 years later (courtesy of J. Vnouček)

3.3.9 Degreasing

Despite the removal of fat during liming, sheepskin parchment can often retain a significant lipid content, which can produce discolouration upon oxidation (yellowing), inhibit the application and drying of ink, as well as potentially damage the collagen (Saxl 1954; Reed 1973; Ghioni et al. 2005; Strlič et al. 2009; Možir et al. 2014). In a particularly greasy skin, yellowing can be seen across the entire sheet of parchment; however, it is more common in localised spots, particularly around the rump. To draw the grease out, parchment recipes from the 15th century onwards recommend the application of various absorbent (predominantly alkali) materials, including lime powder, chalk, gesso, soda, wood ash, and powdered bone and horn (Saxl 1954; Reed 1973). This material would be worked into the skin using a pumice stone and left for a few days, before being brushed off. By the 18th century, presumably in response to increasingly greasier skins, parchment makers often degreased using boiling water, which was known as sizing or scalding (Saxl 1954; Ryder 1964, 395; Hampshire Country Council 2008) (Figure 3.9). In this process, skins would be rehydrated and placed in a warm oven, after which boiling water was poured across both the flesh- and hair-side, followed by scraping with the lunellum (Saxl 1954). This would not only have helped draw out grease, but also partially gelatinise the skin resulting in a uniform surface more receptive to ink.

Figure 3.9: Degreasing: A) Still from *'The Very Idea'*, 1939 (British Pathé) showing boiling water being poured onto both the hair- and flesh-side of the parchment while still on the frame, followed by scraping; B) Photo of degreasing and scraping with the aid of boiling water at Messrs. Russell Leather & Parchment Works, Hitchin, Hertfordshire, c. 1930s (Hampshire County Council 2008); C) In this study, chalk was also applied to the flesh-side to help draw out the grease and improve the whiteness of the finished parchment (by S. Doherty)

3.3.10 Parchment preparation

Parchment for deeds requires little preparation after being removed from the frame, although the role of stationers and the production of blank indentures will be explored further in Chapter 4. The skin would be cut to the required size, pricked and ruled to demarcate the location of the text, and rolled ready for sale. Additional motifs became common by the 19th century, particularly double line borders in vermillion (mercuric sulphide) and ornate headers and crests in iron-gall ink.

3.5 Summary

In sum, sheepskin and the resulting parchment are complex materials which reflect many characteristics unique to the individual animal. The method of parchment production was poorly documented until the late-18th century, and the increased use of industrial machinery and additional alkalies since this period means that we remain remarkably ignorant of the precise means of early parchment production. However, the majority of the skins analysed in this study were produced during the 17th and 19th centuries, during which time the method of production became increasing standardised.

Chapter 4

Species identification of parchment deeds using peptide mass fingerprinting

This chapter presents the species identification of the parchment deeds used in this study via peptide mass fingerprinting. This absolute determination allows for greater confidence in the interpretation of isotopic data in later chapters, as well as providing valuable insight into the selection and provisioning of skins for deeds. Previous visual and biomolecular research into the species used will be examined (4.2), before building upon these studies through the analysis of 663 skins from the 13th to 20th century using peptide mass fingerprinting analysed by MALDI-ToF MS. The results (4.4) will be used to explore the preferential selection of sheepskin, and the provisioning of skins through the analysis of 18th and 19th century legal stationers accounts (4.5).

4.1 Introduction

In conversation with Horatio, Shakespeare's Hamlet ponders legal protection, and the skins on which these legal assurances are written:

> HAMLET: Is not parchment made of sheepskin?
> HORATIO: Ay, my Lord, and of calves' skin too.
> HAMLET: They are sheep and calves which seek out assurances in that …

(Shakespeare 2005, *Hamlet* 5.1.114-7)

Despite the millions of legal parchment deeds produced, uncertainty remains over the species used, with legal documents frequently catalogued as 'vellum' (etymologically meaning calfskin), 'parchment', or even more general 'animal membrane' (Figure 4.1). Previous species identification

via visual, genetic and proteomic analysis (Ryder 1960a; 1962; Teasdale *et al.* 2015; 2017) suggests that Horatio's addition may have been misguided, as deeds appear to have been written almost exclusively on sheepskin parchment. The corpus of identified material is, however, extremely limited, with only eight skins currently identified using biomolecular techniques. Consequently, we know very little about the selection of skins, or how they made their way into the hands of lawyers and their clients.

Figure 4.1: Example of a typical parchment deed. This indenture concerns the renal of land in Ovington, Hampshire, between Messrs. G. Pollard and T. Yeomans. Signed and sealed 8th March 1741 (photo by S. Doherty).

4.2 Previous species identification of British parchment deeds

Previous visual, genetic and proteomic analysis of British medieval and post-medieval deeds indicates that they were predominantly written on sheepskin parchment. Histological and microscopic follicle analysis of 200 deeds from the 13th to 19th century identified 195 as sheep (97.5%); 1 as goat (0.5%); 1 as sheep or goat (0.5%); 1 as calf (from a book cover) (0.5%); and 2 as indeterminate (1%) (Ryder 1960a; 1962, 170). Of those identified as sheep, most contained hair or coarse-wool fibres, with many classified by Ryder as the skins of the Soay and Scottish Blackface breeds (1960a, 131; 1962, 170). This is perhaps unsurprising considering the preferred qualities of the skin that are possessed by breeds of a coarser fleece, such as the Soay's low follicle density and low cutaneous fat content (Ryder 1966; 1971; Clutton-Brock *et al.* 2004, 28), but might suggest that less 'improved' breeds were preferentially selected for parchment. Less rigorous visual assessment of important legal documents such as the Magna Carta (AD 1215) and Treaty of Versailles (1919)

indicate they too were written on sheepskin (Vincent 2012, 1; Cousins 2017; British Library n.d) as well as contemporary Irish deeds (Lévêque 2013).

While the PCR method of mtDNA amplification and sequencing have proved effective in identifying the species used for parchment (Woodward *et al.* 1996; Bar-Gal *et al.* 1999; Poulakakis *et al.* 2007; Burger *et al.* 2000; 2001), its application to British legal deeds has been less successful. Campana *et al.* (2010) found that the majority of 18th and 19th century skins examined produced mtDNA products from multiple species, which was attributed to cross-contamination incurred during their manufacture, preparation or storage. Teasdale *et al.* (2015; 2017) has however, highlighted the PCR method's bias for amplifying less damaged contaminant over endogenous DNA, and demonstrated that high resolution endogenous nuclear DNA can be obtained from parchment. At present, DNA sequences have been obtained from twelve 14th to 19th century deeds, including ecclesiastical oaths attached to the York Gospel, and confirmed that they were all made from sheepskin, but also that they showed genetic affinity to modern blackface-types, such as the Scottish Blackface and Swaledale breeds, and the Border Leicester (Teasdale *et al.* 2015, 4-5; 201). Like DNA-based methods, peptide mass finger printing has been used to provide an absolute determination of the species used for parchment (Kirby *et al.* 2013; Teasdale *et al.* 2015; 2017; Fiddyment *et al.* 2015; *forthcoming*). This technique has been used on legal deeds by Teasdale *et al.* (2015; 2017) in conjunction with their genetic analysis, and confirmed that they all are sheepskin.

4.3 Material & Methods

4.3.1 COL1a1 extraction and analysis

Samples were obtained from 663 individual parchment membranes from a total of 497 legal documents. Of those with multiple membranes, each sheet was of a size to indicate that they came from a single animal (>70x50 cm). The majority were engrossed with the date the legal agreement was signed (day, month and year), apart from 18 dated more broadly to the 13th century. None of the documents had received any conservation, or presented evidence that the parchment had been reused. Destructive sampling was undertaken in conjunction with sampling for isotopic analysis (see Chapter 8). A sample (~1x0.5 cm) was removed from the edge of each membrane, from an area devoid of any ink, pencil, stamp, glue or surface marking to avoid contamination.

Samples were processed following a standard protocol used at the Department of Archaeology, University of York, for the analysis of collagenous archaeological material (see Welker *et al.* 2015a; 2015b; Charlton *et al.* 2016; Fiddyment *et al.* 2015). Samples were placed in individual 15 mL

microcentrifuge tubes, 57 µl of 0.05 M ammonium bicarbonate (NH_5CO_3) buffer added, along with 1 µl of porcine trypsin (0.47 µg/µl) (Promega, WI, USA) and incubated for at 37°C for 18 hrs. After incubation, 1 µl of trifluoroacetic acid (TFA) (5% vol/vol) was added to cease enzymatic digestion. The digest was desalted and purified using C_{18} solid-phase tips (Agilent ZipTip, CA, USA), and the peptides eluted in a final solution of 50 µl, 50% acetonitrile/0.1% TFA (vol/vol). 1 µl of eluted peptides was mixed on a ground steel plate with 1 µl of α-cyano-4-hydroxycinnamic acid matrix solution [1% in 50% ACN/0.1% TFA (vol/vol)] and allowed to co-crystallise. All samples were spotted in triplicate. Samples were analysed using a Bruker Ultraflex II (Bruker Daltonics, Bremen, Germany) MALDI-TOF instrument equipped with a Nd:YAG smart beam laser. Samples spectra were calibrated against an adjacent calibrant spot with six calibration peptides. The resulting mass spectra was analysed within mMass software (www.mmass.org) (Strohalm *et al.* 2010), and individual peptides manually identified according to published markers (Buckley *et al.* 2009; Buckley *et al.* 2010). Separation between sheep and goat, for example, was made using the reported peptide markers observed at *m/z* 3033.3 ± 0.2 (*Ovis aries*) and 3093.3 ± 0.2 (*Capra*) (Figure 4.2).

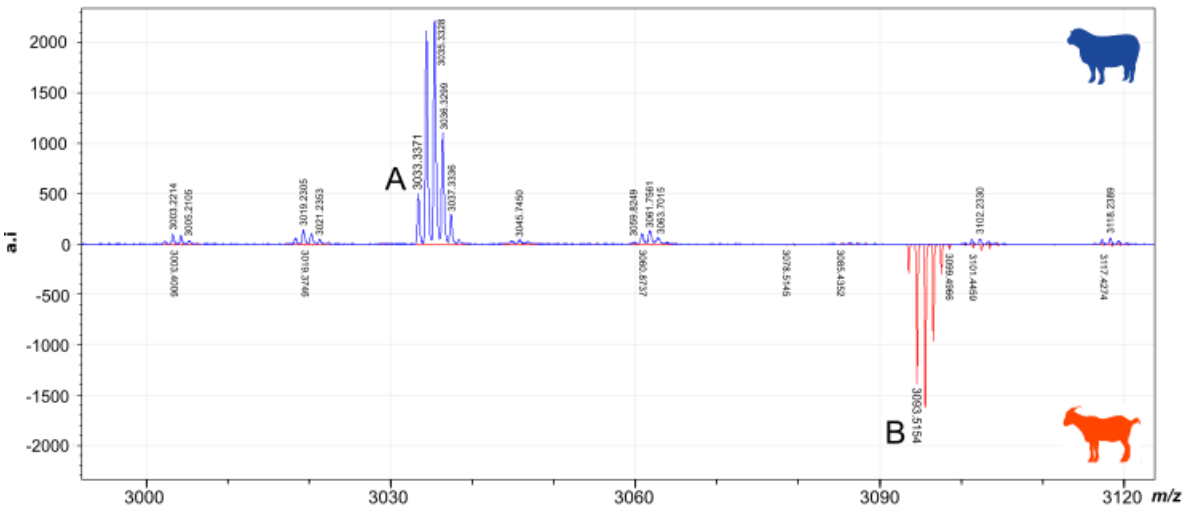


Figure 4.2: Example MALDI-ToF mass spectra demonstrating the separation between sheepskin (top) and goatskin parchment (bottom), using published sheep (A) and goat (B) markers (Buckley *et al.* 2010). Spectra range from 3000 to 3120 m/z, with intensity scales relative to the most intense peak in range. Signal to noise ratio of 3.0

4.4 Results

All 663 samples were identified as animals of the Bovidae family, of which 640 (96.5%) were identified as sheep. The remaining 23 (3.5%) were classified as sheep/goat, as discrimination between the species was not possible due to the lack of diagnostic peptides. Protein survival decreases

over time (Welker 2018, 142) and in parchment can be further degraded via oxidation, hydrolysis and biological attack during storage (Badae *et al.* 2012; Brock 2013, 353-4). In these twenty-three samples, it is likely that protein degradation affected the presence of diagnostic peptides. This highlights the potential limitation of this technique, and the continued role that fibre and follicle analysis has in the identification of historic parchment. It is highly likely that most, if not all samples are sheepskin, but acknowledging the identification of goat fibres by Ryder (1960a) and the suggestion of goat DNA by Campana *et al.* (2010), its presence cannot be ruled out.

4.5 Discussion

4.5.1 The use of sheepskins for legal deeds

The results of this study, along with previous species identification, indicate that for the production of parchment legal deeds, sheepskin was predominantly used. Their use ranged in status and date from the great Magna Carta in AD 1215, to the modest mortgaging of a property in Hull in 1969. Although de Hamel (1992, 8) contends that neither the scribe or recipient knew nor cared what animal it was made from, the evidence suggests otherwise; sheepskin was preferred over that of calf or goat. In the 12th century York Gospels Codex (*York Minster MS. Add. 1*), for example, despite being originally written entirely on calfskin, later 14th century additions, containing ecclesiastical oaths and land surveys, were written on sheepskin parchment (Teasdale *et al.* 2017).

This preference for sheepskin was likely due in no small part to their great abundance and low cost. Throughout the medieval and post-medieval period, domestic sheepskins were of more than sufficiently abundant to meet the demands of British skin processors (Clarkson 1989, 471). Contemporary and recent estimates place the sheep population between 10 and 17 million across the 13th to 17th centuries, increasing to 11 to 14 by the early-18th century, 17 to 20 million in the early 19th century, and continuing to grow to over 25 million by the late-19th century (Fussell & Goodman 1930; Turner 1998; Broadberry *et al.* 2015, 100-5). With an average culling rate of around 20% during this period (Clarkson 1989, 471; Clark 1991, 218), roughly 2-5 million sheepskins would have been yielded annually. In comparison, the goat population of Britain has historically been very low (Albarella 2003, 80-1; Dyer 2004), and the total number of calves is unlikely to have exceeded 1 million until the late-19th century, of which only a few hundred thousand skins may have been yielded annually (Broadberry *et al.* 2015, 106). Despite this abundance, sheepskins were imported from both Ireland and mainland Europe (Armour 1956, 273; Clarkson 1989, 472), as well as finished parchment from France, which was considered superior to that from other parts of Europe (Mortimer 1810; Willement 1845, 42).

The limited supply of calfskin, and its perceived higher quality, meant that vellum was more than double the price of sheepskin parchment (Rogers 1866, 643-3; 1881, 545-604; 1887, 600-2). Even the finest quality sheepskin was cheaper than the poorest quality vellum (Gullick 1991). For example, in 1593, a dozen sheets of parchment cost on average 8*s*, while the same amount of vellum was more than double at 20*s*; by 1660, a dozen sheets of parchment cost 10*s* and vellum 28*s* (Rogers 1881, 607). For common legal documents, cheaper sheepskin parchment presented the ideal inexpensive and durable material.

An additional factor behind its use, specifically for deeds, might be the increased visibility of ink erasure and text alterations afforded by sheepskin. *Dialogues de Scaccario*[6] (c.1178, In Henderson 1912, 47) recommends the use of sheepskin parchment as scraping and shaving to remove ink will leave a visible deformation. This is likely due to the high potential for sheepskin to delaminate as a result of significant cutaneous fat content at the *papillary-reticular* junction (Covington 2009, 33; Michel 2014, 35). The thinner sheepskin is also more likely to produce a transparent blemish with shaving, because there is less dermis to remove. It is unlikely that this was the primary motivation behind the use of sheepskin, particularly during later centuries, but may explain the roots of this preference.

4.5.2 Procurement and distribution of parchment deeds

While parchment has been manufactured in Britain since the 6th century, production was largely restricted to monastic scriptoria, producing religious texts upon the skins of their own livestock (Alexander 1978; Gullick 1991; De Hamel 1992, 5-11). It was not until the growth of the book trade in the 14th century that secular parchment production expanded, often within small villages and towns close to water sources, making use of local skins (Lyall 1989, 11; Da Rold 2011, 17-24). Production flourished across the country, although unlike the extensive distribution of tanneries and associated leather crafts (Sargent 1938; Clarkson 1960), manufacture was heavily concentrated near the urban and educational centres of central and southern England, and around the extensive sheep farming regions of the Cotswolds, Chilterns and North Wessex Downs (Trow-Smith 1957, 78-9; 1959, 11-12, 123-6, 198; Perry 1973, 61; Hall 2014, 56-7) (Figure 4.3). Suitable skins with minimal abrasions and fat were likely sourced locally, and the finished product purchased from the maker. However, by the 16th century, dedicated parchment sellers and stationers were increasingly common, and may have been the primary channels through which parchment was acquired (Peek & Hall 1962, 58; Chance *et al.* 1979; Black 1984, 8).

[6] 'Dialogues concerning The Exchequer', attributed to Richard FitzNeal (1130-1189), Lord Treasurer and Bishop of London.

The role of stationers increased in the late-17th century with the introduction of Stamp Duty, which restricted the supply of stamped parchment and paper to around fifty registered distributors (Hughes 1941). In 1694, during the reign of William & Mary, the Stamp Act came into force *"granting to their Majesties several duties upon vellum, parchment and paper, for four years, towards carrying on the war against France"* (5 & 6 Wm. & Mar. c.21). The Act required a revenue stamp to be fixed to every individual sheet of the deed prior to engrossing (Dagnall 1994). Stamping (or escutcheoning on skin) could only be carried out at the Stamp Office, Lincoln's Inn, London, or one of the regional stamp distributors (Hughes 1941, 246; Dagnall 1994), concentrating the supply to a few dozen stationers who provided deeds and a range of material and service for the legal profession (Figure 4.3).

This taxation coincided with new methods of mortgaging and leasing in the 17th and 18th centuries (North 1981, 158-170), which brought increased business to legal firms, and new orders to stationers, engravers and printers (Raven 2014, 94). To keep up with demand, legal stationers provided blank indentures and copying services to help guarantee accuracy and efficient replication of legal documents (Raven 2014, 94). The method of preparing blank indentures is unknown, but stationers appear to have purchased finished 'skins', which they ruled, pricked and preliminarily engrossed, and then sold to lawyers. The procurement of parchment and distribution of deeds will be explored here through the unpublished accounts of London based stationers Witherby & Co[7], and the regional stationer John Clay, Northamptonshire[8].

[7] London Metropolitan Archives (LMA/4682)
[8] Northamptonshire Records Office (NRO/ML)

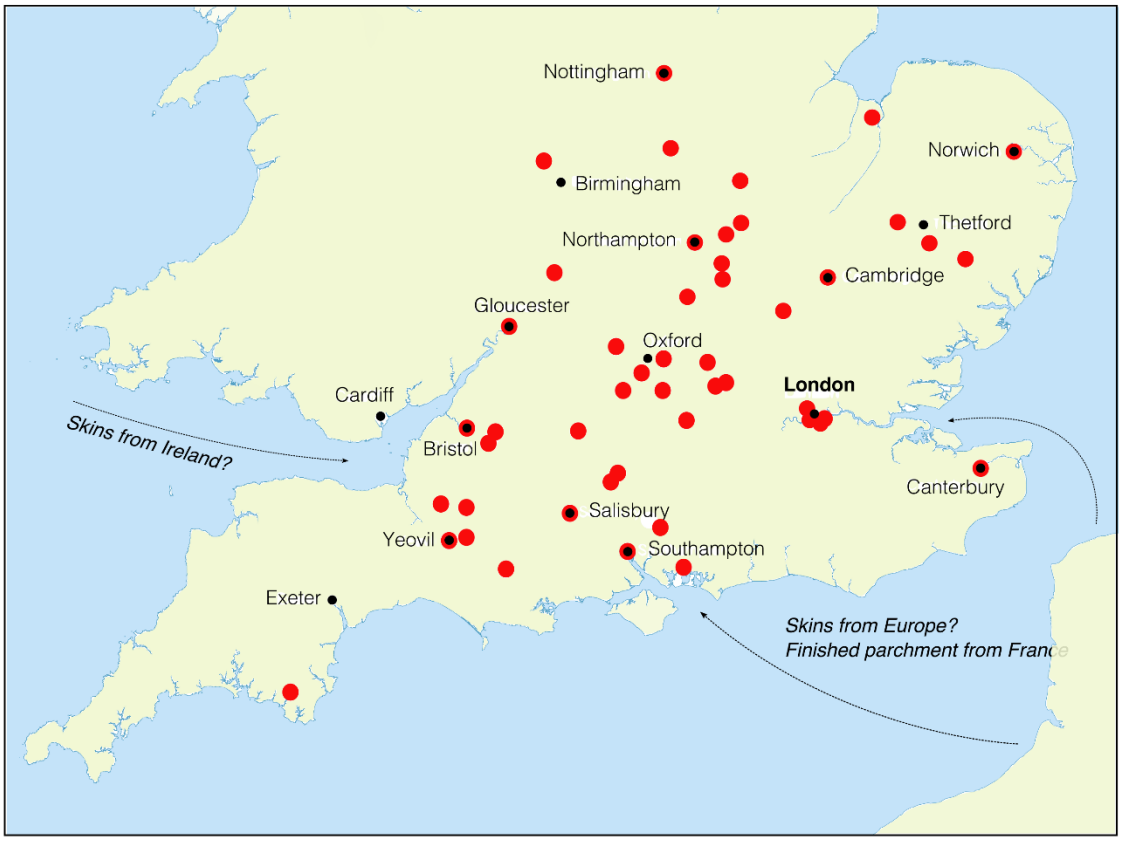


Figure 4.3: Geographical distribution of 16th to 20th century parchment makers (NA/PCC/PROB 11; Bailey 1784; Kelly 1936; Maxted 2014) (by S. Doherty)

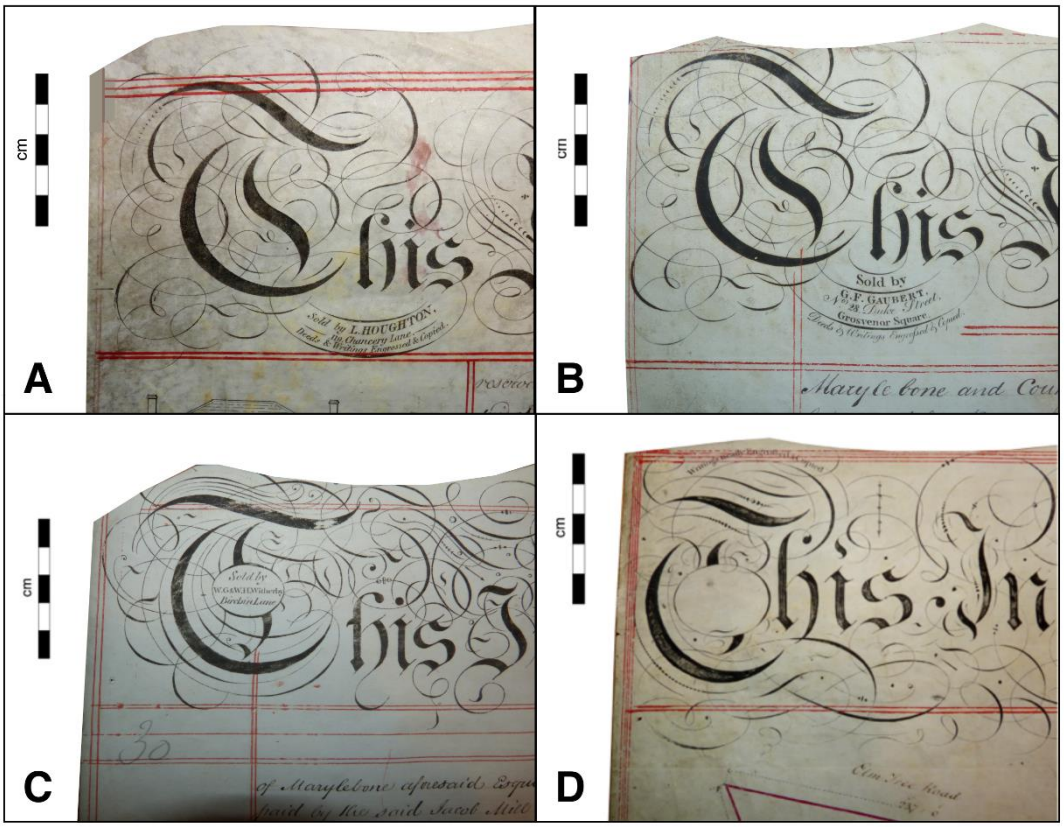


Figure 4.4: Stationers marks on indentures: A) 1822. *'L. Houghton, 119 Chancery Lane. Deeds & Writing Engrossed & Copied';* B) 1822. *'G.F. Gaubert, No. 28 Duke Street, Grosvenor Square. Deeds & Writing Engrossed & Copied';* C) 1824. *'W.G. & W.H. Witherbys, Birchin Lane'.* D) 1821. Space for stationer's mark left blank (photos by S. Doherty)

Witherbys & Co., Birchin Lane, London

Established by Thomas Witherby in 1740, Witherbys & Co. was a legal stationer, which sold parchment, paper and sundries, along with making formal copies of legal documents (LMA/4682/006). Located within the financial district of London, the company grew during the 18th and 19th centuries, undertaking legal work for merchants, ship owners and the Lord Chamberlain of London (LMA/4682/C/06/002; LMA/4682/I/01/001). Witherbys was a considerably large operation, selling in excess of 11,000 stamped parchment skins annually (LMA/4682/E/01/001). Between 1795 and 1806, four parchment makers are recorded in the accounts as supplying the company with finished parchment (LMA/4682/C/04/001; LMA/4682/C/04/002): Noah Crook of Oxfordshire, Thomas Crook and Thomas Rake of Wiltshire, and Samuel Bishop of Bristol (Figure 4.5).

The Crooks of Oxfordshire and Wiltshire were an extended family of skin processors during the 18th and 19th centuries (Maxted 2014; *The Jurist* 1845, 69). Noah Crook was a *"fellmonger, parchment maker and tanner"* who at various times worked in Abingdon and Marlborough, Wiltshire, and in Wheatley, Oxfordshire (NA/PROB/11/1698/337). While in Wheatley, he occupied premises at the eastern of the High Street by the River Thane, an area surrounded by other animal-based industry and butchers (Fox 1997, 22), through which the skins for parchment may have been acquired. These locations were well supplied with skins from the large-scale sheep farming of the North Wessex and Hampshire downs, and well connected to both Oxford and London (Lobel 1957; Crowley 1983). Noah appears to have been a skilled parchment maker, and was supplying skins directly to parliament (Lobel 1957) and large monthly orders to Witherbys. His brother Thomas Cook of Marlborough (N.A./PROB/11/1698/337), also supplied Witherbys with finished parchment around three or four times a year.

Samuel Bishop operated out of Temple Street, Bristol, on the banks of the River Avon, as a skinner, fellmonger and parchment maker (Bailey 1784; Maxted 2014). Bishop is likely to have obtained his skins from the large number of animals processed on Temple Street (Anon. 1833, 123-130), or from sheepskins imported from Ireland through the port of Bristol (Lough 1916; Clarkson 1965; Jones 1998). He supplied Witherbys with parchment two or three times per year, although of a value considerably lower than the Crooks. Little is known about Thomas Rake of Wiltshire, but the ledgers indicate he supplied a significant value of parchment, but only once or twice a year. Supply from these four was staggered throughout the year, with rolls of parchment received most months to keep up with demand.

With regards to Witherbys' customers, the daybooks and ledgers indicate that parchment was purchased regularly, but in limited quantities. Regular customers such as Mr Andrews or Mr Cracraft purchased between two and ten skins every fortnight, including multiple purchases in a single week (LMA/4682/C/06/002; LMA/4682/I/01/001). Purchases greater than a dozen skins were very rare. The largest customer by values was the Office of the Lord Chamberlain of London, who purchased thousands of stamped skins annually, although again, rarely in bulk. In a typical month, such as September 1768, for example, two hundred and ninety-eight stamped skins were purchased across eighteen separate days, ranging from just two on the 13th, to one hundred on the 15th.

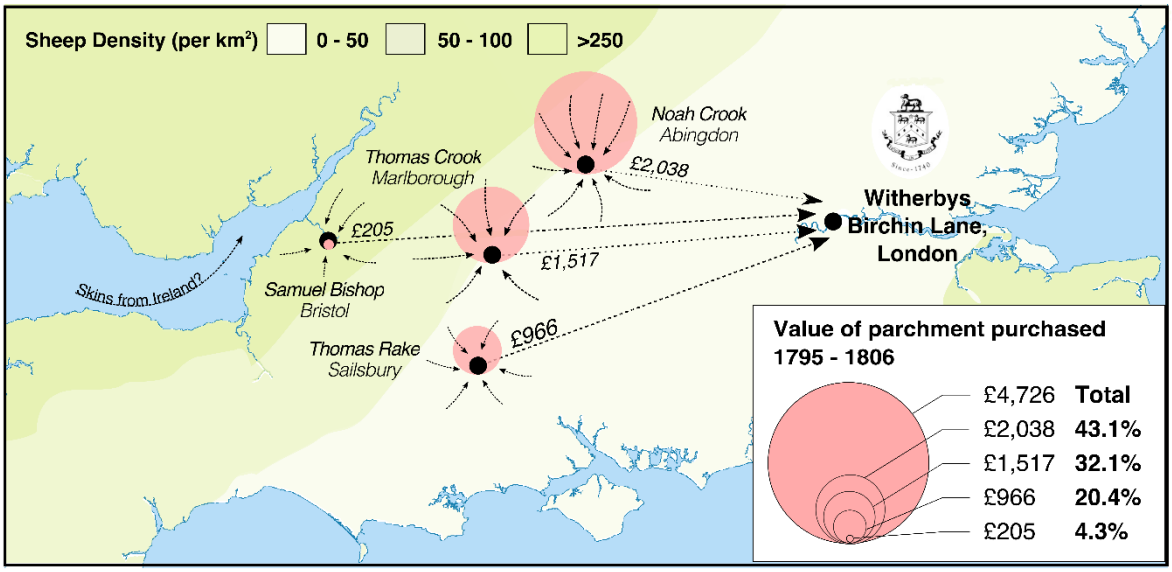


Figure 4.5: Location of parchment makers supplying Witherbys Stationers, London, 1705-1806. Mapped onto modern sheep density data (FAO 2014)

John Clay, Daventry

In contrast to this large London based company, general stationer John Clay operated small premises in Daventry, Lutterworth and Rugby between 1742-1775, supplying paper, books and magazines to the inhabitants of these market towns. While the majority of his business was in paper, Clay was an approved distributor of stamped parchment (Feather 1984, 155), and purchased skins from five parchment makers between 1742-1779[9] (N.R.O. ML/689; D2927) (Figure 4.6). Of these, the two main suppliers were Isaac and Bridgett Abel of Coventry, located on Spon Street on the outskirts of the city, close to the River Sherbourne (N.A./PROB/11/1666/104; Maxted 2014). Spon Street was an area of diverse animal-based industry, as well as a stopping destination for Irish sheepskins which had come through Chester destined for London (West 1830; Armour 1956, 273; Pythian-Adams 1979, 161; Veale 2003, 61), and is therefore likely that the Abel's utilised both imported and local skins for parchment. A small amount of sheepskins were purchased from Charles Franklin of Butcher Row, later Temple Bar, London (Leigh 1759), although he mainly supplied Clay with vellum and 'red Morocco skins' (N.R.O. ML/689). Thomas Grimmit, Shipston-on-Sour, and Francis Smith, Leicester, are also listed in Clay's directory of country suppliers (N.R.O. D2927), but no transactions are recorded in the accounts.

As with Witherbys', John Clay's customers purchased stamped parchment little and often (N.R.O. ML/010; ML/088; ML/089; ML/691; ML/692; ML694). Local lawyers such as Messrs. Caldecott

[9] The shops continued under his son Samuel after his death in 1775.

and Harris[10] purchased one or two skins at a time, with the largest order being for a dozen skins. Another typical customer, Mr Harrison, purchased stamped skins on December 6th 1764, one more on December 22nd, and a further two on January 2nd 1765 (N.R.O. ML/691). The location of Witherbys' or Clay's legal customers, or the final recipients of the engrossed deeds is unknown, however, seven of the skins used for proteomic and isotopic analysis in this study were sold by Witherbys[11]. All concerned the sale or rental of land in St Johns Wood, London, and were once part of the Sun Fire Insurance Archives. This research suggests that although the deeds involved land and clients in London and were purchased from a stationer in Cornhill, the animals were most likely raised and processed many hundreds of miles west of the capital. This disconnect between the location of the live animal and the finished parchment is undoubtedly the case for most deeds, and provides caution for later geographical interpretations of isotopic results. With the transport developments of the 18th-19th centuries, particularly the advent of steamships and steam locomotives, the distance which animals and animal products moved increased exponentially (Trow-Smith 1959, 323-4; Perren 1989, 191-199).

With regards to the time lag between the death of the animal and the date the deed was signed, these accounts indicate that this may have been relatively brief. The regular supply of finished parchment throughout the year, and the purchase of deeds in small quantities, as and when required, suggests that neither the parchment maker or stationer, nor solicitor, stored the skins for any great length of time. For the later isotopic analysis of these deeds, a conservative estimate of no more than two years between the date of death and date of signing should be considered.

[10] Identified as lawyers by Feather (1984, 208), but also in receipt of legal periodicals from Clay (N.R.O. ML/088; ML/089)

[11] 1822: RT086, RT087; 1824: RT071, RT072; 1825: RT049, RT050, RT051,

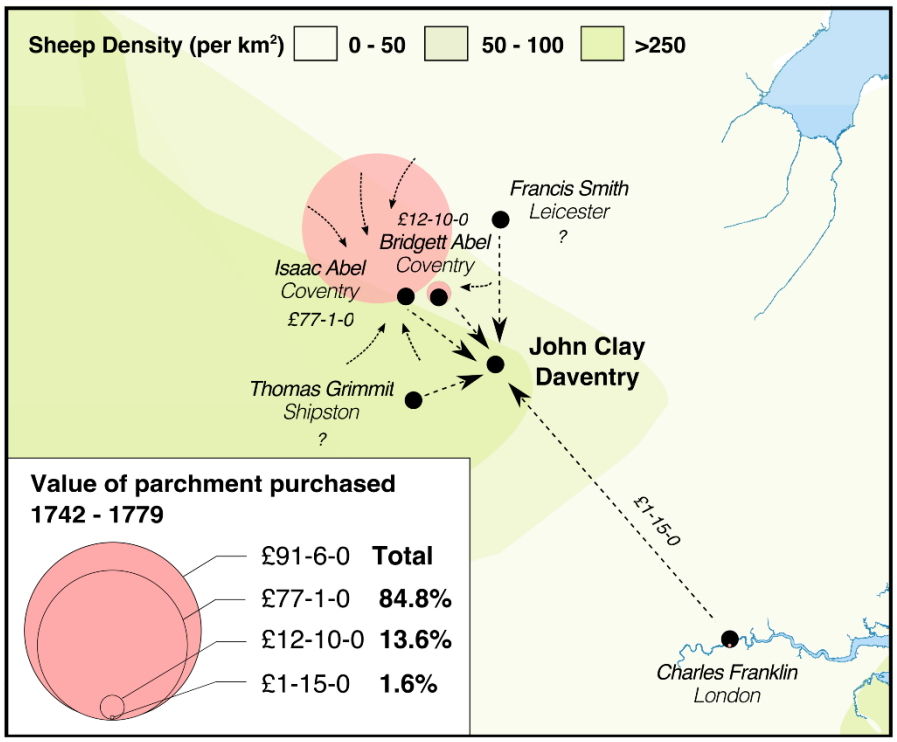


Figure 4.6: Location of parchment makers supplying John Clay, Daventry, 1742-1779, mapped onto modern sheep density data (FAO 2014)

4.6 Summary

The results and analysis presented in this chapter indicate that legal deeds were preferentially written on sheepskin parchment. These findings support the results of previous attempts to identify the species use through visual and genetic analysis, and highlight a continuity of use from the 12th to 20th century. Documentary records concerning the provisioning of skins for deeds indicates that both domestic and imported skins were likely used, and that the finished parchment could travel a considerable distance. This chapter provides greater confidence in the interpretation of later isotopic data through secure species identification, and knowing that the date the deed was signed is likely very close to the date the animal died.

Chapter 5

Examining the impact of parchment production on skin collagen stable $\delta^{13}C$ and $\delta^{15}N$ and isotope values

This chapter examines the impact of parchment production on skin collagen stable isotope values and elemental composition to explore the use of parchment as an isotopic resource. The analysis of 52 paired samples enables an offset between skin and parchment to be calculated, facilitating the interpretation of historic manuscripts. Parchment production (5.2) and the manufacturing technique used in this study will be outlined (5.3), as well as a protocol for the preparation of parchment for analysis. The results (5.4) will then be discussed in relation to the structural and chemical modifications made to the skin during processing (5.5).

5.1 Introduction

Palaeodietary and zooarchaeological stable isotope analysis has traditionally focussed on the analysis of bone collagen and dentine due to their preservation and ubiquity in the archaeological record. While abundant, they are constrained by their archaeological phasing or radiocarbon date, which at best assigns material to a single century, at worst far broader. This is particularly the case for often mixed, truncated, or simply disregarded post-medieval material, which can be phased as broadly as 'post-1550' or 'post-1750'. Parchment in contrast, is both abundant and typically dated to the year of use and, as with unprocessed skin, may offer the possibility of a time-sensitive analysis of dietary and husbandry trends from the weeks and months prior to the animal's death (White & Schwartz 1994; White *et al.* 1995; Abend & Smith 1995; Iacumin *et al.* 1996; 1998; Walker *et al.* 1999; Williams 2008; Finucane 2007; Corr *et al*. 2009; Basha *et al.* 2016; Lamb 2016).

While stable isotope analysis of parchment has been conducted previously (Campana *et al.* 2010; Pollard & Brock 2011), the impact of production on measured values in skin has not been established.

These studies have speculated that values in the parchment may not be representative of the fresh skin, due to isotopic fractionation as a result of the structural and chemical modifications the skin undergoes during liming. Campana *et al.* (2010) and Pollard & Brock (2011) both observed $\delta^{15}N$ values in modern and historic parchment were far higher than those expected from terrestrial herbivores, and suggested a potential impact from production. To address this, the isotopic analysis of 51 paired skin and parchment samples are presented to assess the impact of production on collagen isotope values and elemental composition.

5.2 Parchment production

The traditional methods of parchment production, and the modifications made to the skins structure and chemistry are detailed in Section 3.3. In brief, the skin is unhaired, fleshed, stretched and scraped to produce a dry durable sheet from the collagen-rich dermis layer. The major alterations occur during liming, which: a) breaks the disulphide bonds in the keratinous hair and epidermis, facilitating their removal (Bieńkiewicz 1983, 71-72; Covington 2009, 118-119); b) hydrolyses triglyceride esters (saponification), removing cutaneous lipids (Koppenhoefer 1939); c) hydrolyses some amide groups attached to aspartic and glutamic acid residues (deamidation), decreasing the collagens iso-electric point (Menderes *et al.* 1999); and d) 'opens up' the collagen fibres, removing non-collagenous proteins, and purifying the collagen substrate (Menderes *et al.* 1999, Jones 2004, 31; Maxwell 2007, 123).

5.3 Materials & Methods

5.3.1 Experimental parchment production

Fresh skins were obtained from 47 sheep (*Ovis aires*) (Seaton Ross, E. Yorks. UK; Melton Mowbray, Leics. UK; Newark-on-Trent, Notts. UK; Prague, Czech Republic; Lawrence, Kansas, US), 2 goats (*Capra aegagrus hircus*) (Prague, Czech Republic), 2 calves (*Bos taurus*) (Vienna, Austria) and 5 pigs (*Sus scrofa domesticus*) (Beeston, Notts. UK). The animals were flayed shortly after death, and their skins either salted or frozen –20^{o}C prior to analysis.

To maximise this studies comparability with both medieval and post-medieval parchment, two production methods were used. Method 1 followed traditional pre-19th century European techniques as detailed in historic recipes (Saxl 1954; Ryder 1964; Reed 1973; Hagadorn 2012), with the skins unhaired in a straight lime solution, and delimed in water alone (Figure 5.1). Method 2 used industrial techniques through the addition of chemical depilatory agents during unhairing and acids during deliming.

5.3.1.1 Method 1 (Historical method)

Each skin was washed and rehydrated in water at 8ºC (pH 7.5) for 48 hrs. The water was replenished every 8 hrs, and adhering foreign material removed. The unsplit skins were submerged in a 3.5% calcium hydroxide solution (pH 13.5) at room temperature for 6-18 days, and agitated three times per day to ensure an even exposure across the skin. The ability to remove the fibre was appraised daily, and deemed sufficiently limed when it could be removed at the root by hand with ease. Once unhaired, the skins were fleshed on the beam, with the epidermis and sub-cutaneous tissue remove with a double-handled knife. The skins were subsequently returned to the lime for a further 12 hrs, allowing for a uniform penetration of the solution, which may have been inhibited by hair or fat. The skin was then placed on the beam and mechanically squeezed with the knife to force out as much liquid as possible. To neutralise the alkalinity, each skin was washed vigorously for 30 mins in running water and allowed to soak for 48 hrs, with the water replaced every 8 hrs. Each skin was then tightly stretched with ropes on a wooden frame, and allowed to dry under tension for a minimum of 14 days. While still wet, and then again once dry, each side was shaved with a sharp knife to remove further layers of the dermis and produce a clean even surface.

Figure 5.1: Method 1: A) salted skins are rehydrated and washed; B) skins are submerged in a lime solution; C) unhaired; D) fleshed on the beam; E) delimed in water; F) and dried under tension (by S. Doherty)

5.3.1.2 Method 2 (Modern method)

Due to the costly requirements to treat and remove biological oxygen demand chemicals (BODs) from wastewater if sulphides are used, the parchment produced using Method 2 was supplied by Jesse Meyer of Pergamena, New York, US. Samples of fresh skin and parchment were then sent to the author for analysis.

Each skin was washed and rehydrated as in Method 1. The unsplit skins were treated in a 0.1% sodium hydrosulfide and 3% calcium hydroxide solution at room temperature for 30 mins, and agitated throughout. To this, an additional 3% sodium hydrosulfide and 0.3% sodium hydroxide was then added. The drums were agitated intermittently for 1 hr and then left to stand for 18 hrs, during which time the hair had completely dissolved. The skins were then fleshed on the beam, and neutralised in water and 0.75% formic acid for 6 hrs. The skins were then stretched, shaved and left to dry under tension as in Method 1.

5.3.2 Sample preparation and analysis

Due to the previously limited isotopic analysis of parchment, there is currently no standard method of sample preparation. Parchment is a complex material in which the chemistry and integrity of the collagen is altered during manufacture (and potentially during conservational treatments), and can degrade via oxidation, hydrolysis and biological attack (Hedges *et al.* 1989, 100-1; Badae *et al.* 2012; Brock 2013, 353-4). Surface contaminants, including inks, glues and chalk are common, as well as areas of localised gelatinisation and deterioration (Brock 2013, 353-4). It is also essential that only a small physical sample is taken to minimise the aesthetic change to the manuscript. As such, careful sampling and pretreatment is requited to minimise and remove sources of contamination and ensure accurate analysis.

There is currently no widely accepted method for checking the integrity of collagen from skin or parchment (Lamb 2016); however, C/N rations (a standard for bone collagen and dentine; Ambrose 1990; van Klinken 1999) have been widely used. Modern collagen (COL1a1) has an atypically low C/N of 3.12 due to the abundance of Glycine (C/N 2/1). Ratios higher than this reflect the loss of nitrogen (including deamidation), or the presence of other proteins and lipids (Schoeninger & DeNiro 1984; van Klinken 1999; Kiljunen *et al.* 2006). Brock (2013) examined the impact of different pretreatment protocols in C/N ratio in parchment for carbon-14 dating (Table 5.1), and observed the highest ratios in untreated parchment, highlighting the necessity for some sample preparation, and the lowest in those treated with strong acids or alkalis. The most consistently acceptable ratios were produced from samples which had undergone lipid extraction followed by collagen extraction

(demineralisation, gelatinisation, filtration and freeze-drying). This is consistent with the results from other analyses of parchment and skin, where those that have not undergone collagen extraction have been shown to produce high C/N rations, some in excess of 4 (Iacumin *et al.* 1996; 1998; Williams 2008; Basha *et al.* 2016), while those that have undergone lipid and collagen extraction average around 3.3 (Pollard & Brock 2011; Brock 2013).

Endogenous and exogenous lipids are often present in parchment in significant quantities (Ghioni *et al.* 2005; Strlič *et al.* 2009; Možir *et al.* 2014) and must be removed due to the different isotopic composition of collagen and lipids (Liden 1995). Lipids have more negative $\delta^{13}C$ values than other biochemical compounds due to kinetic isotope effects that occur during lipid synthesis (DeNiro & Epstein 1977; Logan *et al.* 2008). Variability in tissue lipid content can therefore alter bulk $\delta^{13}C$ values, although as they are composed mainly of carbon they have little impact on $\delta^{15}N$ values (Logan *et al.* 2008). The decision was taken to undertake to remove lipids from both skin and parchment samples with DCM/MeOH, a solvent commonly used in tissues where triglycerides dominate (Ferraz *et al.* 2004; Colonese *et al.* 2015; Guiry *et al.* 2016). Due to the potential for residual calcium hydroxide to remain in the skin from liming, a brief demineralisation step was included to remove it. Following the results of Brock (2013), samples were then gelatinised, filtered and freeze-dried to produce purify the collagen substrate for analysis.

None	Acid wash *(CH1)*	Acid-base-acid wash *(HC1, NaOH)*	Lipid Extraction *(Hexane, DCM)*	Demineraliseralisation *(HC1)*	Gelatinisation *(dilute HCl)*	Filter *(60-90 µm)*	C/N	Reference
✓							3.4 - 4.6	1, 2
	✓						3.2 - 3.6	1
		✓					3.2 - 3.7	1, 3, 4
			✓				3.8	1, 3
			✓	✓	✓	✓	3.3	1, 4

Table 5.1: Pretreatment protocols used in the analysis of parchment and their impact on C/N ratios
1 Brock 2013; 2 Campana *et al.* 2010; 3 Donahue *et al.* 2010; 4 Pollard & Brock 2011

5.3.2.1 Skin

Samples were taken from the belly region after soaking, but prior to liming. Adhering hair and fat deposits were removed with a scalpel to leave a dermis rich sample, which was freeze-dried for 48 hrs, and ground to a coarse powder using a ball mill (Retsch MM400). In line with published analyses of modern and archaeological skin (i.e. Finucane 2007; Browning *et al.* 2014; Bergamo *et al.* 2016) samples underwent lipid and collagen extraction prior to analysis. Samples were defatted via solvent extraction, DCM/MeOH (2:1 v/v), by ultrasonication for 1hr, with the supernatant removed and solvent replaced every 15 min. The samples were briefly demineralised in 0.6 M HC1 at 4ºC for 1 hr, rinsed with distilled water and gelatinised in pH 3 0.001M HC1 at 80ºC for 48 hrs. The supernatant containing the collagen was filtered (60-90 µm Ezee-Filter™, Elkay Laboratories, UK), frozen, and freeze-dried.

5.3.2.2 Parchment

Samples were cut from the parchment adjacent to the location sampled for the fresh skin. The lipids were solvent extracted following the same procedure used for skin. Samples were subsequently demineralised in 0.6 M HC1 at 4ºC for 6 hrs to remove residual calcium carbonate/hydroxide, rinsed with distilled water, and gelatinised in pH 3 0.001M HC1 at 80ºC for 48 hrs. The supernatant was then filtered, frozen, and freeze-dried.

5.3.2.3 Stable isotope analysis

Prepared collagen (0.9-1.1 mg) was weighed out in duplicate in 4 x 3.2 mm tin capsules (Elemental Microanalysis, Okehampton, UK) for carbon and nitrogen isotope analysis. Analysis was undertaken at the Natural Environment Research Council Life Sciences Mass Spectrometry Facility (NERC LSMSF) in East Kilbride. Isotope ratio determinations were carried out on a ThermoElectron DeltaPlusXP (Thermo Fisher Scientific, Bremen, Germany) with an Elementar Pyrocube elemental analyser (Elementar, Langenselbold, Germany). The ratios are reported as $\delta^{13}C$ and $\delta^{15}N$ values in per mille (‰) in reference to VPDB and AIR. Samples for bulk isotope analysis were referenced against internal standards of gelatine (Sigma-Aldrich, St. Louis, US), ^{13}C-enriched alanine and ^{15}N-enriched glycine, and the international standard of glutamic acid (USGS40). The isotopic composition and accuracy of control standards are reported in Table 5.2.

Reproducibility between samples was better than ± 0.2‰ (1σ) for $\delta^{13}C$, %C, $\delta^{15}N$ and %N, with the exception of sample USA03 (0.33‰), which was therefore removed from further analysis and calculations. %C ranged from 41.4-46.1 in skin, and from 41.6-44.9 in parchment. %N ranged from

15.1-16.8 in skin, and 15.0-16.1 in parchment. C/N ratios were between 2.9-3.4 in skin, and between 3.2-3.4 in parchment. The quality indicators are all indicative of well-preserved collagen (DeNiro 1985; Ambrose 1990).

5.3.3 Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistic 22.0 software package (IBM 2013). Shapiro-Wilks tests for normality showed that the distribution of $\delta^{15}N$ values in skin and parchment were normal, but that $\delta^{13}C$ values were not (p <0.01). Therefore, non-parametric statistical tests were used in this study. The statistical significance of differences between skin and bone values was determined using a Wilcoxon signed-rank test for paired samples, and difference between Method 1 and Method 2 analysed using a Mann-Whitney U test. Correlation were assessed using the Pearson correlation coefficient test.

		$\delta^{13}C$ (‰ vs. VPDB)		$\delta^{15}N$ (‰ vs. AIR)	
Standard	*n*	Observed	True	Observed	True
^{13}C-enriched alanine	30	–8.47 ± 0.20	–8.36 ±0.13	2.15 ± 0.15	2.08 ± 0.06
^{15}N-enriched glycine	30	–38.54 ± 0.11	–38.58 ±0.09	23.53 ± 0.13	23.54 ± 0.08
Gelatine	78	–20.31 ± 0.09	–20.31 ± 0.18	5.55 ± 0.17	5.54 ± 0.08
USGS40 (glutamic acid)	12	–26.45 ± 0.14	–26.39 ± 0.09	–4.58 ± 0.16	–4.52 ± 0.12
USGS42 (Tibetan hair)	4	-	-	-	-
USGS43 (Indian hair)	4	-	-	-	-
CBS (Caribou hoof)	5	-	-	-	-
KHS (Kudu horn)	4	-	-	-	-

Table 5.2: Isotopic analytical precision of standards run during analysis at NERC LSMSF.
– = not measured

5.4 Results

Isotopic and elemental composition results are reported in Table 5.3 (Method 1) and Table 5.4 (Method 2), and presented in Figure 5.2 (Method 1) and Figure 5.3 (Method 2). Additional compositional results are presented in Table A.1

5.4.1 Carbon/Nitrogen ratios in skin and parchment

All samples produced C/N ratios within the acceptable range of ~2.9-3.5 proposed for bone collagen (DeNiro 1985; Ambrose 1990). Average ratios in skin were 3.2 (2.9-3.4), and 3.2 (3.2-3.4) in parchment, with 15 skins showing higher ratios after processing, 14 lower, and 22 showing no difference. Of those that showed an increased ratio, it was often accompanied by an enrichment in ^{15}N values, potentially due to deamidation and the loss of nitrogen during cleavage of the peptide bond (see 5.5.1). Despite the potential impact from deamidation and the removal of lipids, the C/N ratios indicate little change to the elemental composition of the collagen after processing.

5.4.2 Impact of production

Except for three samples, production resulted in a systematic enrichment in ^{15}N values (Method 1: Mean = +0.23‰, Range = -0.1‰ to 0.9‰; Method 2: Mean = +0.26‰, range = -0.1‰, to +0.5‰). Twenty-nine (56.9%) of these were greater than experimental error, and was observed in both production methods, and across all species, age and sex. The impact of production on $\delta^{13}C$ values was more variable, although on average it resulted in an enrichment in values (Method 1: mean = +0.13 ‰, range = -0.5 to +0.9‰; Method 2: mean = +0.23‰, range = -0.2 to + 0.8‰). Thirty-two (62.7%) showed an impact greater than analytical error. The difference between skin and parchment values was, however, only statistically significant in sheepskin (Table 5.5), although is likely to be due to the far greater sample size. There was no statistically significant difference in the offset between skin and parchment in sheep between either Method 1 or Method 2 (Mann-Witney U test, $\delta^{13}C$: U=72.5, p=0.885; $\delta^{15}N$: U=72.0, p=0.885).

Animal	Species	Age (mo)	Skin				Parchment				Δ (parchment-skin)			
			C/N ratio	δ 13C (‰)	δ15N (‰)	δ18O (‰)	C/N ratio	δ 13C (‰)	δ15N (‰)	δ18O (‰)	Δ13C (‰)	SD	Δ15N (‰)	SD
JSK01	S	6	3.2	–25.4	9.7	-	3.1	–25.0	9.9	-	**+0.4**		+0.1	
JSK02	S	6	3.2	–25.8	9.2	-	3.2	–25.4	9.5	-	**+0.4**		**+0.2**	
JSK03	S	6	3.2	–26.0	10.0	8.71	3.2	–25.7	10.1	8.56	**+0.2**		+0.0	
JSK04	S	11	3.2	–25.8	8.4	9.53	3.2	–25.5	8.5	8.45	**+0.2**		+0.0	
JSK05	S	10	3.2	–25.7	8.4	-	3.2	–25.3	8.6	-	**+0.4**		+0.1	
JSK06	S	10	3.2	–26.0	8.9	-	3.2	–25.6	9.2	-	**+0.3**		**+0.2**	
JSK07	S	10	3.2	–25.8	9.0	-	3.2	–25.6	9.0	-	**+0.2**		+0.0	
JSK08	S	13	3.2	–26.0	9.7	-	3.2	–25.3	9.9	-	**+0.6**		**+0.2**	
JSK09	S	<1	3.2	–24.7	8.4	8.73	3.2	–24.7	8.7	7.59	+0.0		**+0.3**	
JSK10	S	SB	3.2	–25.1	8.7	7.18	3.2	–25.1	8.8	7.82	-0.06		+0.1	
JSK11	S	SB	3.2	–24.9	8.1	-	3.2	–24.4	8.5	-	**+0.4**		**+0.3**	
JSK12	S	60	3.3	–26.0	9.3	-	3.3	–25.5	9.4	-	**+0.5**		+0.1	
JSK13	S	1	3.2	–23.3	7.6	-	3.2	–23.4	7.9	-	-0.09		**+0.2**	
JSK14	S	18	3.2	–25.7	9.3	-	3.2	–25.6	10.0	-	+0.1		**+0.6**	
JSK15	S	18	3.2	–24.6	8.3	-	3.2	–24.8	9.0	-	**-0.21**		**+0.6**	
JSK16	S	18	3.2	–25.4	8.0	-	3.2	–25.2	8.5	-	**+0.2**		**+0.4**	
JSK17	S	9	3.2	–24.9	8.5	-	3.2	–24.7	8.8	-	**+0.2**		**+0.2**	
JSK18	S	25	3.2	–25.3	9.2	-	3.2	–25.8	9.6	-	**-0.50**		**+0.4**	
JSK19	S	16	3.3	–26.0	8.6	-	3.3	–25.8	8.8	-	+0.1		**+0.2**	
JSK20	S	17	3.2	–25.1	8.6	-	3.2	–24.9	9.1	-	+0.1		**+0.4**	
JSK21	S	26	3.3	–25.3	8.9	-	3.2	–24.8	8.5	-	**+0.5**		**-0.36**	
JSK22	S	15	3.1	–25.7	8.3	-	3.2	–24.9	8.5	-	**+0.8**		+0.1	
JSK23	S	15	3.2	–25.8	8.4	-	3.3	–26.0	8.6	-	**-0.25**		**+0.2**	
JSK24	S	15	3.3	–25.7	8.2	-	3.2	–25.4	8.7	-	**+0.3**		**+0.5**	
JSK25	S	10	3.2	–23.8	6.1	-	3.2	–23.8	6.5	-	+0.0		**+0.3**	
JSK26	S	12	3.2	–25.7	5.1	-	3.2	–25.4	5.7	-	**+0.2**		**+0.5**	
LL01	S	20	3.2	–25.3	7.3	-	3.2	–25.3	7.6	-	+0.0		**+0.3**	
LL02	S	<1	3.2	–24.7	8.0	9.23	3.2	–24.6	8.2	9.25	+0.0		**+0.2**	
NW01	S	-	3.3	–25.6	9.7	-	3.3	–26.0	9.8	-	**-0.38**		+0.1	
NW02	S	-	3.2	–25.5	9.5	-	3.2	–25.4	9.4	-	+0.0		-0.04	
NW03	S	-	3.2	–25.4	10.1	-	3.3	–25.9	10.3	-	**-0.44**		+0.1	
NW04	S	-	3.2	–25.6	10.0	-	3.3	–25.5	10.3	-	+0.1		**+0.2**	
NW05	S	-	3.2	–25.8	10.0	-	3.4	–26.1	10.2	-	**-0.29**		**+0.2**	
NW06	S	-	3.2	–26.0	10.5	-	3.3	–25.8	10.8	-	+0.1		**+0.3**	
NW07	S	-	3.2	–25.4	7.4	-	3.4	–25.5	7.8	-	-0.10		**+0.4**	
NW08	S	-	3.2	–25.9	7.2	-	3.4	–25.9	7.6	-	+0.0		**+0.3**	
NW09	S	-	3.3	–26.4	9.1	-	3.3	–25.8	9.4	-	**+0.5**		**+0.3**	
NW10	S	-	3.3	–25.8	9.6	-	3.4	–25.9	9.7	-	-0.10		+0.1	
GSK0	G	9	3.1	–23.8	8.1	-	3.1	–23.8	8.3	-	+0.0		**+0.2**	
GSK0	G	<1	3.1	–23.5	6.7	-	3.2	–24.5	6.9	-	0.00		+0.1	
BEP01	P	-	3.2	–23.3	4.0	-	3.2	–23.4	4.1	-	-0.05		0.00	
BEP02	P	-	3.2	–23.3	4.3	-	3.2	–22.9	4.1	-	**+0.3**		-0.14	
BEP03	P	-	3.3	–23.0	5.6	-	3.2	–22.7	5.7	-	**+0.3**		+0.0	
BEP04	P	-	3.3	–23.0	5.8	-	3.2	–22.5	6.0	-	**+0.5**		**+0.2**	
BEP05	P	-	3.3	–23.6	4.5	-	3.2	–23.3	4.5	-	**+0.3**		+0.0	
CSK01	C	<1	3.1	–24.5	3.9	-	3.1	–24.5	4.0	-	-0.01		+0.0	
CSK02	C	<1	3.2	–24.5	4.7	-	3.2	–24.5	5.2	-	+0.0		**+0.4**	
Sheep mean difference											+0.1	0.3	+0.2	0.2
Goat mean difference											-0.04	-	+0.1	-
Pig mean difference											+0.3	0.2	+0.0	0.1
Calf mean difference											+0.0	-	+0.2	-
Overall mean difference											+0.1	0.3	+0.2	0.2

Table 5.3: $\delta^{13}C$ and $\delta^{15}N$ isotope and elemental composition of skin and parchment samples from Method 1. S = sheep, G = goat, P = pig, C = calf, SB = stillborn, - = no data. Differences greater than experimental error in bold. Standard deviation where >2 samples.

Animal	Species	Age (mo)	Skin			Parchment			Δ (parchment-skin)	
			C/N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C/N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\Delta^{13}C$ (‰)	$\Delta^{15}N$ (‰)
USA01	S	12	3.4	–24.9	6.9	3.3	–24.1	6.8	**+0.79**	-0.07
USA02	S	12	3.2	–21.9	6.6	3.2	–21.7	7.1	**+0.20**	**+0.51**
USA04	S	12	3.2	–20.7	12.3	3.2	–20.6	12.6	+0.10	**+0.28**
USA05	S	12	3.2	–24.2	6.3	3.3	–24.4	6.6	-0.16	**+0.33**
Overall mean difference									+0.23	+0.26
Overall standard deviation									0.4	0.2

Table 5.4: $\delta^{13}C$ and $\delta^{15}N$ isotope and elemental composition of skin and parchment samples from Method 2. S = sheep.

Species	Method	n	$\delta^{13}C$ (‰ vs. VPDB)		$\delta^{15}N$ (‰ vs. AIR)	
			Z	*P*	Z	*P*
All	1 & 2	51	586.5	**0.002**	977.0	**<0.001**
Sheep	1	38	429.0	**0.008**	670.5	**<0.001**
Sheep	2	4	7.5	0.357	9.0	0.141
Goat	1	2	0.0	1.0	3.0	0.157
Calf	1	2	0.0	1.0	3.0	0.180
Pig	1	5	14.0	0.078	6.5	0.577

Table 5.5: Significant differences in isotope values between skin and parchment (Wilcoxon signed-rank test for paired samples). Bold values indicate statistically significant differences *(P<0.05)*

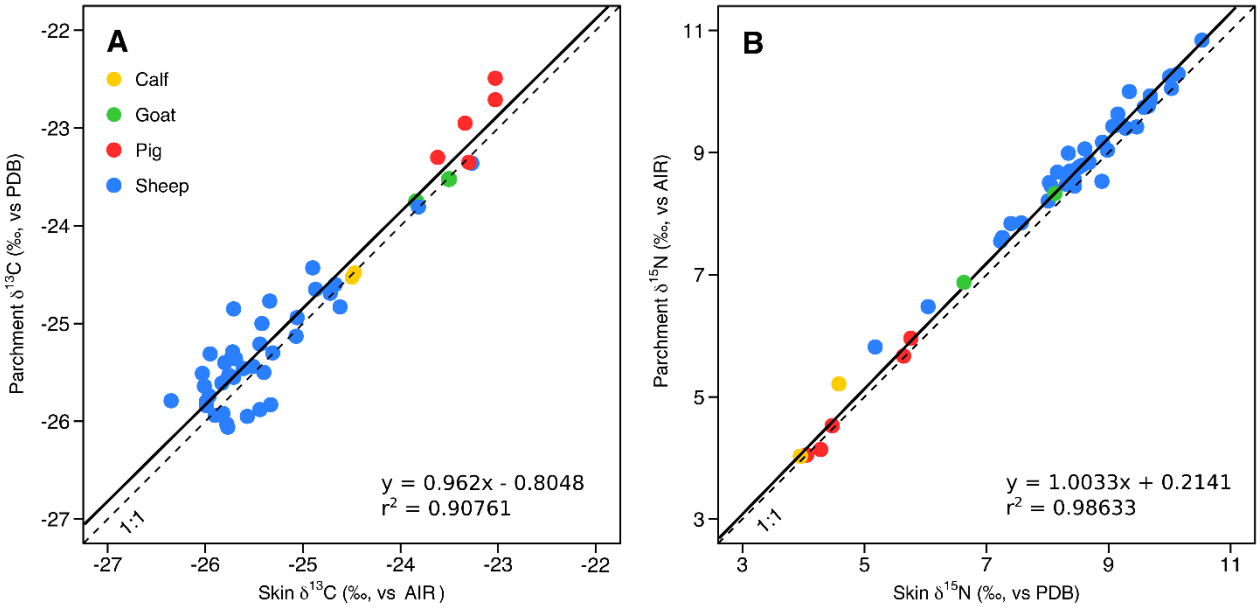


Figure 5.2: Comparison of stable isotope values from skin and parchment produced using Method 1. A) $\delta^{13}C$, B) $\delta^{15}N$, C) $\delta^{18}O$. Solid line = Linear trend line; Dashed line = 1:1

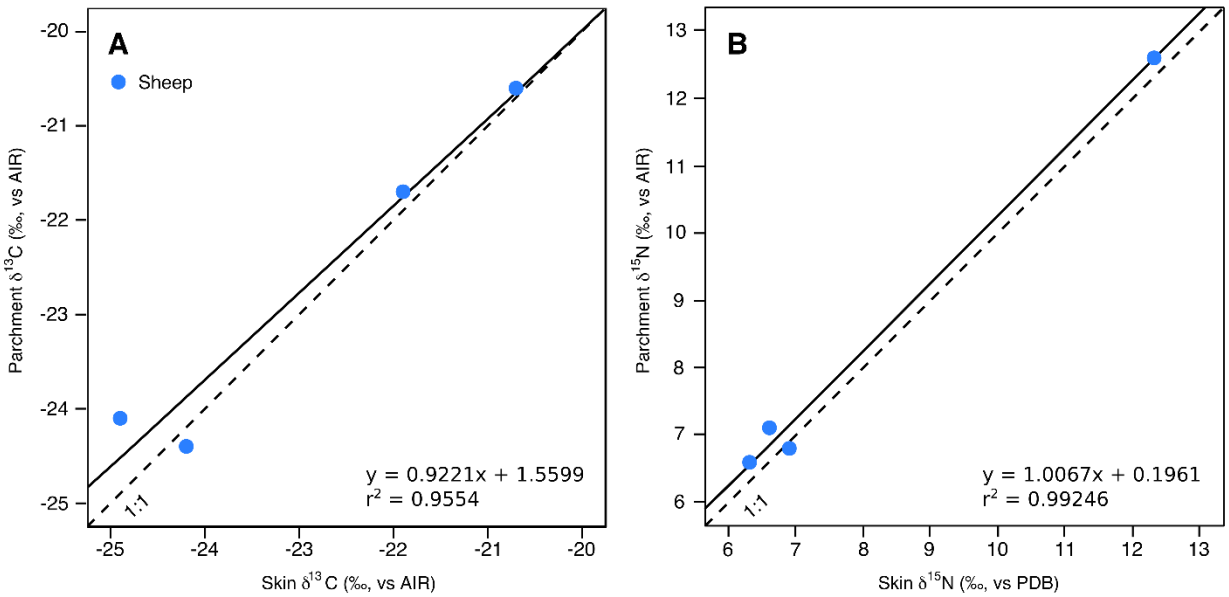


Figure 5.3: Comparison of stable isotope values from skin and parchment produced using Method 2. A) $\delta^{13}C$, B) $\delta^{15}N$. Solid line = Linear trend line; Dashed line = 1:1

5.5 Discussion

The observed variation between skin and parchment $\delta^{13}C$ and $\delta^{15}N$ values is likely the result of a range of factors resulting from changes made to the structure and chemistry of the skin and during parchment production.

5.5.1 Impact of amide sidechain hydrolysis

Forty-seven of the fifty-one skins (92.2%) showed a small, but consistent ^{15}N-enrichment after processing, twenty-nine (56.9%) of which were greater than experimental error (0.2‰). Thirteen skins (25.5%) showed an a ^{13}C-enrichment, although only 6 (11.7%) greater than experimental error. A likely source of this enrichment is the kinetic isotope effect associated with peptide bond hydrolysis, preferentially eliminating the isotopically lighter nitrogenous compounds. During liming, the amide functional groups of asparagine (Asp) and glutamine (Glu) are converted into carboxyl groups through alkaline hydrolysis, producing, respectively, aspartic acid:

$$(CH_2)_1CONH_2 + H_2O + OH^- \rightleftharpoons P - (CH_2)_1 CO_2^- + NH_3 \quad \text{(Eq. 5)}$$

and glutamic acid:

$$(CH_2)_2CONH_2 + H_2O + OH^- \rightleftharpoons P - (CH_2)_2 CO_2^- + NH_3 \quad \text{(Eq. 6)}$$

liberating carbon dioxide and ammonia. This deamidation reaction forms part of the controlled damage collagen undergoes during processing, and is essential in lowering the iso-electric point of collagen, swelling the skin and aiding the removal of non-collagenous proteins (Covington 2009, 132-148). The rate of hydrolysis increases with prolonged liming as the rigidity of the collagen backbone decreases, making the partially deamidated collagen susceptible to further damage (van Duin & Collins 1998; Collins *et al.* 1999). In less than 24 hrs of liming, around 50% of all available sidechains are hydrolysed (Menderes *et al.* 1999).

Cleavage of the carbon-nitrogen bond during hydrolysis has been shown to favour peptide bonds containing the lighter isotopes of ^{12}C and ^{14}N, leading to a retention and enrichment of the heavier isotopes (McClelland & Montoya 2002; Chikaraishi *et al.* 2007; 2009; Macko *et al.* 1986; 1987; Bada *et al.* 1989; Silfer *et al.* 1992; Miura & Goto 2012). Bada *et al.* (1989), for example, observed a ~7‰ enrichment in bulk $\delta^{15}N$ values on bovine collagen after 30% hydrolysis. This enrichment has been observed in deamidation associated with the transfer of amino acids from diet to consumer (Hare *et al.* 1991; Chikaraishi *et al.* 2007; 2009; Popp *et al.* 2007; Miura & Goto 2012) and in the

archaeological degradation of proteins (Dent *et al.* 2004; von Holstein *et al.* 2014). Despite the rate of hydrolysis being linked to stability of the collagen structure, the extent of ^{13}C or ^{15}N enrichment there was no significant correlation with the duration of liming (Pearson correlation coefficient test, $\delta^{13}C$: r=-0.205, p=0.188; $\delta^{15}N$: r=0.065, p=0.665). The skins processed using Method 2 were only in lime for 24 hrs, but displayed identical mean $\delta^{15}N$ enrichment values with sheepskins processed using Method 1, although slightly more enriched $\delta^{13}C$. Further $\delta^{15}N$ enrichment may occur during the hydrolysis of arginine residues to form ornithine and urea, which has shown a preferential bias for releasing the lighter isotope during the urea cycle (Ambrose 2000). In extensively limed skins, such as those in this study, >30% of arginine residues may be converted (Jones 2004, 31).

5.5.2 Removal of keratinous hair and epidermis

During production, the keratinous hair, wool and epidermis layer of the skin are removed chemically and mechanically. This has the potential to influence the isotopic value of the resulting parchment due to the isotopic variation between keratin and collagen (Tieszen & Fagre 1993; O'Connell *et al.* 2012; von Holstein *et al.* 2013). Hair and wool fibres are made predominantly from keratin, which relative to collagen contains less glycine and proline and higher levels of cystine and tyrosine (Robbins 2012). This high cystine content results in an abundance of disulphide bonds, producing a 'hard' keratin, as in nails and horn. The epidermis is composed predominantly of 'soft' epithelial keratins, which have lower cystine and higher methionine and glycine content than hair keratin (Bieńkiewicz 1983; Fuchs 1983). Keratin constitutes <2% of the total composition of the skin (excluding the hair/wool), and in principal is entirely removed during production due to the different behaviour of collagen and keratin during liming (Bieńkiewicz 1983). At high pH values the disulphide bonds undergo hydrolysis, dissolving the prekeratinised base of the hair, so that it is held by friction alone, and also weakens the epidermis (Covington 2009). This chemical attack is followed by mechanical removal of the hair and epidermis during dehairing and shaving, with the resulting parchment made predominantly from the collagen rich dermal/corium layer. The opening-up of the collagen fibres during liming, further results in the removal of non-collagenous components of the skin (such as non-structural proteins and lipids), increasing the relative proportion of collagen in parchment.

The isotopic relationship between collagen and keratin has been examined in a number of studies (DeNiro & Epstein 1978; Tieszen & Fagre 1993; O'Connell *et al.* 2001; Warriner & Tuross 2010; Codron *et al.* 2012; von Holstein *et al.* 2013). Both are typically enriched over diet, with collagen enriched over keratin in both $\delta^{13}C$ (0-4‰) and $\delta^{15}N$ (0-2%). In sheep, von Holstein *et al.* (2013) noted that collagen was enriched over keratin by 2-2.7‰, but $\delta^{15}N$ differences were in experimental error. Isotopic variation between the two tissues are largely due to differences in the routing and

composition of amino acids (O'Connell *et al.* 2001; von Holstein *et al.* 2013). Previous analysis has, however, been conducted on 'hard' keratins, and due to the higher glycine content of epithelial keratins, it is likely that this isotopic variation between 'soft' keratin and collagen is less pronounced. All visible signs of keratinous tissue were removed from the skin sample prior to analysis, but it is possible that further removal during processing contributed to the observed enrichment.

5.5.3 Removal of lipids and non-collagenous proteins

A potential factor being the enrichment of parchment $\delta^{13}C$ values may be the saponification of lipids during liming, reducing the amount of carbon-depleted lipids. Lipids are isotopically lighter (in carbon) than the protein component of animal tissue, and in ecological studies are typically removed through lipid extraction prior to analysis due to their impact on bulk values (i.e. Medeiros *et al.* 2015; Guiry e*t al.* 2016; Elliot *et al.* 2017). During liming, fatty acid esters undergo hydrolysis and are leached out into the lime solution, with as much as 50% of the total lipid content of skin removed (Koppenhoefer 1938; 1939). Lipids are likely to be further lost during deliming and shaving, however sheepskin parchment can still have in high levels of remaining lipids (Ghioni *et al.* 2005). Pollard and Brock (2011) noted an enrichment in $\delta^{13}C$ in defatted parchment relative to that which had not undergone lipid extraction; however, in this analysis lipids were extracted from both the fresh skin and parchment prior to analysis, reducing the influence they may have. Non-collagenous proteins are also removed during processing, reducing the amount of basic amino acids (arginine, lysine, and histidine), resulting in a proportional increase in glycine, which is enriched in ^{13}C relative to other amino acids (McMahon *et al.* 2015). Therefore, purification of the collagen substrate during processing may too influence the resulting bulk isotope values.

5.6 Summary

The alterations made to the structure and chemical composition of skin during parchment production result in a small, but measurable impact upon carbon and nitrogen isotope values. In sheepskin, production resulted in statistically significant mean enrichment in both $\delta^{13}C$ and $\delta^{15}N$. These results confirm Campana *et al.* (2010) and Pollard & Brock's (2011) hypothesis that production alters the isotope value of the skin, and is likely the cause of the high $\delta^{15}N$ values observed in both of these previous studies. Measuring the amino acid composition of paired samples would help clarify the factors driving this mean enrichment, although it is likely the interplay of: deamidation preferentially removing the lighter ^{12}C and ^{14}N isotopes; saponification removing ^{13}C-depleted lipids; and removal of the relatively depleted keratin component during liming and shaving. There was no statistically significant difference in the offset from skin to parchment in those manufactured using historical or modern techniques. This is important for this study as the historical parchment analysed covers this technological change, although is unlikely to have any significant impact.

Chapter 6

Examining the isotopic relationship between diet, bone, skin and parchment through a free-ranging diet study

This chapter explores diet to tissue offset through a free-ranging diet study, and inter-tissue isotopic spacing through the analysis of paired skin and bone samples. This analysis enables the results from historic parchment to be integrated with those from archaeological bone collagen. Previous examinations of diet to tissue enrichment (6.1.1) and inter-tissue spacing (6.1.2) will be presented, before detailing the diet study undertaken (6.2). The results (6.4) will be discussed, focusing on the impact of turnover rate, age, and intra-flock variation.

6.1 Introduction

Stable isotope analysis of consumer tissue is widely applied to ecological, agricultural and archaeological material to reconstruct nutrient acquisition, food webs, trophic relationships, migration and cultural practices (i.e. Hobson 1999; Hobson & Wassenaar 2008; Camin e*t al.* 2016; Alexander *et al.* 2015). As tissue is derived from an animal's dietary consumption over the period of tissue formation, its isotopic composition therefore reflects the signature of the dietary components, which are transferred and retained by the body during the absorption of nutrients (DeNiro & Epstein 1978; 1981).

Due to variations in tissue composition, rate of growth and routing of macromolecules from the diet (carbohydrates, protein, lipids), different tissues within the same individual often display different isotopic signatures (DeNiro & Epstein 1978; Tieszen & Fagre 1993; O'Connell *et al.* 2001; Warriner & Tuross 2010; Codron *et al.* 2012). This has been observed in bone and skin collagen, and thought to largely reflect the different temporal resolution of these tissues; with bone collagen on the scale of years (Hedges e*t al.* 2007) and skin collagen on the scale of weeks and months (Gerber *et al.* 1960; Babraj *et al.* 2005). As ecological studies typically focus on non-invasive sampling (Schwertl *et al.* 2003; Ayliffe *et al.* 2004; Zazzo *et al.* 2015) and agricultural studies on muscle (Camin *et al.* 2007;

Bahar *et al.* 2008; Bontempo *et al.* 2012), much of our understanding on the isotopic relationship between these tissues comes from archaeological investigations (Table 6.1). These indicate that both are typically enriched over diet, with skin $\delta^{13}C$ values usually depleted relative to bone, and $\delta^{15}N$ enriched relative to bone; although with a high degree of individual variability. Interpretation of inter-tissue spacing using archaeological material is complicated by the potential for unknown cultural factors, such as abrupt changes in diet, location and health prior to death to alter the isotopic composition of the tissue. To address this, this chapter presents the first diet to tissue analysis in sheep, through the analysis of paired samples from 26 individuals.

	Contributions	Species	Relative position ($\Delta_{bone\text{-}skin}$)	Reference
$\delta^{13}C$	Dietary carbohydrate, protein, lipid	Human (modern)	Skin collagen enriched c.0.2‰ over bone collagen	Lyon & Baxter 1978
		Human (mummified)	Skin collagen typically depleted 2.2-0.2‰ relative to bone collagen, but high degree of variability and some enriched by 5‰.	White & Schwartz 1994; Iacumin *et al.* 1996; 1998; Finucane 2007; Williams 2008; Norris 2009; Basha *et al.* 2016
		Human (frozen)	Skin collagen depleted 1‰ relative to bone collagen	Corr *et al.* 2009
		Ungulates	Skin collagen depleted 0.6‰ relative to bone collagen	Vogel 1978
		Crocodile	Skin collagen depleted 3.3‰ relative to bone collagen	Iacumin *et al.* 1998
$\delta^{15}N$	Dietary protein	Human (mummified)	Skin collagen enriched 0.2-5.3‰ relative to bone collagen, but high degree of individual variability.	White & Schwartz 1994; Iacumin *et al.* 1996; 1998; Finucane 2007; Williams 2008; Norris 2009; Basha *et al.* 2016
		Human (frozen)	Skin collagen depleted 8‰ relative to bone collagen	Corr et al. 2009
		Crocodile	Skin collagen enriched 1.1‰ relative to bone collagen	Iacumin *et al.* 1998

Table 6.1: Published results on the relationship between bone and skin collagen isotope values

6.1.1 Diet to tissue offset in herbivores

Isotopic fractionation occurs at various stages of the metabolic process, resulting in an offset between the isotopic values of the diet and consumer tissues (DeNiro & Epstein 1978; 1981). Controlled and free-ranging diet studies have observed an enrichment in the heavier isotopes of both carbon and nitrogen in consumer collagen over that of the diet (hereafter referred to as $\Delta^{13}C_{diet\text{-}tissue}$ and $\Delta^{15}N_{diet\text{-}tissue}$). Variable $\Delta^{13}C_{diet\text{-}tissue}$ offsets have been observed, although an enrichment between 2-4‰ is commonly accounted for (DeNiro & Epstein 1978; Sealy *et al.* 1987; Lee-Thorp *et al.* 1989; Ambrose & Norr 1993; Tieszen & Fagre 1993; Bocherens & Drucker 2003; Sponheimer *et al.* 2003a; Hedges *et al.* 2006; Warriner & Tuross 2010). After ingestion, nutrients are not apportioned equally and may be routed to different parts of the body. While collagen carbon can derive from dietary carbohydrate, protein and lipid, single amino acid analysis and feeding studies suggests that collagen carbon is largely routed from dietary protein for use in organ and tissue synthesis (Hedges *et al.* 2006, 120; Voigt *et al.* 2008, 2; Fernandes *et al.* 2012).

Collagen nitrogen derives from dietary protein, and during its assimilation, deamination enzymes preferentially removed amine groups with ^{14}N and generate nitrogen by-products of ammonia (NH_3), uric acid ($C_5H_4N_4O_3$) and urea (CH_4N_2O) which are enriched in ^{14}N relative to animal protein (Perini *et al.* 2009, 2580). Similarly, transamination during the synthesis of amino acids favours the retention of the heavier isotope and excretion of ^{14}N in the form of urea (Styring *et al* 2010, 242). Due to this a $\Delta^{15}N_{diet\text{-}tissue}$ enrichment of 3-5‰ has commonly been observed in feeding studies and is typically accounted for in dietary reconstruction (DeNiro & Epstein 1981; Bocherens & Drucker 2003; Sponheimer *et al.* 2003b; O'Connell *et al.* 2012). Nutritional stress can increase ^{15}N-enrichment, particularly in tissues with a fast turnover rate, where protein within the body is consumed as a source of amino acids (Hobson *et al.* 1993; Hatch *et al.* 2006). Despite this, there is still no accepted explanation for the mechanism by which trophic ^{15}N enrichment occurs (O'Connell 2017, 317).

6.1.2 Isotopic relationship between skin and bone

Due to the complex interplay of variations in tissue composition, the routing of nutrients, remodelling rates, and individual variations in metabolism, bone collagen and skin collagen values in the same individual often vary, with skin collagen typically depleted in ^{13}C relative to bone (c.–2.2-0‰) but enriched in ^{15}N (c.0-5‰). The protein component of bone and skin is dominated by Type 1 collagen (COL1a1), with the two tissues possessing highly similar amino acid compositions despite skin containing a minor elastin (0.6-2.1%; higher glycine abundance) and keratin (<2%; lower glycine abundance) component (Ambrose 1993, 76-77; Corr *et al.* 2009, 14; Covington 2009, 23; 38) (Figure 6.1). Compound specific $\delta^{13}C$ isotope analysis of the frozen remains of Kwäday Dän Ts'ìnchi by

Corr *et al.* (2009), also indicates that the two tissues have analogous biosynthetic/dietary origins of collagen carbon, and ascribed much of the observed isotopic variation between bone and skin collagen in this individual to turnover rate and large geographical movement.

Bone collagen is predominantly laid down in adolescence, but is continually remodelled during life (Hedges *et al.* 2007). In adolescent humans (10-15 years old), turnover rates of approximately 10-30%/year have been modelled, considerably higher than the ~1-4%/year in adults (Hedges et al. 2007). How this translates to short-lived animals such as sheep is unexplored but, in isotopic studies, the values from domesticated animals are typically taken to reflect a whole life average. In contrast, dermal collagen turnover rates are significantly greater, with average turnover rates ≥1%/day reported (Laurent 1982; McAnulty & Laurent 1987; Mays *et al.* 1991; el-Harake *et al.* 1998; Babraj *et al.* 2005; Moore *et al*. 2005), although in animals on a high plane of nutrition, synthesis rates in excess of 12.5%/day have been observed (Li *et al.* 1998; 2007).

Skin collagen, therefore, appears to record clearer seasonal isotopic variation. In individuals preserved by mummification or permafrost, archaeologists have commonly attributed this isotopic variation between tissues to alterations in cultural and environmental conditions, including: dietary changes before death (White & Schwartz 1994; Williams 2008); significant spatial movement prior to death (Corr *et al.* 2009); physiological stress in the final months of life (Finucane 2007; Norris 2009); as well as differential decomposition and diagenesis (Finucane 2007).

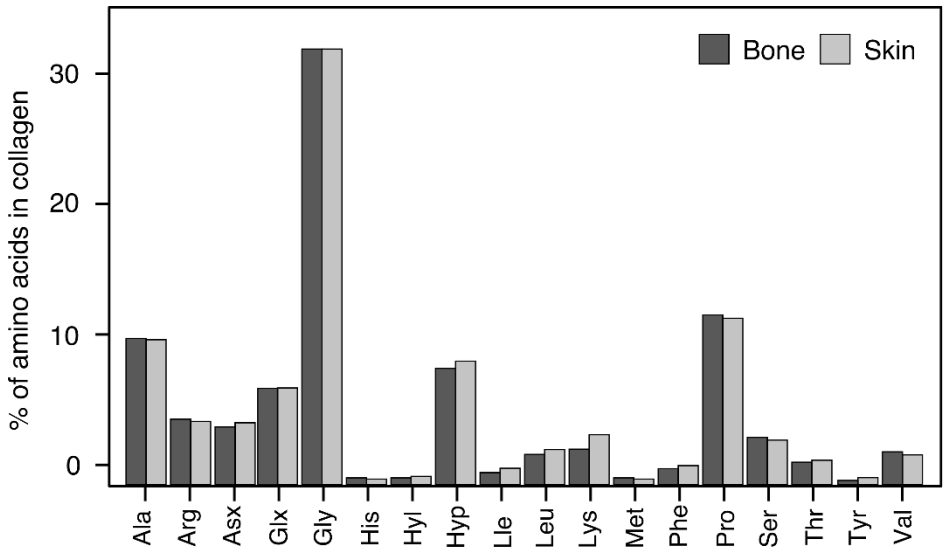


Figure 6.1: Amino acid composition of bone and skin collagen
(Ambrose 1993, 76-77; Covington 2009, 23)

6.2 Material & Methods

This section is separated into the methods and sample preparation used for the diet study (6.2.1) and the isotopic spacing between tissues study (6.2.2), although the animals used for the first study contributed to the second.

6.2.1 Diet study

Twenty-six sheep (*Ovis aries,* Shetland) were born and raised in Seaton Ross, East Yorkshire, UK. The animals were managed under modern conditions, although on a small scale (flock size <60), and were raised primarily for their wool. The sheep grazed across five adjacent fields during the years (approximately 4.5 acres) and were slaughtered at various ages. The animals were not placed on a controlled diet, and consumed the dietary components *ad libitum.* As with other free-ranging studies, samples of each component were taken at different seasons over a two-year period to provide an average isotopic composition of the diet (Heaton *et al.* 1986; Ambrose & DeNiro 1986; Sealy *et al.* 1987; Schoeninger 1989). The major dietary components were: grass pasture; wheat hay (UK, bought-in); and protein concentrates (Lamb Pellets, ARGO, UK; Sheep Concentrate Pellets, BATA, UK). The average dry weight contribution of these components to the diet was estimated to be 85:10:5 (%) (pers. comm. C. Chapman[12]). The isotopic composition of the individual dietary components is provided in Table 6.2. These results are comparable with data from other livestock feeds, and exhibit the relatively higher $\delta^{15}N$ values seen in other UK samples due to the cumulative effect of long-term organic manuring, and relatively depleted $\delta^{13}C$ values due to greater humidity and temperature (González-Martin *et al.* 1999; Piasentier *et al.* 2003; Schwertl *et al.* 2003; Camin *et al.* 2007).

To account for the varying contribution of these component, particularly the dominance of grass, the $\delta^{13}C$ and $\delta^{15}N$ of the diet was calculated using a mass balance equation:

$$\delta X_{diet} = \frac{(W_{grass}) \times (P_{grass}) \times (\delta X_{grass}) + (W_{hay}) \times (P_{hay}) \times (\delta X_{hay}) + (W_{feed}) \times (P_{feed}) \times (\delta X_{feed})}{(W_{grass}) \times (P_{grass}) + (W_{hay}) \times (P_{hay}) + (W_{feed}) \times (P_{feed})} \quad \text{(Eq. 7)}$$

where W is the estimated average contribution of the dietary component to the diet, P is the average measured proportion of X element in the component, and δX is the components average δ-value. The calculated diet isotopic ratios are reported in Table 6.1.

[12] Dr Cluny Chapman is a zooarchaeologists, farmer and embroider who currently manages a small flock of Shetland and Wensleydales in Seaton Ross, East Yorkshire. Further information regarding Cluny and her flock of sheep can be found at Highfield Textiles website

Diet Component	% intake	$\delta^{13}C$	%C	$\delta^{15}N$	%N
Grass [a] (dry)	85	–29.05	42.26	3.35	3.83
Hay [b] (dry)	10	–29.83	44.13	2.69	1.18
Feed [c] (dry)	5	–26.92	44.69	3.05	3.18
Mean Diet		–28.44		3.08	
Eq. 9		–29.02		3.31	

Table 6.2: Isotopic ratios of dietary components

[a] Average of 16 samples from five fields across two years; [b] Average of 6 samples from three bales across two years; [c] Average of 12 samples from two suppliers.

6.2.1.1 Preparation and analysis of food samples

Grass and hay samples were cleaned with distilled water, dried at 40°C for 24 hrs, and subsequently homogenised using a pestle and mortar. Feed samples from each batch were also homogenised before analysis. All samples were defatted via solvent extraction DCM: MeOH (2:1 v/v), by ultrasonication for 1 hr, with the supernatant removed and solvent replaced every 15 mins. 1-2 mg of each sample was weighed out into 4 x 3.2 mm tin capsules (Elemental Microanalysis, Okehampton, UK) for analysis. Isotopic analysis of food samples was undertaken at BioArCh, Department of Archaeology, University of York. $\delta^{13}C$ and $\delta^{15}N$ isotope ratio determinations were carried out on a Sercon GLS analyser coupled to a Sercon 20-22 Mass Spectrometer (Sercon, Crewe, UK), and are reported in per mille (‰) in reference to VPDB and AIR (Eq.1, Eq.2). An internal standard of gelatine (Sigma-Aldrich, St. Louis, US) was used, alongside international IAEA standards. The isotopic composition and accuracy of control standards are reported in Table 6.3.

6.2.1.2 Preparation and analysis of bone samples

Shortly after death, the left metacarpal was removed from the carcass and stored at -20°C prior to analysis. Of the 26 individuals analysed, bone samples were only obtained from 14 due to legal requirements around carcasses entering the meat industry. Adhering tissue was removed, and a 0.5-1.5 g sample taken from the mid-shaft. Bone samples were cleaned with distilled water, freeze-dried for 48 hrs to remove excess moisture, and then ground to a coarse powder using a ball mill (Retsch MM400). The lipids were removed via solvent extraction DCM/MeOH (2:1 v/v) by ultrasonication for 2 hrs, with the supernatant removed and solvent replaced every 15 mins. The samples were demineralised in 0.6M HC1 at 4°C for 4 days, rinsed with distilled water and gelatinised in pH 3

0.001M HC1 at 80°C for 48 hrs. The supernatant containing the soluble collagen was filtered (60-90µm Ezee-Filter™, Elkay Laboratories, UK), frozen, and freeze-dried. 0.8-1.1 mg of each sample was weighed out in duplicate into 4 x 3.2 mm tin capsules (Elemental Microanalysis, Okehampton, UK) for analysis.

Isotopic analysis of bone was carried out at the Natural Environment Research Council Life Sciences Mass Spectrometry Facility (NERC LSMSF) in East Kilbride. $\delta^{13}C$ and $\delta^{15}N$ isotope ratio determinations were carried out on a ThermoElectron DeltaPlusXP (Thermo Fisher Scientific, Bremen, Germany) with an Elementar Pyrocube elemental analyser (Elementar, Langenselbold, Germany). The samples were analysed in the same run as skin and parchment, therefore, please see Chapter 5 for information on the composition and accuracy of control standards. Reproducibility between samples was better than ± 0.2‰ (1σ) for $\delta^{13}C$, %C, $\delta^{15}N$ and %N. %C ranged from 39.6-45.7%, %N from 15.2-16.8%, and C/N rations were between 3.2-3.4, all indicative of well-preserved collagen (Ambrose 1990; van Klinken 1999).

		$\delta^{13}C$ (‰, vs. VPDB)		$\delta^{15}N$ (‰, vs. AIR)	
Standard	*n*	Observed	True	Observed	True
Fish Gelatine (SIGMA)	14	–15.36 ± 0.4	–15.32[a]	15.19 ± 0.3	15.2[a]
Cane Sugar (IA-R006)	3	–11.7 ± 0.06	–11.64 ± 0.03	-	-
Caffeine (IAEA 600)	3	–27.67 ± 0.12	–27.77 ± 0.04	1.4 ± 0.3	1 ± 0.2
Ammonium Sulfate (IAEA N2)	3	-	-	20.56 ± 0.3	20.3 ± 0.3

Table 6.3: Isotopic analytical precision of standards run during analysis at BioArCh, University of York. [a] Standard deviation not defined, - = not measured

6.2.2 Inter-tissue isotopic spacing study

The isotopic relationship between tissues was examined using the same individuals in the diet to tissue analysis, along with paired skin and bone samples from additional 10 sheep obtained from an abattoir in Newark-on-Trent, Nottinghamshire, UK, and 2 from a flock in Leicestershire. For those from the abattoir, husbandry information could not be confirmed from the farmer, but they were believed to be from a single commercial flock, slaughtered at around 9 months of age all on the same day. The two from Melton Mowbray, Leicestershire, UK, were Leicester Longwool's, farmed under small-scale conditions, and aged 20 months (LL01) and <7 days (LL02).

6.2.2.1 Preparation and analysis of bone, skin and parchment samples

Bone samples were prepared and analysed as those in Section 6.3.1.2. See Chapter 5 for the preparation and isotopic analysis of skin and parchment samples.

6.2.3 Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistics 22.0 software package (IBM 2013). Shapiro-Wilk tests for normality showed that the distribution of $\delta^{15}N$ values in all tissues were normal, as were $\delta^{13}C$ values in bone, but that $\delta^{13}C$ values in skin and parchment were not (p <0.01). Therefore, non-parametric statistical tests were used in this study. The statistical significance of differences between group and tissues were determined using a Mann-Whitney U test for paired samples, and a Kruskal-Wallis test for multiple samples. Correlations were assessed using the Pearson correlation coefficient test.

6.3 Results & Discussion

6.3.1 Diet to tissue spacing

$\delta^{13}C$ and $\delta^{15}N$ values of the analysed tissues are presented in Table 6.4, and $\Delta_{diet\text{-}tissue}$ offsets summaries in Table 6.5 Additional elemental results are presented in Table A1. Mean and range $\Delta_{diet\text{-}tissue}$ values were calculated excluding foetal and new born lambs due to the potential enrichment resulting from nutrients received through the ewe *in utero* and the consumption of milk (Hedges & Reynard 2007; Jay 2009, 169; de Luca *et al.* 2012). The distribution of $\Delta_{diet\text{-}tissue}$ values in sheep <1-month-old was not significantly different from older individuals (Mann-Whitney U test, p >0.05), as they had either not suckled at all or only for a couple of days (pers. comm. C. Chapman). However, as they had not directly consumed the dietary components being analysed they were not included in the calculations.

Consistent with the results of previous studies, consumer tissue was enriched over diet. This was greatest in parchment, along with a considerable degree of variability ($\Delta^{13}C_{diet\text{-}parchment}$ +3.9‰ ± 2.23, $\Delta^{15}N_{diet\text{-}parchment}$ +5.1‰ ± 4.38), although as demonstrated in the previous chapter, this is likely due in part to the impact of production. Statistical analysis will therefore focus on bone and skin. The range in $\Delta_{diet\text{-}tissue}$ values was greater in skin than in bone, likely reflecting the faster turnover rate of skin collagen than bone collagen, but the smaller samples size for bone must also be acknowledged.

The enrichment between diet and collagen observed in this study are on the upper range of those seen in controlled feeding studies ($\delta^{13}C$: 2-4‰, $\delta^{15}N$: 3-5‰) (Bocherens & Drucker 2003), although slightly lower than those observed in other free-ranging studies (5-6‰) (DeNiro & Epstein 1978; Vogel 1978; Heaton *et al.* 1986; Ambrose & DeNiro 1986; Sealy *et al.* 1987; Lee-Thorp *et al.* 1989; Schoeninger 1989; Schwartz & Schoeninger 1991). This likely reflect the increasing uncertainty over the 'true' isotopic signature of the diet, which is at its most variable in wild animals. Two skins, JSK25 and JSK26, presented lower $\delta^{15}N$ than the rest of the flock and were much closer to the isotope value of the diet. The driving factor behind this depletion is unknown, as they grazed over the same location, but may highlight the significant degree of individual metabolic variation. Using the husbandry information available, the impact of sex, age and season of slaughter on $\Delta_{diet\text{-}tissue}$ values was explored:

- There was no significant difference in $\Delta_{diet\text{-}tissue}$ enrichment between male (n=14) and females (n=6):
  $\Delta^{13}C_{diet\text{-}bone}$ – Mann-Whitney U test, U=20.0, p=0.797
  $\Delta^{15}N_{diet\text{-}bone}$ – Mann-Whitney U test, U=22.0, p=1.000
  $\Delta^{13}C_{diet\text{-}skin}$ – Mann-Whitney U test, U=29.0, p=0.958
  $\Delta^{15}N_{diet\text{-}skin}$ – Mann-Whitney U test, U=25.5, p=0.635

- There was no significant difference in $\Delta_{diet\text{-}tissue}$ enrichment between juveniles (6-17 months) (n=15) and adults (>18 months old) (n=7):
  $\Delta^{13}C_{diet\text{-}bone}$ – Mann-Whitney U test, U=9.0, p=0.833
  $\Delta^{15}N_{diet\text{-}bone}$ – Mann-Whitney U test, U=7.0, p=0.515
  $\Delta^{13}C_{diet\text{-}skin}$ – Mann-Whitney U test, U=25.0, p=0.462
  $\Delta^{15}N_{diet\text{-}skin}$ – Mann-Whitney U test, U=28.0, p=0.635

Grass $\delta^{13}C$ values display seasonal variation consistent with changes in water availability (Smedley *et al.* 1991), which is mirrored in perpetually remodelling hair and wool (Zazzo *et al.* 2015; Mosbacher e*t al.* 2016). Despite the rapid turnover of skin, there was no statistically significant variation in values for either isotope depending on the season of slaughter, with the slower turnover of skin likely smoothing out the seasonal signal:

- Winter–DJF (n=4); Spring – MAM (n=16); Summer – JJA (n=2); Autumn – SOC (n=10):
  $\Delta^{13}C_{diet\text{-}bone}$ – Kruskal-Wallis test, p=0.140
  $\Delta^{15}N_{diet\text{-}bone}$ – Kruskal-Wallis test, p=0.537
  $\Delta^{13}C_{diet\text{-}skin}$ – Kruskal-Wallis test, p=0.075
  $\Delta^{15}N_{diet\text{-}skin}$ – Kruskal-Wallis test, p=0.195

Animal	Age (mo)	C/N Bone	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
			Bone	Skin	Parchment	Bone	Skin	Parchment
JSK01	6	-	-	–25.4	–25.0	-	9.7	9.9
JSK02	6	3.2	–24.7	–25.8	–25.4	8.2	9.2	9.5
JSK03	6	3.2	–24.2	–26.0	–25.7	7.9	10.0	10.1
JSK04	11	3.2	–24.5	–25.8	–25.5	7.9	8.4	8.5
JSK05	10	3.2	–24.6	–25.7	–25.3	7.4	8.4	8.6
JSK06	10	3.2	–24.6	–26.0	–25.6	7.2	8.9	9.2
JSK07	10	3.2	–24.7	–25.8	–25.6	8.4	9.0	9.0
JSK08	13	3.1	–24.8	–26.0	–25.3	8.5	9.7	9.9
JSK09	<1	3.2	–23.9	–24.7	–24.7	7.7	8.4	8.7
JSK10	SB	3.2	–24.4	–25.1	–25.1	7.7	8.7	8.8
JSK11	SB	3.2	–23.9	–24.9	–24.4	7.4	8.1	8.5
JSK12	60	3.2	–24.6	–26.0	–25.5	6.4	9.3	9.4
JSK13	<1	3.2	–23.3	–23.3	–23.4	7.0	7.6	7.9
JSK14	18	-	-	–25.7	–25.6	-	9.3	10.0
JSK15	18	-	-	–24.6	–24.8	-	8.3	9.0
JSK16	18	3.2	–24.5	–25.4	–25.2	3.6	8.0	8.5
JSK17	9	-	-	–24.9	–24.7	-	8.5	8.8
JSK18	25	-	-	–25.3	–25.8	-	9.2	9.6
JSK19	16	-	-	–26.0	–25.8	-	8.6	8.8
JSK20	17	-	-	–25.1	–24.9	-	8.6	9.1
JSK21	26	3.2	–25.3	–25.3	–24.8	5.6	8.9	8.5
JSK22	15	-	-	–25.7	–24.9	-	8.3	8.5
JSK23	15	-	-	–25.8	–26.0	-	8.4	8.6
JSK24	15	-	-	–25.7	–25.4	-	8.2	8.7
JSK25	10	-	-	–23.8	–23.8	-	6.1	6.5
JSK26	120	-	-	–25.7	–25.4	-	5.1	5.7

Table 6.4: Isotopic composition of tissues from a single flock used for diet to tissue analysis. SB = Stillborn, - = not available for analysis.

	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
	Bone	Skin	Parchment	Bone	Skin	Parchment
Raw:						
Mean	–24.47	–25.36	–25.14	7.67	8.49	8.77
Range	± 0.5	± 1.1	± 1.1	± 1.3	± 2.5	± 2.2
ΔTissue-Diet:						
Mean	+4.1	+3.7	+3.9	+4.6	+4.8	+5.1
Range	± 1.1	± 2.23	± 2.23	± 2.51	± 4.93	± 4.38

Table 6.5: Summary of isotope data and tissue to diet offset. Mean $\Delta_{Tissue\text{-}Diet}$ calculated from sheep >6 months of age.

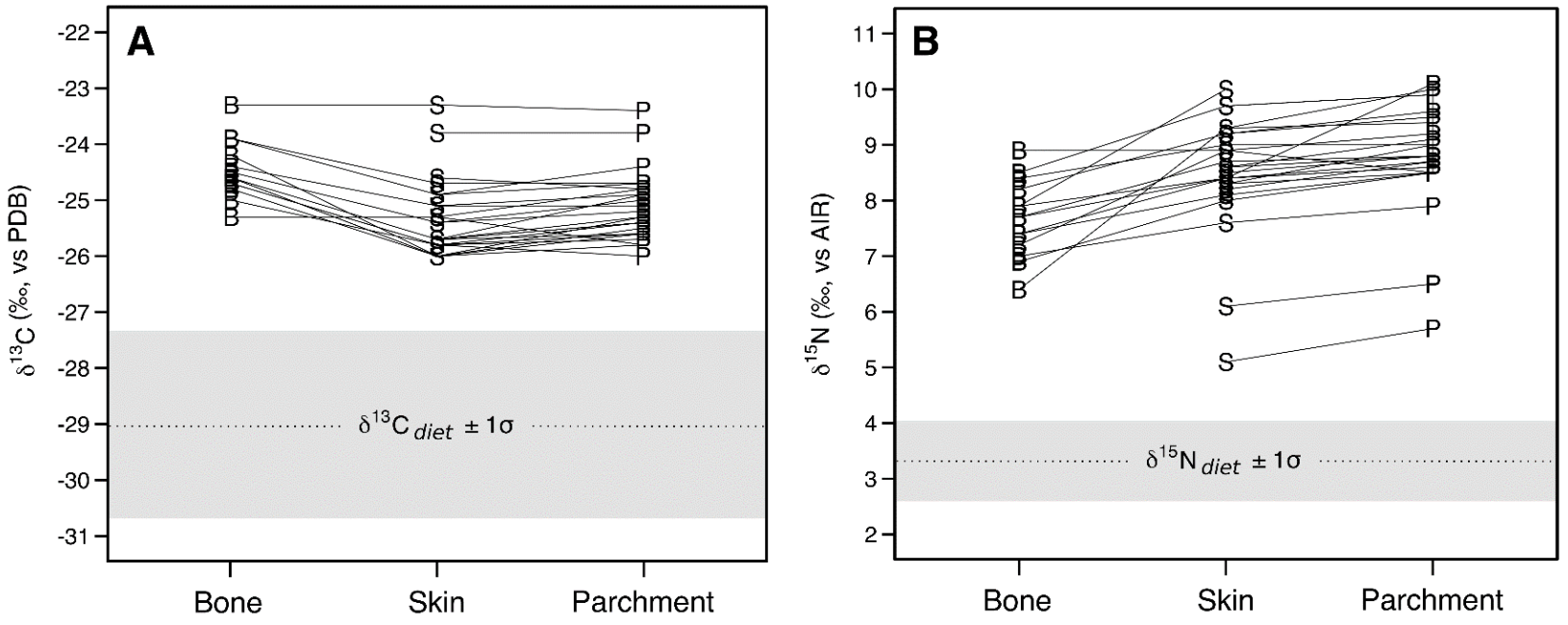


Figure 6.2: Isotopic relationship between diet and tissues, A) $\delta^{13}C$, and B) $\delta^{15}N$ (Seaton Ross sheep only)

6.3.2 Isotopic relationship between bone and skin

The isotopic composition of tissues used to explore the relationship between bone and skin are detailed in Table 6.4 and Table 6.6, and summarised in Table 6.7. Pairwise comparison reveals that skin is more depleted in ^{13}C than bone (0.8 ± 0.8‰), but more enriched in ^{15}N (1.1 ± 1.9‰). This pattern is consistent with data from archaeological populations, and modern grass eating ruminants (Vogel 1978). Parchment was depleted in ^{13}C relative to bone, though not to the same extent as skin (0.6 ± 0.8‰), and more enriched in ^{15}N than both (1.3 ± 1.8‰), although as noted earlier, this likely due to the loss of lighter compounds through deamidation and saponification during production. Using the husbandry information available, statistical analysis indicated:

- There was no significant difference in $\Delta_{bone\text{-}skin}$ offset between male (n=11) and females (n=5):
  $\Delta^{13}C_{bone\text{-}skin}$ – Mann-Whitney U test, U=65.5, p=0.724
  $\Delta^{15}N_{bone\text{-}skin}$ – Mann-Whitney U test, U=51.5, p=0.261

- There was no significant difference in $\Delta_{bone\text{-}skin}$ offset between juveniles (6-17 months) (n=12) and adults (>18 months old) (n=4):
  $\Delta^{13}C_{bone\text{-}skin}$ – Mann-Whitney U test, U=43.5, p=0.324
  $\Delta^{15}N_{bone\text{-}skin}$ – Mann-Whitney U test, U=54.0, p=0.794

- There was no significant difference in $\Delta_{bone\text{-}skin}$ offset depending on the season of slaughter Winter – DJF (n=4); Spring – MAM (n=17); Summer – JJA (n=2); Autumn – SOC (n=4):
  $\Delta^{13}C_{bone\text{-}skin}$ – Kruskal-Wallis test, p=0.106
  $\Delta^{15}N_{bone\text{-}skin}$ – Kruskal-Wallis test, p=0.787

Although the $\delta^{13}C$ and $\delta^{15}N$ values of bone and skin are significantly correlated ($\delta^{13}C$: r=0.877, p=<0.001; $\delta^{15}N$: r=0.908, p=<0.001), they are not identical, as would be expected if the amino acids from the diet were similarly apportioned to both tissues (Finucane 2007, 2121). The protein component of both is dominated by Type 1 collagen (COL1a1) (Ambrose 1993, 76-77; Covington 2009, 23), and are thought to have analogous biosynthetic/dietary origins of amino acids (Corr *et al.* 2009). Part of this ^{13}C-depletion in skin may be due to the greater quantity of carbon-depleted lipids relative to bone, although all samples underwent lipid extraction prior to analysis reducing its potential impact (Liden 1995; Guiry et al. 2016). While the faster turnover rate of dermal collagen than that of bone collagen (el-Harake *et al.* 1998; Babraj *et al.* 2005; Hedges *et al.* 2007) undoubtedly influences this difference, the skins of two stillborn lambs and a two-day old lamb all showed a depletion in ^{13}C (0.7 to 1.1‰) and enrichment in ^{15}N (0.7 to 1.1‰) consistent with the wider pattern. These individuals had received all of their nutrition from their mother *in utero* and could not have

developed any turnover-related difference between these tissues. The observed difference in paired samples of modern sheep skin and bone cannot be attributed to short-term variation in location (Corr *et al.* 2009), diet (White & Schwartz 1994; Finucane 2007; Williams 2009; Corr *et al.* 2009), health (Finucane 2007; Norris 2009), or differential decomposition of the collagen (Finucane 2007). Therefore, some doubt is cast upon the interpretations presented by these archaeological studies. This analysis suggests that some of the observed isotopic variation likely results from small variation in the amino acid composition of these tissues, and the routing of nutrients from the diet.

While the presence of elastin and keratin in the skin may alter the amino acid composition between these two tissues, it is unlikely that they significantly impact on the isotope offset between skin and bone collagen. The higher glycine content in elastin has the potential to elevate ^{13}C values (Hare *et al.* 1991; Corr *et al.* 2009), but this was not seen. Moreover, keratin is depleted in ^{15}N relative to collagen (Tieszen & Fagre 1993; O'Connell *et al.* 2001), but skin nitrogen values were higher than bone. At the moment we do not fully understand the factors underlying this difference, however, amino acid profiles of paired samples may shed light on the importance of composition differences, as well as monitoring the incorporation of radio-labelled amino acids to examine nutrient routing.

Animal	Age (mo)	C/N Bone	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
			Bone	Skin	Parchment	Bone	Skin	Parchment
LL01	20	3.2	–25.2	–25.3	–25.3	5.8	7.3	7.6
LL02	<1	3.2	–24.0	–24.7	–24.6	7.1	8.0	8.2
NW01	~9	3.2	–25.3	–25.6	–26.0	8.5	9.7	9.8
NW02	~9	3.2	–25.1	–25.5	–25.4	8.0	9.5	9.4
NW03	~9	3.2	–25.0	–25.4	–25.9	9.1	10.1	10.3
NW04	~9	3.2	–25.3	–25.6	–25.5	9.0	10.0	10.3
NW05	~9	3.2	–25.3	–25.8	–26.1	8.8	10.0	10.2
NW06	~9	3.2	–25.5	–26.0	–25.8	9.4	10.5	10.8
NW07	~9	3.2	–24.8	–25.4	–25.5	7.7	7.4	7.8
NW08	~9	3.2	–24.8	–25.9	–25.9	8.2	7.2	7.6
NW09	~9	3.2	–24.9	–26.4	–25.8	6.6	9.1	9.4
NW10	~9	3.2	–25.4	–25.8	–25.9	8.0	9.6	9.7

Table 6.6: Isotopic composition of tissues from additional sheep remains, used in the analysis of inter-tissue spacing

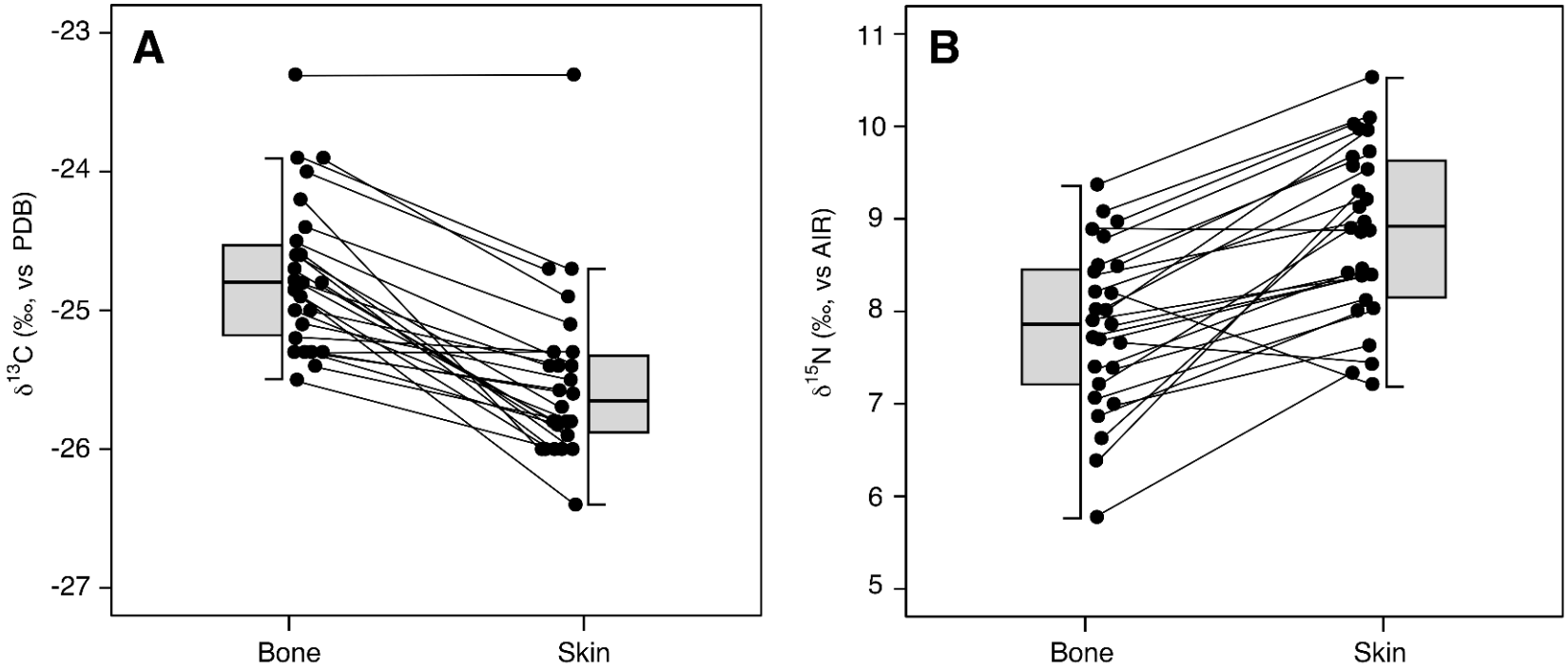


Figure 6.3: Isotopic relationship between bone and skin collagen isotope values in sheep, A) $\delta^{13}C$, and B) $\delta^{15}N$ (all sheep)

	n	$\delta^{13}C$ (‰)	Range	$\delta^{15}N$ (‰)	Range
$\Delta_{bone\text{-}skin}$	26	0.8	± 0.8	-1.1	± 1.9
$\Delta_{bone\text{-}parchment}$	38	0.6	± 0.8	-1.3	± 1.8

Table 6.7: Summary of inter-tissue isotopic spacing (all sheep)

6.3.3 Implications for parchment

The results from this study confirm that ^{13}C and ^{15}N isotope values in skin and parchment are higher than values for the sheep's diet. The offset between diet and skin collagen is comparable with that of bone collagen, although show substantial individual variability. Parchment $\delta^{13}C$ values can be elevated over diet by as much as 5.2‰ (JSK25), although is more likely to be around 4‰. Parchment values were closer to diet than bone in this study, and therefore should enable dietary patterns to be inferred from historic skins. The results from this analysis indicate that $\delta^{15}N$ values in parchment may be elevated by as much as 3‰ over that of bone collagen (JSK12), and as much as 6.7‰ over diet (JSK01). Both Campana *et al.* (2010) and Pollard & Brock (2011) have observed considerably enriched $\delta^{15}N$ values in parchment, with one early-19th century skin as high as 14.2‰. While these high values may reflect farming practices such as organic manuring (Bogaard *et al.* 2007; Bateman & Kelly 2007) and or the limited consumption of leguminous crops (Treasure *et al.* 2016), or physiological issues such as nutritional stress (Hobson *et al.* 1993; Hatch *et al.* 2006), this elevation is likely due in part to the preferential loss of ^{14}N during metabolism, the loss of ^{14}N through deamidation during liming, as well as variations in the amino acid composition and routing in skin.

The isotopic variability within a flock increases with the turnover rate of the tissue analysed, due to the increasing potential to incorporate short-term seasonal variation. Based upon the analysis of modern sheep, isotopic variability is seen to follow the pattern: wool > skin > muscle > bone (Camin *et al.* 2007; Perini *et al.* 2009; von Holstein *et al.* 2013; Zazzo *et al.* 2015), a progression that parallels increasing turnover rates. The sheep raised in Seaton Ross grazed an area of ~4.5 acres, and consumed a restricted range of food. Nevertheless, bone collagen $\delta^{13}C$ and $\delta^{15}N$ values displayed a range of 2.1‰ and 2.5‰, respectively, highly comparable with those from the English Heritage Sheep Project ($\delta^{13}C$ – 2.2‰; $\delta^{15}N$ – 2.5‰) (von Holstein *et al.* 2013). The resulting parchment from these sheep displayed a range in $\delta^{13}C$ values of 2.7‰ and $\delta^{15}N$ values of 4.3‰. This high degree of individual variation is perhaps not surprising, because terrestrial herbivores consuming identical diets can vary in $\delta^{13}C$ by up to 0.8‰ and $\delta^{15}N$ by 3.6‰ (Sponheimer e*t al.* 2003a; 2003b), and plants can

vary by over 10‰ over a small area due to varying mycorrhizal activity and areas of localised defecation (Hobbie *et al.* 2000; Dawson *et al.* 2002).

6.4 Summary

This study examined diet to tissue offset through a free-ranging diet study, and inter-tissue isotopic spacing through the analysis of paired skin and bone samples. Consistent with the result from other feeding studies, sheep tissue was isotopically enriched over diet. The extent of enrichment varied between tissues, with bone ^{13}C values more enriched than skin and parchment, while ^{15}N values were most enriched in parchment. The study revealed no relationship between diet to tissue offset with sex, age, or season of slaughter. This enrichment therefore, most likely reflects variations in the amino acid composition of tissue, the routing of amino acids, and individual metabolism. This study indicates that parchment $\delta^{15}N$ values can be enriched by as much as 6.7‰ over diet, due both to the diet to skin offset and the impact of production. This may, however, caution the interpretation of high $\delta^{15}N$ values which have previously been observed in parchment.

Skin and bone are both dominated by Type 1 collagen, display highly similar amino acid compositions, and are through to have analogous dietary/biosynthetic origins of amino acids. Despite this, skin $\delta^{13}C$ values were typically depleted relative to bone (mean = 0.8‰), and $\delta^{15}N$ values were enriched (mean 1.1‰). This pattern has been observed in archaeological human remains, and previously explained through a range of environmental and cultural factors due to the different turnover rates of these tissues. These explanations are not possible for these modern sheep, whose location and diet did not differ, and suggests that variations in the amino acid composition of these tissue and the routing of amino acids contributes to this isotopic difference.

Chapter 7

$\delta^{13}C$ and $\delta^{15}N$ isotope analysis of post-medieval sheep bone collagen

This chapter presents the isotopic analysis sheep bones from 15th to 20th century York and London, which are synthesised with published results to explore broad temporal trends in $\delta^{13}C$ and $\delta^{15}N$ values. These results are integrated with zooarchaeological and historical evidence to explore the influence of nutritional and husbandry change on conformational change.

7.1 Introduction

Large-scale meta-analyses of archaeological data have the potential to address issues typically beyond the scope of site-level studies. Many of the big themes in contemporary archaeology, such as human evolution and migration, the adoption and adaption of agriculture, and trading network, necessitate the synthesis of data on a large scale (Kintigh *et al.* 2014; Orton & Morris 2016; Orton *et al.* 2016; Slatkin & Racimo 2016; Cooper & Green 2017; Olalde *et al.* 2018). Zooarchaeology has long embraced inter-site comparison and online repositories for data (e.g. ABMAP 2003), and in the era of 'big data' has led the field in highlighting the value of meta-analysis in addressing archaeological questions (e.g. Thomas *et al.* 2013; Orton *et al.* 2014; 2016). In archaeological stable isotope studies, inter-site comparison is routinely undertaken, but the large synthesis of data has been less common, and has so far focussed on human mobility through oxygen ($\delta^{18}O$) and strontium ($\delta^{87}Sr$) isotope analysis (Evans *et al.* 2012; Pellegrini *et al.* 2016). Meta-analyses and shared databases have been championed (Britton 2017; Pauli *et al.* 2017) but, perhaps unsurprisingly, the major temporal and inter-site studies have been those which integrate zooarchaeological and stable isotope analysis (e.g. Barrett *et al.* 2011; Fisher & Thomas 2013; Madgwick e*t al.* 2012; Sykes *et al.* 2016). Building upon these studies, this chapter presents the analysis of forty-seven sheep bones

from 15th to 20th century York and London, and integrates them with published isotope and zooarchaeological data to examine long-term trends in sheep management and diet. Unfortunately, this dataset remains limited, with much of the published data broadly dated, and few pertaining solely to the post-medieval period. In an effort to address this, the remain analysed in this study were selected from tightly dated contexts and focus on the post-16th century.

7.1.1 Stable isotopes and post-medieval zooarchaeology

Post-medieval faunal remains are a particularly profitable source of isotopic information due to the significant agricultural and landscape changes of the period, as well as the ability to integrate results with textual sources (Guiry *et al.* 2012). A range of historically important husbandry and farming practices have the potential to alter the isotopic value of sheep tissue, including: organic manuring (Commisso & Nelson 2006; Bogaard *et al.* 2007; Heaton *et al.* 2009; Fraser *et al.* 2011; Szpak *et al.* 2009); intensive folding (Aranibar *et al.* 2008); the use of synthetic fertilisers (Choi *et al.* 2002; 2003; Bateman & Kelly 2007); foddering (Balasse *et al.* 2006; Madgwick *et al.* 2012); deeper ploughing (Ledgard *et al.* 1984; Koopmans *et al.* 1997); the cultivation of nitrogen-fixing crops (DeNiro & Epstein 1981); the introduction of maize (O'Leary 1988); as well as the increasing mobility of livestock and prepared meat due to transport improvements.

While this technique has been widely applied to North American and Australian historical remains, particularly in the analysis of urban provisioning and long-distance meat trade (e.g. Guiry *et al.* 2012; 2014; 2017), much of the available post-medieval faunal isotope data from the UK comes from the construction of terrestrial herbivore baselines to aid the interpretation of human collagen data (Table 7.1). These results, however, show considerable inter-site variability in sheep $\delta^{13}C$ and $\delta^{15}N$ values, but little temporal variation in mean values until the 21st century. Unfortunately, most of these data are broadly phased, and is therefore difficult to integrate with documentary sources, but may indicate that the agricultural developments of this period had little effect on the isotopic composition of sheep tissues. As Trow-Smith noted, even in the 19th century, sheep were 'less responsive to improvement', and were managed under more 'natural' and simple practices (1959, 319).

At Dudley Castle, West Midlands, Fisher & Thomas (2012) integrated zooarchaeological, textual and stable isotope evidence to examine potential nutritional and husbandry factors behind conformational change in cattle, and a reduction in slaughter age. Biometrical analysis indicated a statistically significant increase in the size of post-cranial elements between Phases 5 (1262–1321) and 6 (1321–1397), and 8 (1533–1647) and 9 (1647–1750), as well as a gradual reduction in cattle

age between Phases 6 and 8, with an increasing emphasis on the consumption of 'prime' young adults. These changes could not be linked to shifts in the sexual composition of the herd, and the paucity of teeth measurements made it difficult to determine if there was any genetic change. $\delta^{13}C$ and $\delta^{15}N$ isotope analysis was conducted on 135 targeted bones covering this period, but revealed generally static values, with no significant variation in either isotope, aside from a period of more $\delta^{13}C$-depleted values in Phase 7 (1397–1533). They concluded that the gradual increase in size and reduction in slaughter age was not accompanied by and major dietary or environmental change, or they were insufficiently distinct to alter the isotopic values of the cattle tissue. With conformation change noted in post-medieval sheep from York (O'Connor 1995) and London (Thomas *et al.* 2013), stable isotope analysis here may prove useful in exploring potential nutritional and husbandry factors.

			$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)			
Site	Period	n	Mean	SD	Range	Mean	SD	Range	Reference
Swinegate, York	13th century	16	–21.88	0.44	1.63	6.98	1.67	5.45	Cooper 2011*
Wharram Percy, Yorkshire	10th-16th century	5	–21.82	0.32	0.80	5.88	1.01	2.60	Müldner & Richards 2005
St Giles, Yorkshire	12th-15th century	9	–21.67	0.27	0.90	6.05	1.23	4.30	Müldner & Richards 2005
Fishergate, York	13th-16th century	3	–21.03	0.89	1.60	4.86	0.12	0.20	Müldner & Richards 2007
Portmahomack, Ross	15th century	1	–22.00	-	-	8.80	-	-	Curtis-Summers *et al.* 2014
Durham	16th-17th century	15	–21.51	0.81	2.63	5.36	1.10	3.94	Millard *et al.* 2015
Hungate, York	19th century	3	–22.63	1.62	2.90	4.50	0.14	0.20	Brown 2014*
Queen's Chapel of the Savoy, London	19th century	5	–21.82	0.27	0.70	7.24	2.16	5.70	Bleasdale 2016*
Modern	21st century	20	–26.31	0.47	2.20	7.03	0.62	2.50	von Holstein *et al.* 2014
Modern	21st century	26	–24.73	0.54	2.22	7.83	0.88	3.60	This study (Chapter 6)

Table 7.1: Available sheep bone collagen $\delta^{13}C$ and $\delta^{15}N$ values from late-medieval and post-medieval Britain.

*MSc theses held at the Department of Archaeology, University of York

7.2 Material & Methods

7.2.1 Sites

Samples of sheep bone were taken from multiple post-medieval sites in York (Figure 7.1) from contexts dating from the 15th to 20th century, and from 16th to 19th century contexts from Prescot Street, London (Figure 7.2). Samples were taken from bones which were fused. Unfortunately, available comparative data does not indicate if sampled bones were fused or unfused (Müldner & Richards 2005; 2007; Brown 2014 Millard *et al.* 2015), therefore there is the potential for some young animals in the data, potentially exhibiting a weaning signal. No attempt was made to distinguish between sheep or goat, as goat bones were not previously identified at any of these sites (O'Connor 1984b; Scott 1985; Reilly 2010; Rainsford 2012). Every effort was taken to ensure that the same individual was not sampled multiple times, by ensuring a range of contexts were analysed and sampling elements from a single side.

Hungate, York (YAT 2006-11; YORM5000)

Located adjacent to the River Foss, within in the historic walled centre of York, Hungate is a large complex site with a sequence of use from a 3rd century Roman cemetery through to early-20th century tenements and industrial complexes (YAT 2015). Between the 15th and 17th century, the area was used for refuse pits, horticulture and the Cordwainers Hall, before more intensive use from the 18th century onwards, with dense terraced housing, gas works and warehouses. By the 19th and early-20th century, the tenements of Hungate had proliferated to become *"one of the main slum districts in York"* (Rowntree 1901, 199), before being cleared in the 1930s (Harrison 2015). Preliminary assessment of the post-medieval faunal material indicates that they reflect this domestic and industrial land use, with a diverse range of taxa and elements present, along with multiple caches of sheep metacarpals indicative of skin processing (Rainsford 2012). Metrical analysis was conducted on 86 sheep metacarpals from the 16th-20th century (measurements according to von den Driesch 1976), and are presented later in the discussion. As with other multi-period sites, the excavation presented complex stratigraphy, with residual and intrusive material observed in the pottery and finds assemblages; an issue likely to also affect the faunal material (Rainsford *et al.* 2016). Only the most securely dated and least mixed material was retained after assessment, and samples with the tightest dating were utilised in this analysis.

Walmgate, York (YAT 1978-9; 1978.9.8)

18th and 19th century Walmgate was also a slum area, and considered *"one of the poorest in the city"* (Rowntree 1901, 199). Located in the south of the walled centre, extensive deposits of sheep metapodia were recovered from clay lined pits. They were associated with industrial rather than

domestic activity, and believed to be the refuse from nearby tanners and skin processors (O'Connor 1984b).

The Bedern, York (YAT 1976-83; 1983.13)
Located east of York Minster, the Bedern was an area of residential occupation and light industry (Richards 1993), with faunal evidence for domestic activity and some skin processing recovered from pits and garden soil deposits (Scott 1985).

Prescot Street, London (LP Archaeology; PCO06)
Located just to the east of City of London, Prescot Street, is a multi-period site, which like Hungate, saw use from a 2nd century Roman cemetery through to dense terraced housing of the 19th century (Hunt 2008). In the 16th century the area was farmed by the Abbey of St Clare, before taking on an increasingly residential and commercial nature after the Dissolution of the Monasteries. As the capital grew, Prescot Street and the East End became an area of dense poor-quality housing, with a less affluent population (Hunt *et al.* 2008). Assessment of the faunal remains shows a diverse range of taxa, along with caches of sheep metapodia presenting evidence for sheepskin processing (Reilly 2010).

7.2.2 Stable isotope analysis

Samples were taken from a range of contexts, and effort made to ensure that each sample represented a single individual. Samples of bone (~5-10 g) were cleaned to remove the outer surface and any contaminant, and then demineralised in 0.6M HC1 at 4ºC for up to 10 days. The resulting insoluble fraction was gelatinised in pH 3 0.001M HC1 at 80ºC for 48 hrs and the supernatant filtered (60-90 µm Ezee-Filter™, Elkay Laboratories, UK), frozen, and freeze-dried. 0.8-1.1 mg of collagen was weighed out in 4 x 3.2 mm tin capsules (Elemental Microanalysis, Okehampton, UK) and analysed in duplicate. Isotopic analysis was conducted at BioArCh, Department of Archaeology, University of York. $\delta^{13}C$ and $\delta^{15}N$ isotope ratio determinations were carried out on a Sercon GLS analyser coupled to a Sercon 20-22 Mass Spectrometer (Sercon, Crewe, UK), and are reported in per mille (‰) in reference to VPDB and AIR. An internal standard of gelatine (Sigma-Aldrich, St. Louis, US) was used, alongside international IAEA standards. The isotopic composition and accuracy of control standards are reported in Table 7.2

Reproducibility between samples was better than ± 0.2‰ (1σ) for $\delta^{13}C$, %C, $\delta^{15}N$ and %N. %C ranged from 32.0-44.5%, %N from 11.7-16.3%, and C/N rations were between 3.1-3.2, all indicative of well-preserved collagen (DeNiro 1985; Ambrose 1990).

The 20th-century bones from Hungate are thought to date before the slums clearance in the 1930s. To account for the introduction of ^{12}C into the atmosphere through the burning of fossil fuels during this time, a Suess Effect (Friedeli *et al.* 1986) correction value of 0.15‰ was calculated from Hellevang & Aagaard's (2015) model for these four samples. To aid the integration of 21st century bone, and correction value of 1.7‰ was used (see Section 8.2.2).

7.2.3 Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistics 22.0 software package (IBM 2013). The statistical significant of inter-site and temporal variation in $\delta^{13}C$ and $\delta^{15}N$ values was determined using the non-parametric Mann-Whitney U tests for paired samples, and Kruskal-Wallis test for multiple samples. The equality of variance between samples was calculated using a Levene's test.

		$\delta^{13}C$ values (‰, vs. VPDB)		$\delta^{15}N$ values (‰, vs. AIR)	
Standard	*n*	Observed	True	Observed	True
Fish Gelatine (SIGMA)	26	-15.32 ± 0.2	-15.32[a]	15.17 ± 0.1	15.2[a]
Fish Gelatine (Bradford)	14	-15.58 ± 0.4	-15.5[a]	14.40 ± 0.2	14.4[a]
Cane Sugar (IA-R006)	9	-11.78 ± 0.2	-11.64 ± 0.03	-	-
Caffeine (IAEA 600)	9	-27.63 ± 0.2	-27.77 ± 0.04	0.7 ± 0.2	1 ± 0.2
Ammonium Sulfate (IAEA N2)	9	-	-	20.47 ± 0.3	20.3 ± 0.3

Table 7.2: Isotopic analytical precision of standards run during analysis at BioArCh, University of York. [a] Standard deviation not defined, - = not measured

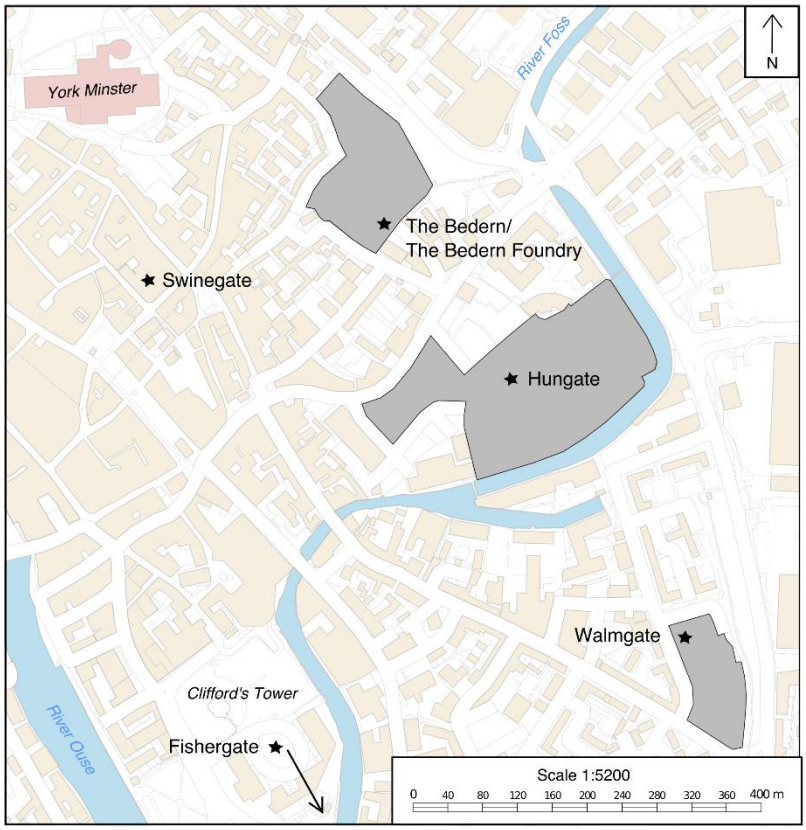


Figure 7.1: Location of sites in York sites from which material was obtained, along with previous published material. OS Data © Crown Copyright 2018

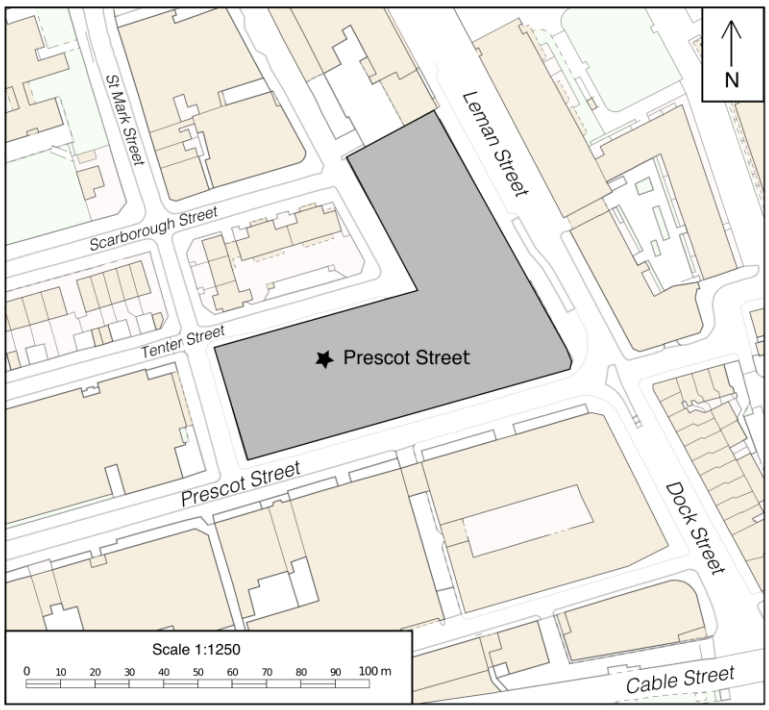


Figure 7.2: Location of Prescot Street excavations. OS Data © Crown Copyright 2018

7.3 Results

Full $\delta^{13}C$ and $\delta^{15}N$ results are presented in Table 7.3, and Figures 7.3 (York) and 7.4 (London). Three samples showed a deviation greater than 0.2 ‰ (1σ) between duplicates and were removed from further analysis. A significant outlier was observed in the 18th-19th century material recovered from Hungate (Brown 2014), possessing a $\delta^{13}C$ value of –24.5, far more comparable with 21st-century values. Recognising the stratigraphic complexities and mixing at this site (Rainsford 2012; Rainsford *et al.* 2016) it is highly likely that this bone is intrusive, and was therefore removed from any statistical analysis. Statistical analysis revealed no significant difference between samples from the three York sites for either isotope (Kruskal-Wallis test, $\delta^{13}C$: p = >0.05; $\delta^{15}N$: p = >0.05). There was no statistically significant variation in $\delta^{15}N$ between the 13th and 20th century in York, but 20th century $\delta^{13}C$ values were significantly more depleted than those from the 18th- 19th century (Table 7.4). The sheep from York were comparable with those from London, but despite being geographically closer, the $\delta^{13}C$ values of the 15th-17th century material was significantly different to the 16th-17th century Durham (Millard *et al.* 2015) (Table 7.5).

There was no significant difference between the London samples from Prescott Street and Queen's Chapel of the Savoy, and the values from both sites were comparable with those from contemporary York and London (Table 7.6). There was no significant temporal difference in $\delta^{13}C$ values, but there was a statistically significant enrichment in $\delta^{15}N$ values between the 16th-17th and 18th-19th century. In both York and London, variability was greater in $\delta^{15}N$ values than $\delta^{13}C$ values, with all periods in York and the 18th-19th century material in London showing variations on the scale of trophic differences (3-5 ‰) (Bocherens & Drucker 2003).

Sample	Site	Phase	Period	Element	$\delta^{13}C$ (‰)	%C	$\delta^{15}N$ (‰)	%N	C/N
PCSG02	PCS	EPM	16th-17th C	SCAP	-21.9	41.4	5.1	15.4	3.1
PCSG03	PCS	EPM	16th-17th C	MAN	-21.1	43.2	5.5	15.8	3.2
PCSG10	PCS	EPM	16th-17th C	MT	-22.0	42.7	5.4	15.8	3.2
PCSG12	PCS	EPM	16th-17th C	MC	-22.1	43.2	5.1	16.1	3.1
PCSG16	PCS	EPM	16th-17th C	HUM	-21.2	44.1	5.6	16.1	3.2
PCSG33	PCS	EPM	16th-17th C	SCAP	-22.2	43.8	5.3	16.3	3.2
PCSG36	PCS	LPM	18th-19th C	MAN	-21.7	41.9	6.2	15.3	3.2
PCSG38	PCS	LPM	18th-19th C	HUM	-22.3	32.0	8.0	11.5	3.2
PCSG39	PCS	LPM	18th-19th C	FEM	-22.4	34.1	5.9	12.4	3.2
PCSG40	PCS	LPM	18th-19th C	MC	-21.6	40.6	6.0	14.9	3.2
PCSG45	PCS	LPM	18th-19th C	TIB	-22.4	35.9	7.9	13.1	3.2
PCSG46	PCS	LPM	18th-19th C	HUM	-22.4	35.3	5.4	12.7	3.2
HUN01	HUN	3905	19th C	MAN	-22.3	39.8	7.2	14.3	3.2
HUN02	HUN	3905	19th C	MAN	-21.9	43.3	4.5	15.9	3.1
HUN03	HUN	3905	19th C	MAN	-22.1	41.0	9.2	15.3	3.1
HUN027	HUN	3946	15th C	MC	-22.6	39.3	6.2	14.3	3.2
HUN064	HUN	3917	18th-19th C	MC	-21.6	39.3	4.6	14.1	3.2
HUN080	HUN	3914	18th-19th C	MC	-22.1	39.2	6.1	14.2	3.2
HUN082	HUN	3914	18th-19th C	MC	-22.2	40.0	6.5	14.4	3.2
HUN101	HUN	3904	20th C	MC	-22.2	39.2	4.1	14.3	3.2
HUN102	HUN	3904	20th C	MC	-22.3	39.3	5.3	14.2	3.2
HUN105	HUN	3904	20th C	MC	-22.2	38.8	7.0	14.3	3.2
HUN108	HUN	3904	20th C	MC	-23.0	40.0	7.0	14.5	3.2
HUN160	HUN	3911	19th C	MC	-22.1	39.0	4.7	14.0	3.2
HUN161	HUN	3991	19th C	MC	-22.5	38.4	6.7	13.9	3.2
HUN162	HUN	3911	18th-19th C	MC	-22.3	43.0	6.1	15.5	3.2
HUN163	HUN	3911	18th-19th C	MC	-22.0	36.2	8.9	13.2	3.2
HUN165	HUN	3911	18th-19th C	MC	-22.7	43.0	8.2	15.6	3.2
HUN241	HUN	3910	16th-17th C	MT	-22.5	44.5	6.4	16.1	3.2
HUN267	HUN	3910	16th-17th C	MT	-21.5	43.1	6.1	15.6	3.2
HUN422	HUN	3912	18th-19th C	MC	-21.8	42.7	3.6	15.6	3.2
HUN426	HUN	3912	18th-19th C	MT	-21.7	39.6	6.9	14.5	3.2
HUN331	HUN	3912	18th-19th C	MC	-22.1	38.9	3.9	14.3	3.2
BED01	BED	14.II	15th C	MC	-22.1	37.6	3.9	13.9	3.2
BED02	BED	14.II	15th C	PEL	-22.3	40.8	8.8	15.2	3.1
BED03	BED	14.II	15th C	MC	-21.8	35.8	5.5	13.5	3.1
BED04	BED	14.II	15th C	SCAP	-22.3	38.7	9.0	14.2	3.2
BED05	BED	14.II	15th C	MT	-22.1	35.7	5.0	13.6	3.1
BED07	BED	13.X	18th-19th C	TIB	-21.5	38.5	5.6	14.5	3.1
BED08	BED	13.X	18th-19th C	HUM	-21.8	37.2	5.4	14.2	3.1
BED09	BED	13.X	18th-19th C	TIB	-21.5	38.4	4.8	14.5	3.1
BED15	BED	13.X	18th-19th C	MC	-20.8	37.9	8.7	14.3	3.1
BED16	BED	13.X	18th-19th C	MC	-22.2	36.2	8.0	13.7	3.1
BED18	BED	13X	18th-19th C	MC	-22.3	34.5	5.2	12.9	3.1
WAL01	WAL	WB	18th-19th C	MAN	-20.8	43.0	6.3	15.5	3.2
WAL02	WAL	WB	18th-19th C	MAN	-21.7	39.1	4.4	13.3	3.2
WAL03	WAL	WB	18th-19th C	MAN	-21.7	42.8	5.4	15.6	3.2

Table 7.3: $\delta^{13}C$ and $\delta^{15}N$ values from post-medieval sheep bones analysed in this study. Sites: PCS – Prescot Street (PCC06); HUN – Hungate (YORM5000); BED – The Bedern (YAT 1976.13/.14); WAL – Walmgate (YAT 1978.8.9)

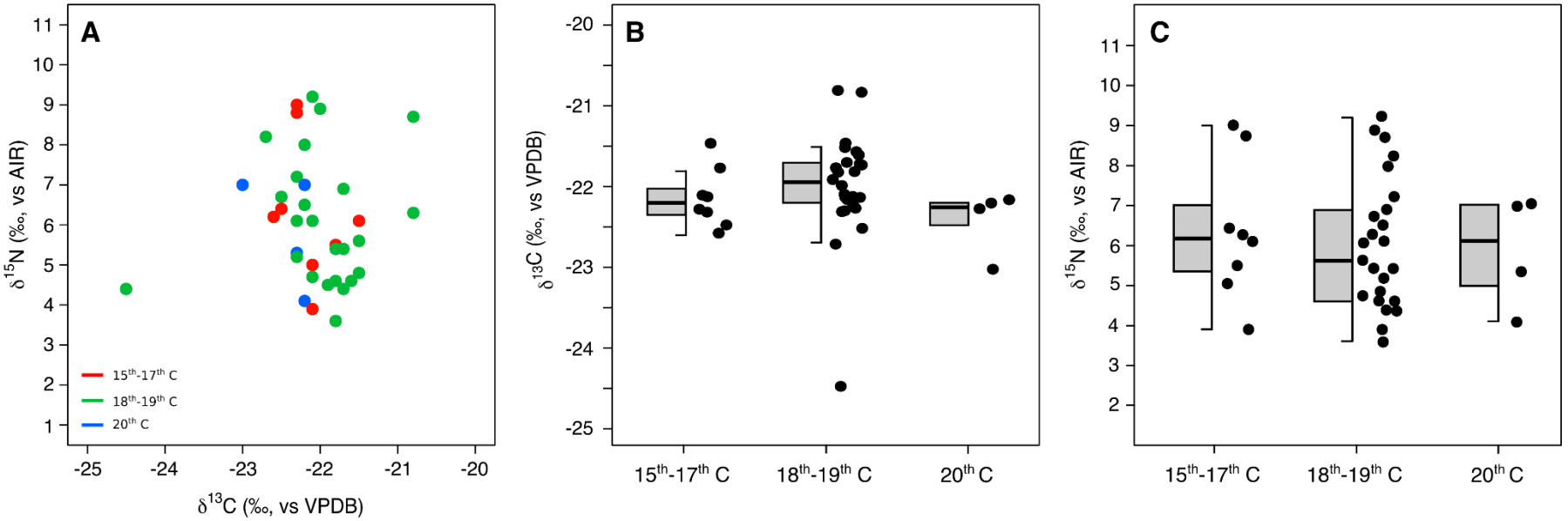


Figure 7.3: Post-medieval York. A) $\delta^{13}C$ and $\delta^{15}N$ values by period; B) Temporal variation in $\delta^{13}C$ values; C) Temporal variation in $\delta^{15}N$ values. Additional data from Brown 2014. Boxplots calculated using R package ggplot 2, presenting the median, maximum, minimum, first and third quartile

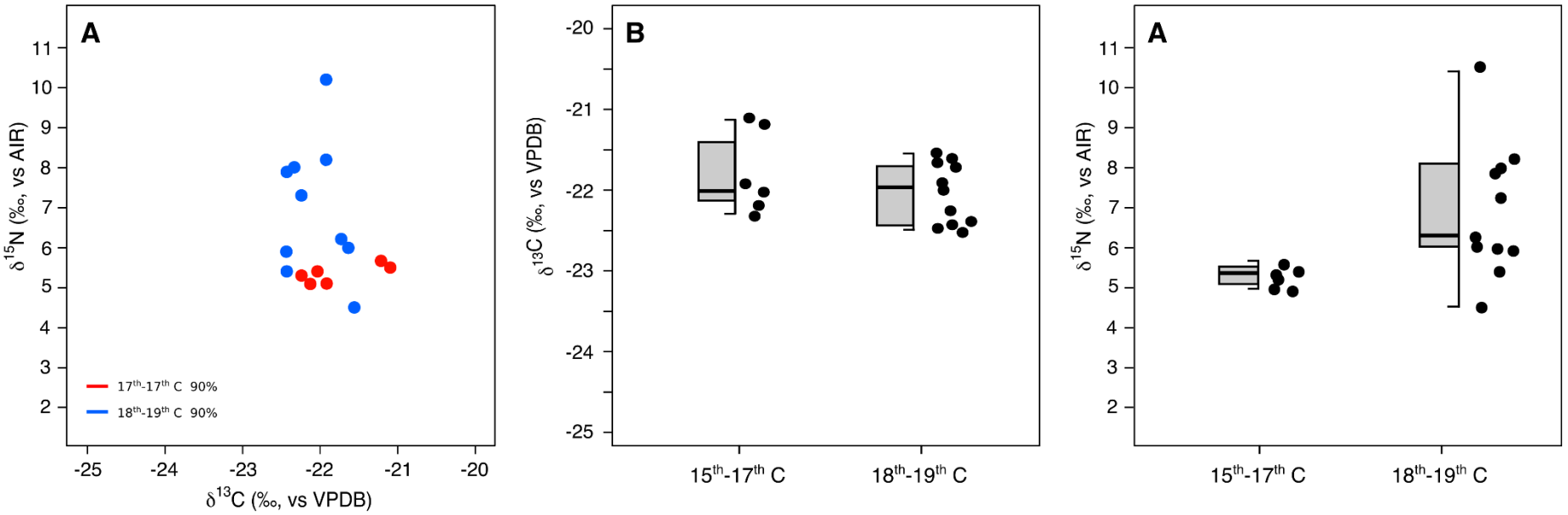


Figure 7.4: Post-medieval London. A) $\delta^{13}C$ and $\delta^{15}N$ values by period; B) Temporal variation in $\delta^{13}C$ values; C) Temporal variation in $\delta^{15}N$ values. Additional data from Bleasdale 2016. Boxplots calculated using R package ggplot 2, presenting the median, maximum, minimum, first and third quartile.

Isotope	Period Comparison	U	*P*
$\delta^{13}C$	13th Century (n=16) – 15th-17th Century (n=11)	40.5	0.15
	15th-17th Century (n=11) – 18th-19th Century (n=26)	58.5	0.17
	18th-19th Century (n=26)– 20th Century (n=4)	15.5	**0.04**
$\delta^{15}N$	13th Century – 15th-17th Century	45.5	0.26
	15th-17th Century – 18th-19th Century	79.0	0.70
	18th-19th Century – 20th Century	40.0	0.91

Table 7.4: Mann-Whitney U tests of $\delta^{13}C$ and $\delta^{15}N$ values from York by phase. Data pooled from this study; Cooper 2011; Brown 2014. Bold values indicate statistically significant differences *(P<0.05)*

Isotope	Period Comparison	U	*P*
$\delta^{13}C$	York (15th-17th Century) (n=11) – Durham (16th-17th Century) (n=15)	27.0	**0.03**
	York (15th-17th Century) (n=11) – London (16th-17th Century) (n=6)	11.0	0.11
	York (18th-19th Century) (n=26) – London (18th-19th Century) (n=11)	117.0	0.90
	London – Prescott St. (18th-19th Century) (n=6) – London – Queen's Chapel of the Savoy (19th Century) (n=5)	11.5	0.54
	London (16th-17th Century) – Durham (16th-17th Century)	41.0	0.80
$\delta^{15}N$	York (15th-17th Century) – Durham (16th-17th Century)	38.5	0.17
	York (15th-17th Century) – London (16th-17th Century)	13.5	0.19
	York (18th-19th Century) – London (18th-19th Century)	92.5	0.30
	London – Prescott St. (18th-19th Century) – London – Queen's Chapel of the Savoy (19th Century)	11.5	0.92

Table 7.5: Mann-Whitney U tests of $\delta^{13}C$ and $\delta^{15}N$ values from York, London and Durham. Data from this study; Brown 2014; Millard *et al.* 2015; Bleasdale 2016. Bold values indicate statistically significant differences *(P<0.05)*

Isotope	Period Comparison	U	*P*
$\delta^{13}C$	16th-17th Century (n=6) – 18th-19th Century (n=11)	29.5	0.73
$\delta^{15}N$	16th-17th Century – 18th-19th Century	8.5	**0.01**

Table 7.6: Mann-Whitney U tests of $\delta^{13}C$ and $\delta^{15}N$ values from London by phase. Data pooled from this study and Bleasdale 2016. Bold values indicate statistically significant differences *(P<0.05)*

The results from this study were integrated with those from published analyses and grouped into chronological periods (Table 7.7-7.8, Figures 7.5-7.6). It must be noted, however, that this pools statistically different datasets, which may affect the reliability of this data and the interpretations that can be drawn. However, statistical analysis indicated the following significant patterns:

- $\delta^{13}C$ values from the 20th century are significantly more depleted (mean difference: 0.7‰) than those from the 18th-19th centuries (p=0.05)
- $\delta^{13}C$ values from the 21st century are significantly more depleted (mean difference: 1.4‰) than those from the 20th century (p=<0.001)
- $\delta^{15}N$ values from the 21st century are significantly more enriched (mean difference: 1.6‰) than those from the 20th century (p=0.03)
- Variation in $\delta^{13}C$ values decreases significantly between the 16th-17th centuries to the 18th-19th centuries (p=0.05) and then again to the 20th century (p=0.04), before significantly increasing in the 21st century (p=<0.01). Variation in $\delta^{15}N$ values increases significantly between the 16th-17th centuries to the 18th-19th centuries (p=0.02), and again between the 20th and 21st century (p=<0.01). Despite this, interpretation of these results is cautioned by the considerable variation in sample size between periods.

Isotope	Period Comparison	U	*P*
$\delta^{13}C$	15th Century (n=7) – 16th-17th Century (n=21)	37.0	0.33
	16th-17th Century (n=21) – 18th-19th Century (n=31)	321.0	0.15
	18th-19th Century (n=31) – 20th Century (n=4)	28.0	**0.05**
	20th Century (n=4) – 21st Century (n=46)	0.0	**0.00**
$\delta^{15}N$	15th Century – 16th-17th Century	52.5	0.17
	16th-17th Century – 18th-19th Century	298.5	0.07
	18th-19th Century – 20th Century	63.0	0.71
	20th Century – 21st Century	32.0	**0.03**

Table 7.7: Mann-Whitney U tests of $\delta^{13}C$ and $\delta^{15}N$ values from across Britain by phase. Data pooled from this study; Mülder & Richards 2007; Cooper 2011; Brown 2014; von Holstein *et al.* 2013; Curtis-Summers *et al.* 2014; Millard *et al.* 2015; Bleasdale 2016. Bold values indicate statistically significant differences *(P<0.05)*

Phase	$\delta^{13}C$ (‰ v. VPDB)	$\delta^{15}N$ (‰ v. AIR)
	P	*P*
15th century (n=7) – 16th-17th centuries (n=21)	0.06	0.09
16th-17th centuries (n=21) – 18th-19th centuries (31)	**0.05**	**0.02**
18th-19th centuries (n=31) – 20th centuries (n=4)	**0.04**	0.55
20th century (n=4) – 21st century (n=46)	**<0.01**	**<0.01**

Table 7.8: Results from Levene's Tests of homogeneity of variance. Top between centuries, bottom over 50-year periods *(P<0.05)*

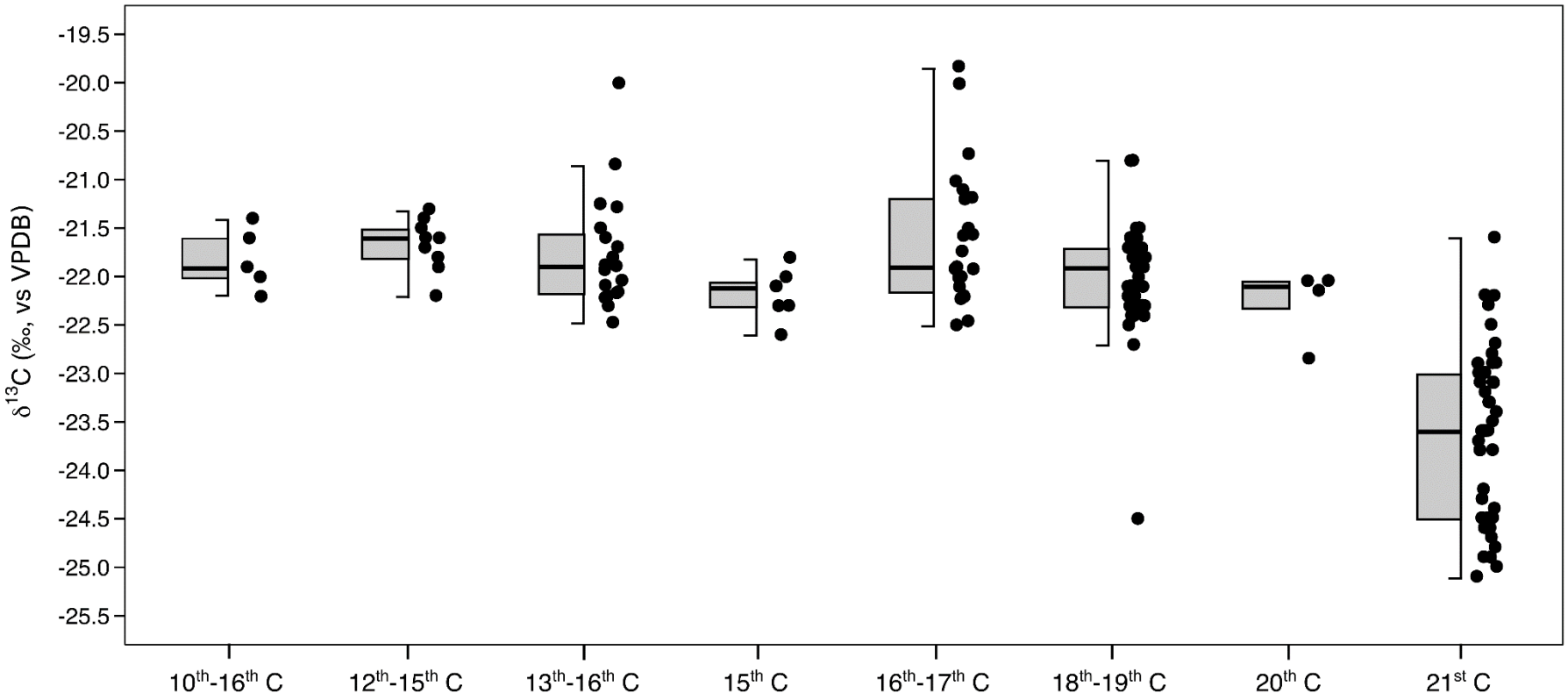


Figure 7.5: Temporal variation in sheep bone collagen $\delta^{13}C$ values from 13th to 21st century Britain. Post-1900 values corrected for Suess Effect. Data from: this study; Mülder & Richards 2005; 2007; Cooper 2011; Brown 2014; von Holstein *et al.* 2013; Curtis-Summers *et al.* 2014; Millard *et al.* 2015; Bleasdale 2016

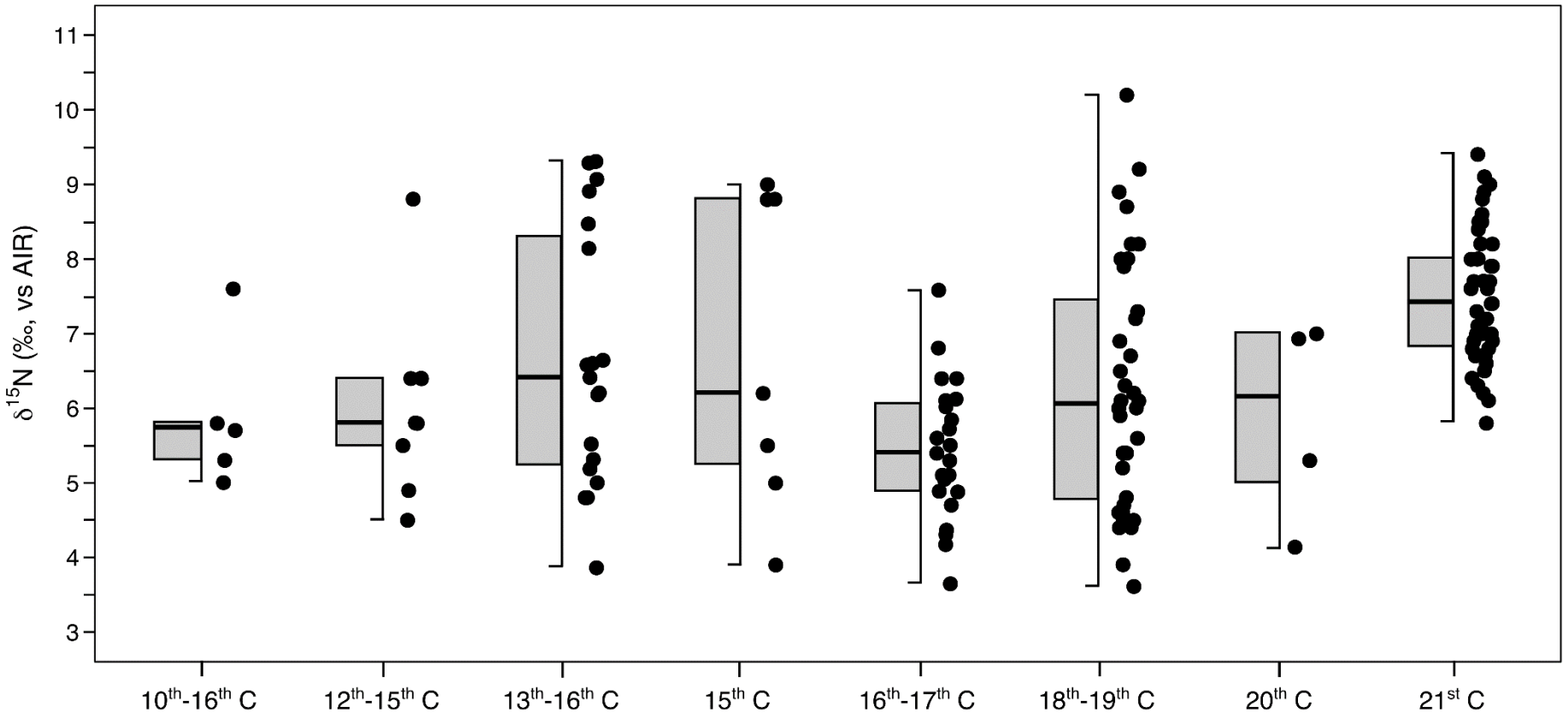


Figure 7.6: Chronological variation in sheep bone collagen $\delta^{15}N$ values from 13th to 21st century Britain. Data from: this study; Mülder & Richards 2005; 2007; Cooper 2011; Brown 2014; von Holstein *et al.* 2013; Curtis-Summers *et al.* 2014; Millard *et al.* 2015; Bleasdale 2016

7.4 Discussion

Integrating biometrical and isotopic evidence has been shown by Fisher & Thomas (2012) to provide valuable insight into potential factors driving size change and the reduction in slaughter age of cattle at Dudley Castle. It is therefore worth applying this to the sheep processed in York and London. Biometrical data from late-medieval and post-medieval York has highlighted considerable continuity in the size of sheep entering the city, with the presence of larger individuals not seen until the early-19th century (Table 7.9) (O'Connor 1984a; 1984b; 1995; Scott 1985). The size and shape of 16th to 20th century sheep metacarpals are presented in Figure 7.7 expressed as log-ratios relative to mean Soay and Clun Forest measurements. Relative to the Soay sample, greatest length ratios are relatively lower than for the other medio-lateral measurements, suggesting that the greatest development away from the 'unimproved' form is the developing cross-sectional dimensions of the bone, not length (O'Connor 1995, 87). Relatively short but robust metacarpals may be taken as potential evidence for faster growth and early maturing due to foreshortening of the limb with earlier maturation (O'Connor 1982). The log ratios indicate appreciably more robust individuals recovered from early-19th century deposits at The Bedern. Relative to the more improved Clun Forest, the sheep from 15th to 20th century York are notably smaller and slenderer, but again the individuals from the early-19th century Bedern display greater comparability with this breed, and indicate that in 19th century York there were sheep which possessed a more improved conformation (O'Connor 1995). Despite this, contemporary and later metacarpals from Hungate analysed in this study are comparable with those from earlier deposits, indicating some continuity in form. Due to only summary metrical data available for some sites, the statistical significance of any changes is unknown.

Bone $\delta^{13}C$ and $\delta^{15}N$ values from York also reveal no statistical change in values until the 20th century. $\delta^{13}C$ values from the 20th century were significantly more depleted, although most were comparable with other British material. In this analysis, bone from 19th-century context at The Bedern identified by O'Connor (1995) as possessing distinctly larger individuals were samples, but they presented values highly similar to those from the same site dating to the 15th century. As at Dudley Castle, the conformational changes do not appear to have coincided with any nutritional change that can be detected isotopically. This apparent lack of change, likely reflects the continuity in the economic role of sheep. At Walmgate and The Bedern, the vast majority of sheep survived beyond three years of age, some to as old as seven (O'Connor 1984a; 33; Scott 1985, 16), most likely raised primarily for their wool. As such there may have been little economic or financial incentive to alter existing feeding or husbandry strategies.

Site (Period)		Mean (mm)	SD	*n*
The Bedern	GL	119.0	7.1	40
15th Century	Bp	21.9	0.9	40
	BFd	25.0	1.0	40
	SD	13.3	0.8	40
The Bedern	GL	120.5	14	25
15th - 16th Century	Bp	22.5	1.2	25
	BFd	24.7	1.1	25
	SD	13.5	0.9	25
The Bedern	GL	129.5	8.2	25
19th Century	Bp	25.2	1.4	28
	BFd	27.2	1.7	22
	SD	15.3	1.3	28
Aldwark	GL	115.8	8.9	60
16th Century	Bp	21.7	1.1	60
	BFd	24.3	1.3	60
	SD	13.1	1.0	60
Walmgate	GL	120.3	8.2	50
18th Century	Bp	22.4	1.3	50
	BFd	25.3	1.5	50
	SD	13.4	1.2	50
Hungate	GL	117.9	8.3	49
16th - 17th Century	Bp	22.6	1.5	98
	BFd	24.5	1.4	52
	SD	13.6	1.0	88
Hungate	GL	119	5.7	18
18th-19th Century	Bp	23	1.2	38
	BFd	24.5	0.9	18
	SD	13.8	1.0	33
Hungate	GL	118.7	6.1	4
20th Century	Bp	22.8	1.1	11
	BFd	24.8	1.1	3
	SD	13.3	0.7	10
Soay	GL	116.2	4.9	69
Modern	Bp	19.8	1.0	70
	BFd	22.2	1.1	70
	SD	12.4	0.9	70
Clun Forest	GL	131.0	8.2	22
Modern	Bp	26.2	2.3	22
	BFd	29.2	2.4	22
	SD	17.6	1.7	22

Table 7.9: Mean, standard deviation, and number of cases for measurements of sheep metacarpals from York post-medieval sites. Data from modern Soay and Clun Forest sheep for comparison (O'Connor 1995, 86; Hungate data from this study). GL = Greatest Length, Bp = Proximal medio-lateral width, BFd = Distal medio-lateral epiphysial width, SD = Minimum medio-lateral shaft width

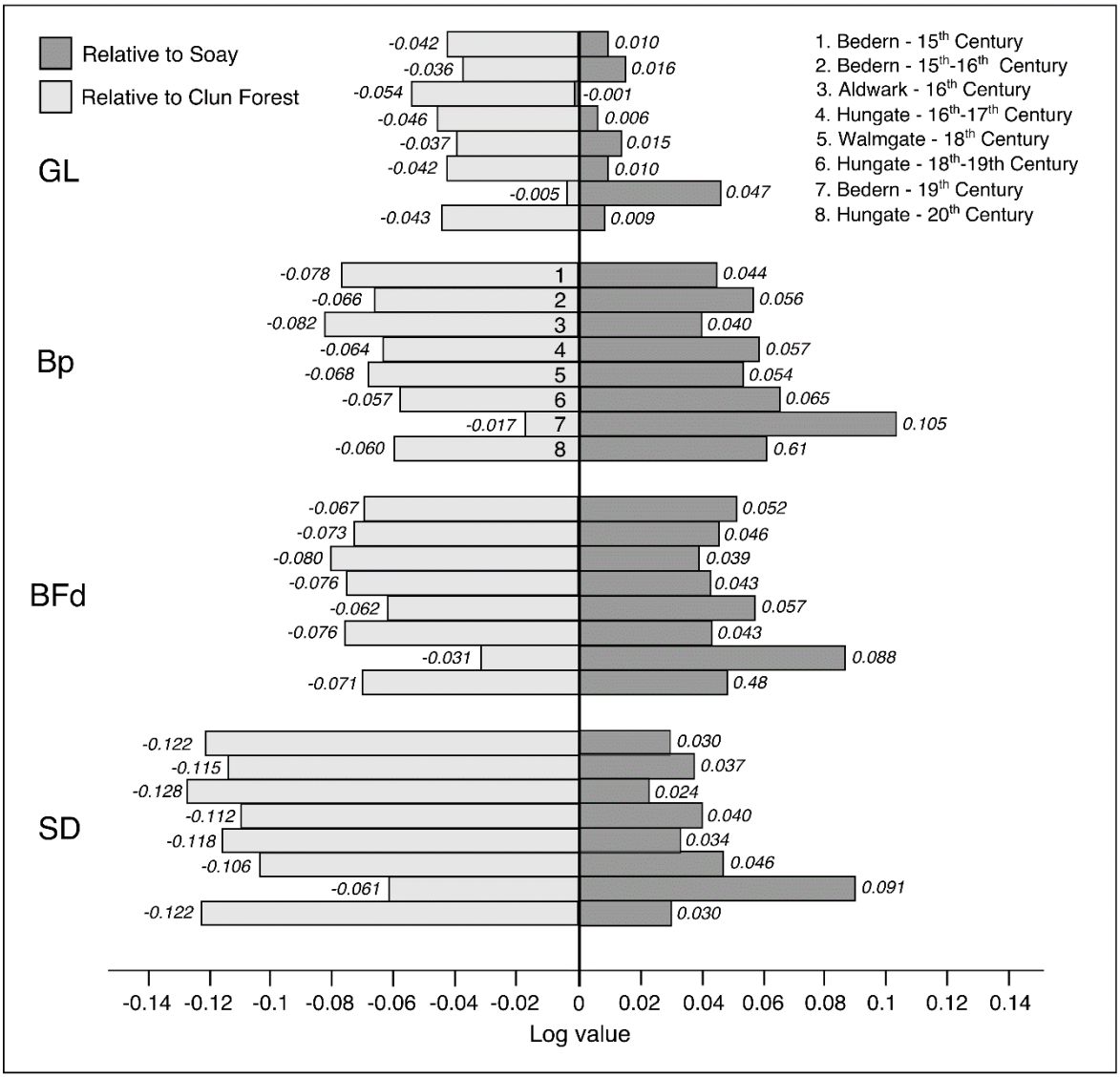


Figure 7.7: Log-scaled comparison of mean sheep metacarpals measurements from York post-medieval sites, relative to Soay and Clun Forest sheep (data from O'Connor 1995, 87; this study)

Meta-analysis of post-cranial sheep bones from London had demonstrated an increase in their breadth, depth and to a lesser extent length, from the 14th to 19th century (Thomas *et al.* 2013). Between periods D (1550-1600) and E (1600-1700), F (1650-1725) and G (1700-1800), and between G and H (1800-1900), statistically significant increases were seen, as they became more robust and broad. In the 19th century, sheep bones appear to have decreased in size, perhaps due to the historically documented refinement in low-profit bone and the desire for a lower bone:muscle ratio (Parkinson 1810; Thomas *et al.* 2013, 3316-7). $\delta^{15}N$ values from 18-19th century were significantly higher than those from the 16th-17th century (mean difference: 1.5‰), which suggests some nutritional change contemporary with increase in size.

This ^{15}N-enrichment may be due to the increased consumption of manured crops, with the improving farmer known to have fed their sheep a range of root vegetables and cereal grains (Trow Smith 1959, 200-5; Ryder 1983, 508; Williamson 1998, 14; Brassley 2000). Adequate nutrition is required for an animal to achieve its genetic potential (Hossner 2005, 4-10), and the consumption of these additional foodstuff may have aided the growth of these sheep. The rising population of the early-modern era, combined with the limited availability of additional good-quality farmland, necessitated the need for raising crop yields; and with it the need for maintaining and improving soil fertility (Allen 1992; Broadberry *et al.* 2015, 73-79). Prior to the emergence of artificial fertilisers in the early-19th century (Turner *et al.* 2001, 87; Smil 2005), the primary methods of maintaining and supplying nitrogen – the most common limiting element in agricultural systems (Touraine *et al.* 2001) – was through the recycling of organic wastes (human and animal waste, crop residues/stubble) or adopting crop rotations which included atmospheric nitrogen-fixing leguminous plants (beans, peas, clover, alfalfa) which returned nitrogen via livestock manure (Allen 1992; Smil 2005). Of these, manure was the most important source of fertility to the post-medieval farmer, with mixed farming practiced where possible (Turner *et al.* 2001, 66-88). The necessity to increase livestock numbers and the quantity of manure, thus raising soil nitrogen and crop yields, was central to the agricultural development of the period (Ryder 1983, 495-6; Overton 1996, 111-121; Williamson 1998; Turner *et al.* 2001, 222; Allen 2008), with Kerridge (1967, 311) regarding it as the primary driver behind all farming innovations from the 16th century onwards. An increase in livestock numbers and the intensity of manuring and folding, is likely to have altered the isotopic composition of the soil and plants, and therefore influenced the nitrogen values of the consumer tissue.

In the nitrogen cycle, the nitrogenous compounds in decomposing plant and animal matter are mineralised, and converted into inorganic forms of nitrogen, mainly ammonia (NH_3) and ammonium (NH_4^+). During nitrification, these compounds are oxidised to produce nitrite (NO_2^-) and nitrate (NO_3), respectively, which can themselves be reconverted to gaseous form of nitrogen (such as N_2) through denitrification (Lewis 1986). In plants which assimilate nitrogen from the soil via their roots, ammonium and nitrate are incorporated and used in the formation of plant proteins. Nitrogen can be added to the soil system through manuring, which often has a higher nitrogen isotope value than the endogenous soil (Szpak 2014, 4). The application of animal excreta has therefore been shown to elevate $\delta^{15}N$ values in plants, relative to those untreated or fertilised with synthetic fertilisers (Choi *et al.* 2002; 2003 Bateman *et al.* 2005; Bogaard *et al.* 2007; Lim *et al.* 2007; Fraser e*t al.* 2011; Szpak *et al.* 2009). Organic fertilisers have variable but positive $\delta^{15}N$ values (Table 7.9), with mean manure values placed at around 8‰ (Bateman & Kelly 2007, 242), although the extent to which it elevates plant nitrogen values is dependent upon the amount, frequency and duration, and the amount of mineralised nitrogen available (Szpak 2014, 4). Around 20-40% of the mineralised nitrogen in sheep

and cattle manure is available to the plants (Eghball *et al.* 2002; Castellanos & Pratt 1981). Livestock urine, however, is typically depleted in $\delta^{15}N$ to the diet by around 2-3‰. This is due to variations in animal metabolism and the excretion of urea, although the difference between urine and manure can be increased by the latter's storage and composting which further elevates its $\delta^{15}N$ value (Choi *et al.* 2007). Utilising long-term manuring experiments, Bogaard *et al.* (2007) and Fraser *et al.* (2011) have demonstrated that the application of livestock manure can raise cereal grain $\delta^{15}N$ values by as much as 8‰ and those of the rachis by 6‰. Nitrogen values in the soil and plant can also be raised by management practices including shifting between pasture and arable cultivation, such as the 'convertible husbandry' of the 17th-18th centuries (Kerridge 1967) and deeper ploughing (Szpak 2014). Synthetic fertilisers in contrast, have low $\delta^{15}N$ values. Modern synthetic fertiliser fix nitrogen through the Haber-Bosch process, which reacts atmospheric nitrogen (N_2) with hydrogen (H_2) at high pressure to form ammonia (NH_3) (Michalski *et al.* 2015). There is limited fractionation during this process, and therefore, typically display $\delta^{15}N$ (‰ relative to AIR) around 0‰ (Table 7.10). There is no evidence from these the more modern sheep bones to indicate the use of synthetic fertilisers.

Although the use of organic fertilisers dramatically increased in the 19th-century (Turner *et al.* 2001, 86), there was no significant variation in mean $\delta^{15}N$ values across all sites until the 21st century. It is likely that the majority of sheep were not consuming crops from fields which has been intensively manured, and were instead pastured on fields that received no more than casual droppings. The systematic application of manure is often restricted to cropped field, cultivating foodstuffs for human consumption. Elevated nitrogen isotope values, such as those seen in 18th-19th century London, may reflect animals which have been grazing on stubble after harvest, although access to this crop was often reserved for cattle (Clark 1993, 250; Lord 2009, 2). Due to livestock typically only consuming pasture, Müldner & Richards (2005) have suggested that a manuring effect may be invisible in herbivores. An alternative explanation to the elevation in nitrogen values in 18th-19th century sheep entering London is that it reflects the increasing geographical range from which they were drawn due to the increasing population and the improvement in transport (Bagwell 1974). This, however is not supported by the $\delta^{13}C$ values which show considerable continuity.

The only temporal variation in $\delta^{13}C$ values were in samples from the 20th and 21st century which were more depleted than those from previous centuries. While the recycling of CO_2 in canopied environments can lead to the depletion of $\delta^{13}C$ in plants and the animals that consume them (Tieszen 1991; Heaton 1999), this is not the case in the modern sheep and unlikely to be true of those from the 20th century. This most likely reflects the considerably depleted values of modern livestock feeds due to the introduction of lighter ^{12}C through the burning of fossil fuels (Friedeli *et al.* 1986; Hellevang & Aagaard 2015) and increasing humidity (Madhavan *et al.* 1991) (Table 7.10).

Type	Fertiliser	$\delta^{15}N$ (‰ v. AIR)	Reference
Organic			
Manure	Cattle manure	5.0 ± 0.8	Choi *et al.* 2002
	Cattle manure	2.9 ± 0.5	Kerley & Jarvis *et al.* 1996
	Cattle manure	3.1 ± 0.2	Ma & Dwyer 1998
	Cattle manure	4.5	Rogers 2008
	Pig manure	13.9	Choi *et al.* 2002
	Pig manure	16.9	Lim *et al.* 2007
	Pig manure	16.4	Yun *et al.* 2006
	Pig manure	11.3	Rogers 2008
	Pig manure	6.5	Rogers 2008
	Farmyard manure	9.4 ± 6.0	Bateman & Kelly 2007
	Farmyard manure	9.5 ± 4.4	Verenitch & Mazumder 2012
	Chicken manure	6.2 ± 1.9	Bateman & Kelly 2007
	Poultry manure	8.6 ± 0.3	Rapisarda *et al.* 2010
	Poultry manure	2.7	Rogers 2008
	Seabird guano	26.7 ± 0.6	Szpak *et al.* 2009
	Seabird guano	24.5 ± 0.5	Verenitch & Mazumder 2012
Non-manure	Blood	5.9 ± 1.3	Bateman & Kelly 2007
	Bonemeal	4.9 ± 0.3	Bateman & Kelly 2007
	Cattle urine	-3.2 ± 0.5	Kobbe *et al.* 2006
	Cattle urine	-1.8 ± 0.8	Sutoh *et al.* 1987
	Feathers	3.4 ± 1.5	Verenitch & Mazumder 2012
	Horn and Hoof	6.4 ± 0.2	Bateman & Kelly 2007
	Seaweed based	2.5 ± 1.5	Bateman & Kelly 2007
Fish	Fishmeal	8.8 ± 2.0	Bateman & Kelly 2007
Synthetic	Ammonium dihydrogen phosphate -$(NH_4)H_2PO_4$	-0.6 ± 0.4	Bateman & Kelly 2007
	Ammonium-nitrate - NH_4NO_3	-0.6 ± 1.7	Bateman & Kelly 2007
	Ammonium-nitrate - NH_4NO_3	1.6 ± 0.7	Vitoária *et al.* 2004
	Ammonium-nitrate - NH_4NO_3	-1.7	Rogers 2008
	Ammonium-sulfate - $(NH_4)_2SO_4$	1.7 ± 3.4	Bateman & Kelly 2007
	Ammonium-sulfate - $(NH_4)_2SO_4$	-1.6	Rogers 2008
	Ammonium-sulfate - $(NH_4)_2SO_4$	-2.6	Choi *et al.* 2003
	Ammonium-sulfate - $(NH_4)_2SO_4$	-0.2 ± 1.5	Vitoária *et al.* 2004
	Nitrate of soda – $NaNO_3$	-2.1 ± 1.0	Michalski *et al.* 2015
	Magnesium nitrate $(Mg(NO_3)_2$	-0.1 ± 0.1	Vitoária *et al.* 2004
	Potassium-nitrate (KNO_3)	-1.2 ± 0.2	Bateman & Kelly 2007
	Synthetic urea	-2.4 ± 2.1	Bateman & Kelly 2007
	Synthetic urea	-1.7	Rogers 2008
	Synthetic urea	-0.7	Yun *et al.* 2006
	Urea-ammonium-nitrate (UAN)	-0.2 ± 3.6	Michalski et al. 2015
	General ‘synthetics’	0.6 ± 1.6	Verenitch & Mazumder 2012

Table 7.10: Nitrogen isotope composition of common organic and inorganic fertilisers

Livestock Feed	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Whole pant	–29.3 to –22.9	–0.3 to 4.3
Seeds	–35.6 to –21	0.8 to 6.3
Silage and meal	–29.6 to –25.1	–0.2 to 5.2
Corn-based	–14.0 to –11	–4.5 to 7.2

Table 7.11: Carbon and nitrogen stable isotope composition of animal feeds (after Jahren & Kraft 2008)

7.5 Summary

Stable isotope analysis of 47 sheep bones from York and London were integrated with a further 150 from across Britain between the 10th and 21st centuries. This study identified considerable variation, but only signification temporal changes in carbon and nitrogen values in sheep from the 20th and 21st century, although these are through to reflect broad environmental changes, and the considerably difference in modern farming techniques and those of previous centuries.

Substantially larger sheep of a more 'improved' conformation were processed at The Bedern, York, during the 19th century. Despite this morphological variation, sheep bone collagen $\delta^{13}C$ and $\delta^{15}N$ values from these contexts showed no statistically significant difference to those from other 15th to 20th century sites across the city. These results suggest that conformational improvement in these animals was not accompanied by a significant environmental or dietary change, or that they are not detectable isotopically. The $\delta^{15}N$ values of sheep from London in the 18th and 19th century, however, are significantly higher than those processed in the 16th-17th centuries (mean difference = 1.5‰). This is contemporary with an increase in the size of sheep entering the capital, and indicates a potential nutritional and environmental driver. A possible factor in this enrichment could be the greater consumption of crops which have been cultivated in well manured fields. Livestock in the 18th and 19th century increasingly consumed fodder crops such as turnips and swede which formed part of new field rotation systems. Unlike pasture, these fields often received the intensive application of manure, which in experimental studies has been shown to elevate $\delta^{15}N$ values.

Chapter 8

$\delta^{13}C$ and $\delta^{15}N$ isotope analysis of post-medieval sheepskin parchment deeds

This chapter presents the isotopic analysis of 663 sheepskin parchment deeds from the 12th to 20st century. The results will be used to explore the provisioning of skins for deeds, as well as temporal variation in animal and land management. The discussion will consider broad chronological trends in the geographical origin and age of skins, as well as more focussed analysis on areas such as the impact of the French Revolutionary and Napoleonic Wars, the emergence of 'High Farming' in the 1830-40s, and the advent of the railways.

8.1 Introduction

In 1696, the *"first great economic statistician"* Gregory King (Stone 1997, xxii) predicted that Britain's population would grow from 5.5 to 6.4 million by 1800 (actually 10.6 million) and again to 7.4 million in 1900 (in fact 41.5 million) (King 1696, In Laslett 1973; Jefferies 2005; Broadberry *et al.* 2015, 29). Despite his eminence, King drastically underestimated the population boom of the following centuries, a surge that was underpinned by the contemporary improvements in British Agriculture. Between the 16th and 19th centuries, grain yields increased by 320%, the sheep population grew by 170%, and mutton and wool yields all grew by more than 500% (Broadberry e*t al.* 2015, 80-124). Yet despite the recognised centrality of agricultural developments to industrialisation and growth (Deane 1965; Moykr 1985; Williamson 2002; 2013; A'Hern 2004), there is remarkably little consensus on when, or how rapidly these changes occurred (Overton 1996, 1). Historical enquiry has been hampered the scarcity of farming data, particularly for the pastoral economy (Williamson 2002, 165), which is typically not of the chronological resolution required to explore this period of rapid change (Turner *et al.* 2001). While valuable insight into animal management has been made by zooarchaeological research, it too has been hampered by the broad

phasing of post-medieval remains. Sheepskin parchment, dated to the year of use, may therefore be a unique resource for a time-sensitive analysis of changes in animal and land management through stable isotope analysis.

8.2.1 Sample preparation and stable isotope analysis

Samples for destructive analysis were obtained from 663 individual parchment skins from a total of 497 legal documents. Species identification by peptide mass finger printing (Chapter 4) identified 640 (96.5%) of these as sheepskin and the remaining 23 (3.5%) as sheep or goat. The majority were engrossed with the date the agreement was signed (day, month and year), apart from 18 dated more broadly to the 13th century (Figure 8.1). None of the manuscripts had received any conservational treatment, or presented any evidence that they had been reused. To avoid possible contamination, samples were removed from the edge of each skin (~1 x 0.5 cm), from an area devoid of any ink, pencil, glue, revenue stamp or surface marking. Samples were defatted via solvent extraction, DCM/MeOH (2:1 v/v), by ultrasonication for 1 hr with the supernatant removed and replaced every 15 mins. The samples were demineralised in 0.6 M HC1 at 4^{o}C for 1 hr, rinsed with distilled water, and gelatinised in pH 3 0.001 M HC1 at 80^{o}C for 48 hrs. The supernatant containing the soluble collagen was filtered (60-90 µm Ezee-Filter™, Elkay Laboratories, UK), frozen, and freeze-dried.

The prepared collagen (0.9-1.1 mg) was weighed out in duplicate in 4 x 3.2 mm tin capsules (Elemental Microanalysis, Okehampton, UK), and isotopic analysis performed at the NERC LSMSF in East Kilbride. Isotope ratio determinations were carried out on a ThermoElectron DeltaPlusXP (Thermo Fisher Scientific, Bremen, Germany) with an Elementar Pyrocube elemental analyser (Elementar, Langenselbold, Germany). The ratios are reported as $\delta^{13}C$ and $\delta^{15}N$ values in per mille (‰) in reference to VPDB and AIR. Samples were referenced against internal standards of gelatine (Sigma-Aldrich, St. Louis, US), ^{13}C-enriched alanine and ^{15}N-enriched glycine, and the international standard of glutamic acid (USGS40). The isotopic composition and accuracy of control standards are reported in Table 8.1. Sample reproducibility was better than 0.2‰ for $\delta^{13}C$. %C, $\delta^{15}N$ and %N for 640 skins. %C ranged from 37.1-47.3, %N ranged from 12.3-16.9, and C/N ratios were between 3.1 and 3.6, indicative of well-preserved collagen (DeNiro 1985; Ambrose 1990). Twenty-three samples showed a deviation between duplicated >0.2‰, or elemental composition outside the accepted range (particularly low %N potentially due to deamidation), and were therefore removed from further analysis.

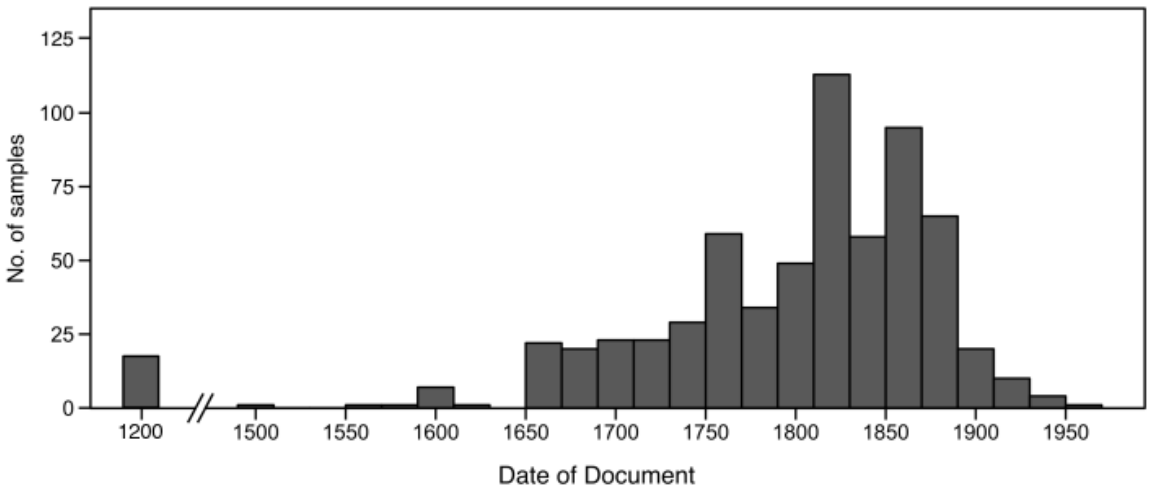


Figure 8.1: Chronological distribution of 663 historic parchment deeds analysed in this study

		$\delta^{13}C$ (‰, vs. VPDB)		$\delta^{15}N$ (‰, vs. AIR)	
Standard	*n*	Observed	True	Observed	True
^{13}C-enriched alanine	44	–8.47 ± 0.20	–8.36 ± 0.13	2.15 ± 0.15	2.08 ± 0.06
^{15}N-enriched glycine	44	–38.54 ± 0.11	–38.58 ± 0.09	23.53 ± 0.13	23.54 ± 0.08
Gelatine	117	–20.31 ± 0.09	–20.31 ± 0.18	5.55 ± 0.17	5.54 ± 0.08
USGS40 (glutamic acid)	20	–26.45 ± 0.14	–26.39 ± 0.09	-4.58 ± 0.16	-4.52 ± 0.12

Table 8.1: Isotopic composition and precision of control standards run at NERC LSMSF

8.2.2 Suess effect

The extensive burning of fossil fuels since the 19th century has introduced lighter ^{12}C to the atmosphere, decreasing the $\delta^{13}C$ composition of atmospheric CO_2 from around -6.4‰ at the end of the 18th century to around =7.6‰ in 1980 (Friedeli *et al.* 1986), -8.1‰ in 2000 (Keeling *et al.* 2005), and around -8.3‰ in 2015 (Hellevang & Aagaard 2015). To account for these emissions and enable the accurate comparison of pre- and post-industrial period material, Friedeli *et al.* (1986) suggested a correction value of 1.1‰, however, with the increased burning of coal, gas and oil over the past three decades, Hellevang & Aagaard's (2015) data now places this at 1.7‰. Using their model, a 5-year correction offset was calculated for seventeen 20th century parchment samples, ranging from 0.1‰ in 1905 to 0.7 in 1970 (Table 8.2). Samples were corrected to the closest 5-year date. Raw and corrected values are presented in Table A.2. A correction value of 1.7 was applied to the modern parchment manufactured in this study to aid its integration.

Date	Modelled atmospheric CO_2 $\delta^{13}C$ value without historic emissions	Real atmospheric CO_2 $\delta^{13}C$ value with historic emissions	Calculated $\delta^{13}C$ offset
1905	-6.55	-6.59	+ 0.05
1910	-6.55	-6.63	+ 0.08
1915	-6.55	-6.67	+ 0.12
1920	-6.56	-6.70	+ 0.15
1925	-6.56	-6.71	+ 0.15
1930	-6.57	-6.73	+ 0.16
1935	-6.57	-6.75	+ 0.18
1940	-6.57	-6.78	+ 0.22
1945	-6.58	-6.79	+ 0.22
1950	-6.59	-6.82	+ 0.23
1955	-6.62	-6.89	+ 0.27
1960	-6.65	-7.05	+ 0.40
1965	-6.66	-7.16	+ 0.49
1970	-6.66	-7.38	+ 0.73
2015	-6.64	-8.35	+ 1.71

Table 8.2: Suess correction offsets used in this study. Offset calculated using Hellevang & Aagaard's (2015) model of atmospheric CO_2 models of with and without historic fossil fuel emissions

8.2.3 Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistics 22.0 software package (IBM 2013). While both $\delta^{13}C$ and $\delta^{15}N$ values were normally distributed (Shapiro-Wilks test, $\delta^{13}C$: p=<0.01; $\delta^{15}N$: p=<0.01), non-parametric tests were used due to the potential for non-normal distributions to occur as the data was broken down into smaller chronological intervals. The statistical significance of inter-collection and temporal variation in $\delta^{13}C$ and $\delta^{15}N$ values was determined using a Mann-Whitney U test for paired samples, and a Kruskal-Wallis test for multiple samples. The equality of variance between samples was calculated using a Levene's test.

8.3 Results

Full $\delta^{13}C$ and $\delta^{15}N$ and elemental composition results are presented in Table A.2, and summary statistics provided in Table 8.3. For statistical analysis, samples were grouped by century, and then into 50-year intervals if the sample size was >50, and into 25-year periods if >20 (Table 8.4-8.7). As the only skin from the 15th century dated to 1499, it was included in the 16th century grouping for statistical analysis.

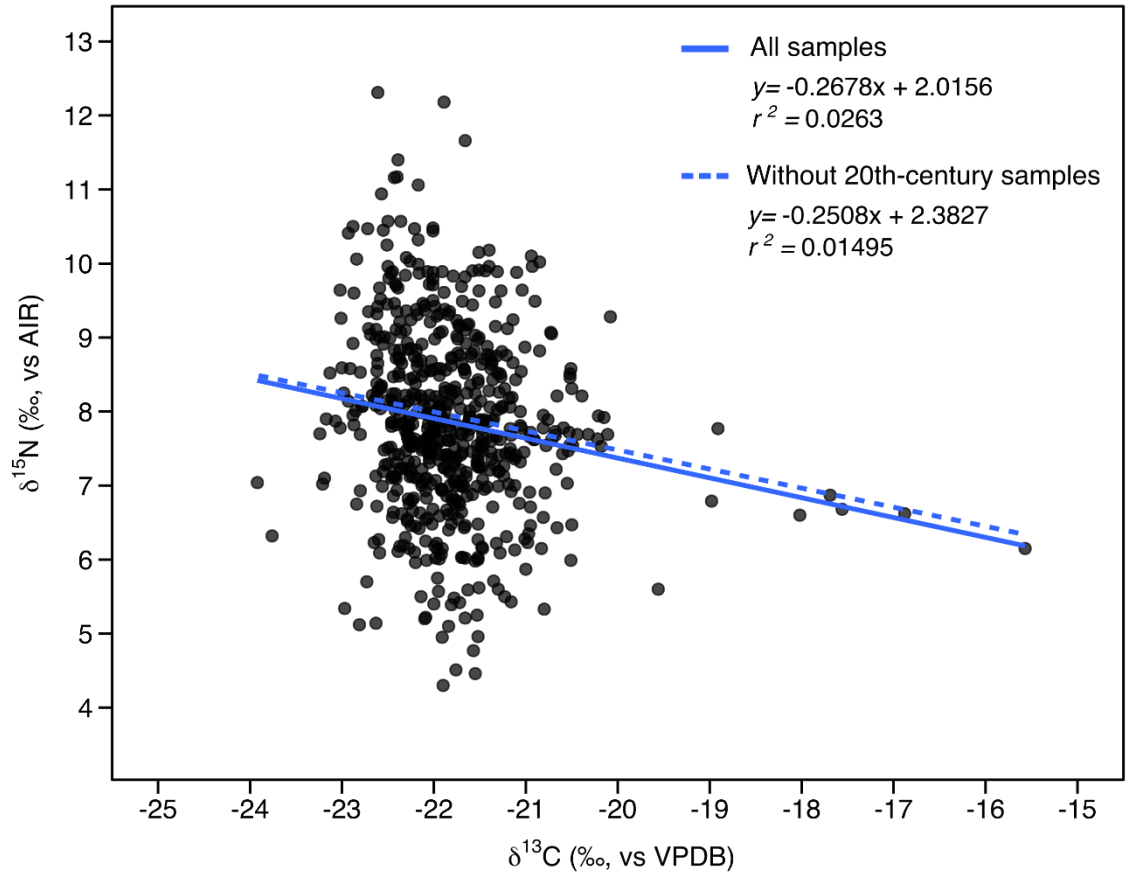


Figure 8.2: Bivariate plot of $\delta^{13}C$ and $\delta^{15}N$ values for all historic samples, 1499-1969. Linear trendlines for all samples, and without ^{13}C-enriched 20th century samples. R package 'ggplot2' used

A negative correlation between $\delta^{13}C$ and $\delta^{15}N$ values was observed (Figure 8.2, Pearson correlation coefficient, p=<0.01), with skins displaying the most enriched carbon values displaying some of the lowest nitrogen values. This trend is true across all the data, and not just in a number of isotopically distinct late-19th and 20th century skins. When examined chronologically (Figure 8.3) $\delta^{13}C$ values are seen to be relatively consistent between the 13th and early-19th century, with mean values falling between -22.8‰ and -22.4‰. In the 19th century however, there is a significant increase in variability and the presence of skins with higher $\delta^{13}C$ values. Mean $\delta^{13}C$ values increase from -22.8‰ in the 18th century to -22.4‰ in the 19th century, and to -21.0‰ in the early-20th century (Table 8.4). These chronological differences in carbon values are statistically significant over 50 and 25-year intervals (Table 8.5-8.6). $\delta^{13}C$ values from 1800-49 were significantly more enriched (0.4‰, p=<0.01) than those from 1750-99, and values from 1850-99, were significantly more enriched again (0.4‰, p=<0.01). At a finer resolution, carbon values from 1800-24 were significantly more enriched (0.3‰, p=0.038) than those from 1775-99, and values from 1850-75 more enriched (0.3‰, p=0.020) than those from 1825-49. Across the same period, variability increase from 1.8‰ in the 18th century to

5‰ in the 19th century, to 6.8‰ in the 20th century (Table 8.7). The most isotopically different skins are 7 skins from the late-19th and 20th century which present $\delta^{13}C$ between -19.6‰ and -16.2‰. $\delta^{15}N$ values show considerable variability across the 13th to 20th century, ranging between 5‰ and 13‰ (Figure 8.4). This variability, however, decreases from 7.8‰ in the 18th century to 6.1‰ in the 19th century, to 4.1‰ in the 20th century, and again to 3.2‰ in the 20th century (Table 8.7). Mean nitrogen values show a small but statistically significant increase in the 19th century between 1800-49 and 1850-99 (0.4‰, p=0.001), which is significant over 25-year intervals between 1800-24 and 1825-49 (0.4‰, p=0.002).

Phase	Period	*n*	$\delta^{13}C$ (‰ v. VPDB)			$\delta^{15}N$ (‰ v. AIR)		
			Mean	SD	Range	Mean	SD	Range
A	1200	18	–22.66	0.38	1.59	10.09	1.52	5.16
B	1500-1599	4	–22.40	0.41	0.80	8.43	1.32	3.14
C	1600-1699	65	–22.81	0.45	2.05	9.11	1.62	7.88
C1	*1650-1699*	*50*	*–22.79*	*0.45*	*2.05*	*9.25*	*1.55*	*7.88*
D	1700-1799	173	–22.80	0.37	1.88	9.14	1.33	7.87
D1	*1700-1749*	*68*	*–22.77*	*0.35*	*1.50*	*9.12*	*1.40*	*6.94*
D2	*1750-1799*	*105*	*–22.81*	*0.38*	*1.88*	*9.16*	*1.30*	*7.36*
E	1800-1899	364	–22.41	0.67	5.01	9.20	1.11	6.10
E1	*1800-1849*	*200*	*–22.56*	*0.56*	*3.32*	*9.02*	*1.08*	*5.61*
E2	*1850-1899*	*164*	*–22.25*	*0.74*	*5.01*	*9.40*	*1.12*	*5.86*
F	1900-1999	21	–21.02	2.07	6.84	8.88	1.32	4.53
G	2000-	38	–23.67	0.56	2.70	9.07	0.82	3.29

Table 8.3: Mean, standard deviation and range of isotope results by period

Phase	$\delta^{13}C$ (‰ v. VPDB)		$\delta^{15}N$ (‰ v. AIR)	
	U	*P*	U	*P*
A (1200) (n=18) – B (1500-1599) (n=4)	25.5	0.386	14.0	0.066
B (1500-1599) (n=4) – C (1600-1699) (n=65)	67.0	0.111	100.0	0.464
C (1600-1699) (n=65) – D (1700-1799) (n=173)	5464.5	0.738	5534.0	0.854
D (1700-1799) (n=173) – E (1800-1899) (n=364)	19506.0	**<0.001**	30239.5	0.458
E (1800-1899) (n=364) – F (1900-1999) (n=21)	2405.0	**0.004**	3175.0	0.192
F (1900-1999) (n=21) – G (2000-) (n=38)	27.0	**<0.001**	332.0	0.447

Table 8.4: Mann-Whitney U tests of $\delta^{13}C$ and $\delta^{15}N$ values by century. Bold values indicate statistically significant differences *(P<0.05)*

Phase	$\delta^{13}C$ (‰ v. VPDB)		$\delta^{15}N$ (‰ v. AIR)	
	U	*P*	U	*P*
C1 (1650-1699) (n=50) – D1 (1700-1749) (n=68)	1522.5	0.792	1488.5	0.645
D1 (1700-1749) (n=68) – D2 (1750-1799) (n=105)	3287.0	0.527	3472.5	0.961
D2 (1750-1799) (n=105) – E1 (1800-1849) (n=200)	7329.0	**<0.001**	9902.5	0.439
E1 (1800-1849) (n=200) – E2 (1850-1899) (n=164)	12167.0	**<0.001**	13201.5	**0.001**

Table 8.5: Mann-Whitney U tests of $\delta^{13}C$ and $\delta^{15}N$ values by 50-year periods. Bold values indicate statistically significant differences *(P<0.05)*

Phase	$\delta^{13}C$ (‰ v. VPDB)		$\delta^{15}N$ (‰ v. AIR)	
	U	*P*	U	*P*
D1a (1700-1724) (n=28) – D1b (1725-1749) (n=21)	465.0	0.588	419.9	0.250
D1b (1725-1749) (n=21) – D2a (1750-1774) (n=64)	1034.0	0.546	1063.0	0.696
D2a (1750-1774) (n=64) – D2b (1775-1799) (n=41)	1271.5	0.256	1367.0	0.582
D2b (1775-1799) (n=41) – E1a (1800-1824) (n=117)	2255.5	**0.038**	2577.0	0.346
E1a (1800-1824) (n=117) – E1b (1825-1849) (n=83)	3913.0	0.382	3118.5	**0.002**
E1b (1825-1849) (n=83) – E2a (1850-1874) (n=102)	2612.0	**0.020**	3088.0	0.295
E2a (1850-1874) (n=102) – E2b (1875-1900) (n=62)	3303.0	0.377	3088.0	0.121

Table 8.6: Mann-Whitney U tests of $\delta^{13}C$ and $\delta^{15}N$ values by 25-year periods. Bold values indicate statistically significant differences *(P<0.05)*

Phase	$\delta^{13}C$ (‰ v. VPDB)	$\delta^{15}N$ (‰ v. AIR)
	P	*P*
A (1200) – B (1500-1599)	0.552	0.624
B (1500-1599) – C (1600-1699)	0.989	0.493
C (1600-1699) – D (1700-1799)	0.110	0.460
D (1700-1799) – E (1800-1899)	**<0.001**	**0.024**
E (1800-1899) – F (1900-1999)	**<0.001**	0.131
F (1900-1999) – G (2000-)	**<0.001**	**0.007**
B1 (1500-1549) – B2 (1550-1599)	0.959	0.353
B2 (1550-1599) – C1 (1600-1649)	0.961	0.360
C1 (1600-1649) – C2 (1650-1699)	0.202	0.494
C2 (1650-1699) – D1 (1700-1749)	0.720	0.480
D1 (1700-1749) – D2 (1750-1799)	**0.002**	0.251
D2 (1750-1799) – E1 (1800-1849)	**0.001**	0.482
E1 (1800-1849) – E2 (1850-1899)	**<0.001**	0.177

Table 8.7: Results from Levene's Tests of homogeneity of variance. Top between centuries, bottom over 50-year periods *(P<0.05).* See Table 8.3 for sample sizes

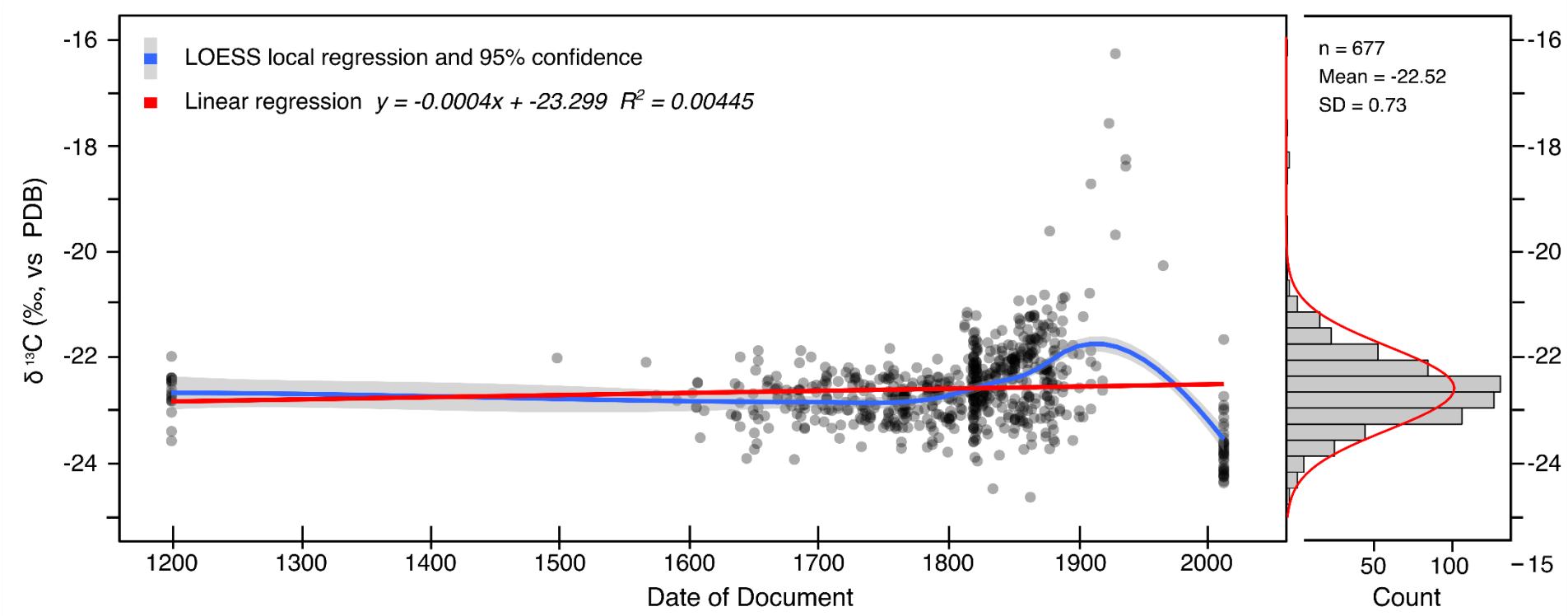


Figure 8.3: Chronological variation in $\delta^{13}C$ values, 13th to 21st century. R package 'ggplot 2' used for graph

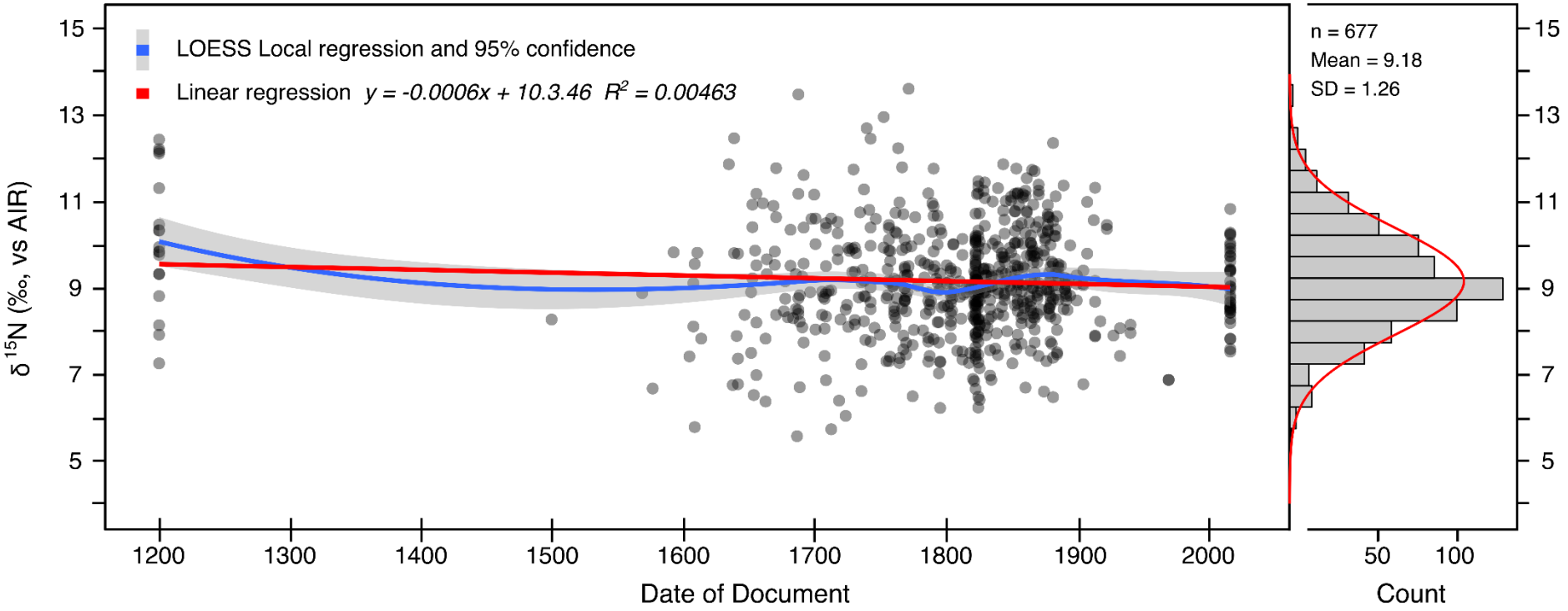


Figure 8.4: Chronological variation in $\delta^{15}N$ values, 13th to 21st century. R package 'ggplot 2' used for graph

8.4 Discussion

It has been repeatedly noted throughout this thesis that previous stable isotope analyses of parchment have observed higher $\delta^{15}N$ values than would be typically expected for domestic herbivores (Campana *et al.* 2010; Pollard & Brock 2011).). This pattern is again seen here in 13th to 20th century British sheepskins. While this is likely due in part to the impact of production (Chapter 5) and the isotopic spacing between diet and parchment (Chapter 6), it is worth considering the use of young lambskins, as this may affect the interpretation of the data with regards to post-medieval husbandry and the procurement of skins.

Over 160 skins display $\delta^{15}N \geq 10‰$, higher than those reported in modern lambs which are still suckling (5 to 8‰) (Piasentier *et al.* 2003; Camin *et al.* 2007; Perini *et al.* 2009). This is even more pronounced in British herbivorous livestock which typically display higher nitrogen values than other parts of Europe, a feature attributed to ^{15}N-enrichment in the plant-soil-system from intensive farming and organic fertilisation (Piasentier *et al.* 2003; Schmidt *et al.* 2004; Camin *et al.* 2007; Heaton *et al.* 2008). The 15th to 20th century (fused) sheep bones analysed in Chapter 7 presented a range in $\delta^{15}N$ values of 3.9 to 9.2‰ (mean = 6.1‰), while 15th to 20th century parchment ranged from 5.6 to 13.6‰ (mean = 9.2‰). Considering a mean $\Delta^{15}N_{bone\text{-}parchment}$ offset of 1.3‰, it is clear that the nitrogen values in parchment are considerably higher, it is likely that many of these skins are from young lambs displaying a trophic level effect due to the consumption of the ewe's milk. The ingestion of milk results in an enrichment in the $\delta^{15}N$ value of the offspring by around 2-3‰ over that of the mother due to consumption of the mother's tissue (Fogel *et al.* 1989; Tieszen & Fagre 1993; Fuller *et al.* 2006). Due to the preferential removal of amide groups with ^{14}N during the assimilation of nitrogen (Styring *et al* 2010, 242), suckling lambs are theoretically one trophic level above the ewe. Milk consumption also results in an enrichment in $\delta^{13}C$ values by around 1‰, although in sheep, carbon isotope ratio were found to be more reflective of wider geographical and environmental factors (Piasentier *et al.* 2003; Camin *et al.* 2007; Perini *et al.* 2009). During the weaning process, the consumption of other dietary components typically results in a depletion in $\delta^{13}C$ and $\delta^{15}N$ values as the animal's tissues begin to reflect their wider diet, i.e. pasture, although this occurs more rapidly for carbon than nitrogen values (Fuller *et al.* 2006).

Although sheep of any age can be used for parchment, the visibility of imperfections makes the skins of younger animals more desirable, as the risk of dermatological issues increases with age. Sheep are susceptible to external parasitic attack, such as flystrike, sheep scab and lice (Bates 2012; Scott 2018), as well as general injuries, which can all leave visible flaws in the resulting parchment (Ryan 1987). Moreover, as the widespread docking of tails appears to be a relatively recent practice (Ryder 1983,

480, 500), the risk of infection in previous centuries may have been even greater. Documentary and zooarchaeological evidence indicates an increasing consumption and utilisation of young sheep, including suckling lambs, from the 16th century onwards (e.g. Fitzherbert 1523, 42; Mascall 1627, 220-9; Markham 1631, 105, 108, 113; Beeton 1861, 176-7; O'Connor 1984a; Gordon 2015; Rackham n.d.). This was driven by the increasing importance of meat over wool production, dietary preference for lamb, and their faster maturation. In Chester, a city with a large skin processing industry, domestic and imported lambskins were utilised, with faunal remains from potential skin processors showing a deliberate selection for younger animals (Gordon 2015, 85-6). By the early-19th century, around 50% of the sheep processed at 25 Bridge Street, Chester, were killed before six months old, with some as young as two months (Gordon 2015, 85-6).

There is little documentary evidence on the age of sheep use for parchment, although Ryder (1964, 391; 1983, 732) states that the finest parchment was always made from either young calf or lambskins. In the 16th-19th century, sheep were typically weaned around 16-18 weeks old (Thirsk 1967b, 187), although recognising their smaller size in this period, it is unlikely that a four months old lamb could yield a stretched skin of ~70x50 cm, notwithstanding the skill of the parchment maker. Although as Fuller *et al.* (2006) noted, the elevated $\delta^{15}N$ may have persisted for some time after weaning. The $\delta^{15}N$ value in the suckling lambs tissue would have been even higher if the ewe has been receiving supplementary feed during pregnancy, which was a common practice by the 18th century thanks to the greater availability of fodder crops (Trow-Smith 1959, 199-201). In a practice known as flushing, the ewe's plane of nutrition is elevated prior, during and after lambing to promote the rate of ovulation; increasing lambing and twinning rates (Clark 1934); improved nutrient quantity for the foetus; and increase milk yields (National Academy of Sciences 1975, 23). Root vegetables and grains, were routinely given to pregnant ewes (Trow-Smith 1957, 200-5; Thirsk 1967b, 188; Ryder 1983, 479, 496), and as these were typically cultivated in well manured field they may have had elevated $\delta^{15}N$ values. This preferential selection of young skins, and efforts to improve the condition of ewes during the lambing season, may go some way to explain the high nitrogen isotope values observed in many parchment samples.

8.4.1 Chronological variation in $\delta^{13}C$ and $\delta^{15}N$ values

Between the 13th and 18th centuries, parchment $\delta^{13}C$ values display considerable continuity, with almost all falling between -24‰ and -22‰. This restricted range and limited change likely reflects the use of British and Irish skins from sheep primarily grazing on C3 grasses. The $\delta^{13}C$ value of animal tissue is highly influenced by the composition of the diet, with sheep largely reflecting the location and nature of the pasture on which they graze (Piasentier *et al.* 2003; Camin e*t al.* 2007;

Perini e*t al.* 2009; von Holstein e*t al.* 2013; Zazzo *et al.* 2015). The most fundamental influence on $^{12}C/^{13}C$ ratios in plans are the photosynthetic pathway used (C3 and C4), due to different isotopic discrimination capabilities of the carboxylase enzymes involved in CO_2 fixation (Camin *et al.* 2016, 869) and atmospheric variation in CO_2 (Korner e*t al.* 1988; van Klinken e*t al.* 2000; Kohn 2010). Plants utilising the C3 pathway have an average $\delta^{13}C$ value of -27‰, with a range between -34‰ and -22‰, while C4 plants have an average value of -13‰, ranging from -20‰ to -9‰ (O'Leary 1988; Heaton 1999). Considering the mean $\Delta^{13}C_{\text{diet-parchment}}$ offset of 3.9‰ (± 2.2), it can be concluded that except for seven 20th-century skins, all the sheep in this study predominantly consumed C3 plant protein, and fall within the range typically expected for the UK and parts of north-west temperate Europe.

Contemporary $\delta^{15}N$ values show a high degree of variability, ranging from 6‰ to 14‰, although with no statistically significant difference between centuries. Disentangling the impact of suckling lamb is challenging, but it does demonstrate the diversity of farming systems during this period, likely reflecting varying levels of manuring, legume cultivation and stocking densities (Thirsk 1967a; Turner *et al.* 2001). Increasing the quantity of manure and fertilisers available to arable land was a central tenet of the periods agricultural developments (Kerridge 1967; Overton 1996; Williamson 2002; Turner *et al.* 2001; Allen 2008). The earliest British agricultural writers were well aware of their value, and recommended a diverse range of animal substances to "fatten the land" (Blith 1649, 100), including dung, blood, fish, ground bone and shavings of horn (Fitzherbert 1534; Tusser 1580; Markham 1631; Hartlib 1652). Organic fertilisers such as these typically display more positive $\delta^{15}N$ values than endogenous soil, and their application has been shown to elevate nitrogen values in plants, and in the tissue of animals that consume them (Bogaard *et al.* 2007; 2013; Bateman & Kelly 2007; Heaton *et al.* 2009; Fraser *et al.* 2011; Szpak *et al.* 2009; Szpak 2014; Treasure *et al.* 2016). With their increasing use in the post-medieval period, it is perhaps surprising that parchment from the 13th century display the highest mean $\delta^{15}N$ values (10.1‰). However, Campbell (1983; 2006) suggest that organic fertilisers were assiduously used at this period, with nutrients transferred to the land through intensive sheep-folding and the application of human waste.

At the start of the 19th century, carbon values show a significant increase in variability, which is matched by a constriction in the range of nitrogen values. The increased variability in $\delta^{15}N$ values seen from the early-19th century, may reflect a potential change in the location from which skins were sourced or the diet of the animals. Identifying the origin of skins can be very challenging (see Chapter 4), with finished parchment travelling from Ely to Cambridge; Scotland to Bury St Edmunds and from France to Britain (Gullick 1991; Gransden 2013, 295; Mortimer 1810; Willement 1845, 42). However, as in the provenancing of modern animal products (see Camin *et al.* 2016 for a review),

stable isotope analysis may prove useful. Defining spatial isotopic variation, or isoscape, using ratios derived from domestic livestock is difficult due to the potential for anthropogenic factor, such as the consumption of bought-in feeds or manuring, to alter isotope values in the tissue. Despite this, terrestrial herbivore bone and muscle collagen have been used to examine country-level variation in $\delta^{13}C$ and $\delta^{13}C$ values, often for assessing the provenance of meat (Table 8.8). Modern UK sheep displays a range in $\delta^{13}C$ values of 8.6‰ (-27.7‰ to -19.1‰), and a range in $\delta^{15}N$ values of 4.7‰ (6.1‰ to 10.8‰). These ranges are comparable to those in parchment of 8.3‰ (-24.6‰ to -16.2‰) and 8‰ (5.6‰ to 13.6‰), respectively, although the range in nitrogen values in parchment is greater. Parchment and muscle are more viable than bone, which might reflect differences in turnover rates, with skin and muscle on the scale of weeks and months, and bone on the scale of years (McAnulty & Laurent 1987; Mays e*t al.* 1991; Moore e*t al.* 2005; Waterlow 2006; Hedges *et al.* 2007), enabling the former to display greater seasonal variability (Lamb 2016). With such broad ranges and inter-flock variability, it is difficult to separate animals from different countries, let alone on regionally, even with the additional use of hydrogen, oxygen and sulphur isotope analysis (Piasentier *et al.* 2003; Schmidt *et al.* 2004; Perini *et al.* 2009; Kelly 2010). Plants and animals raised under 'organic' conditions often display a broader range in isotope values than those farmed under modern 'conventional' practices (Schmidt *et al.* 2004; Boner & Forstel 2004; Bateman *et al.* 2007; Jahren & Kraft 2008), likely making the variability even greater in previous centuries. Grouping parchment together by the stationer through which it was sold, highlights this large range, and potentially similar sources (Figure 8.4), with no significant difference between suppliers for either isotope (Kruskal-Wallis test, $\delta^{13}C$: $p=0.062$; $\delta^{15}N$: $p=0.197$). However, the distinctiveness of three skins from supplier G.F Gaubert suggests some regional differences in where skins were being obtained from. Despite this, $\delta^{13}C$ and $\delta^{15}N$ isotope analysis may only be useful for identifying those of a non-domestic origin.

County	Type	Period	*n*	$\delta^{13}C$ (‰ v. VPDB)			$\delta^{15}N$ (‰ v. AIR)		
				Range	Min	Max	Range	Min	Max
UK[1]	Beef (muscle)	Modern	-	4.3	–27.2	–22.9	2.2	5.9	8.1
UK[2]	Lamb (muscle)	Modern	30	9.5	–28.6	–19.1	3.2	7.3	10.5
England[3]	Beef (muscle)	Modern	101	8.0	–27.6	–19.6	5.2	4.9	9.8
Wales[3]	Beef (muscle)	Modern	101	8.4	–28.0	–19.6	4.0	5.7	9.7
Scotland[3]	Beef (muscle)	Modern	100	3.0	–27.6	–24.5	4.9	4.9	9.8
Ireland[4]	Beef (muscle)	Modern	31	3.9	–26.7	–21.3	5.4	5.5	9.8
France[5]	Cattle (bone)	Neolithic	94	5.9	–23.5	–17.6	1.1	7.2	8.3
France[5]	Sheep (bone)	Neolithic	67	2.1	–20.1	–18.0	2.4	7.7	10.1
France[2]	Sheep (muscle)	Modern	31	1.3	–23.4	–22.1	2.6	4.1	6.7
Italy[6]	Lamb (muscle)	Modern	95	3.4	-	-	4.0	-	-
Italy[2]	Lamb (muscle)	Modern	55	3.1	–26.8	–23.7	4.2	2.2	6.4
UK[7]	Sheep (bone)	Modern	56	4.3	–27.7	–23.4	4.7	6.1	10.8
16th-20th century sheep bone			70	4.7	–24.5	–19.8	6.6	3.6	10.2
16th-20th century sheepskin parchment			640	8.3	–24.6	–16.3	8.0	5.6	13.6

Table 8.8: Country-wide isotopic variability in various livestock collagen samples. [1]FERA 2015; [2]Camin e*t al.* 2007; [3]Kelly 2010; [4]Schmidt *et al.* 2004; [5]Goude & Fontugne 2017; [6]Perini *et al.* 2009; [7]von Holstein *et al.* 2013 and this study

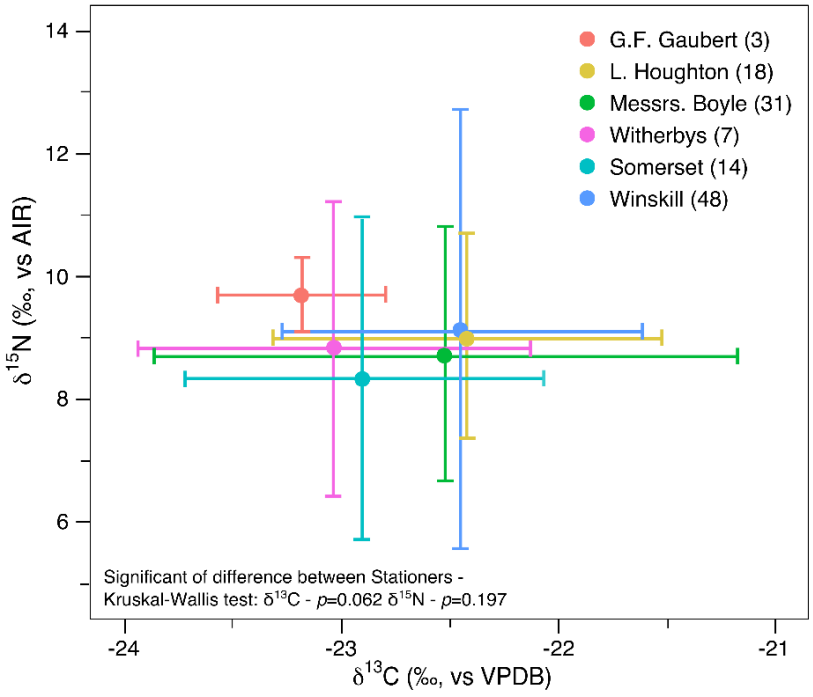


Figure 8.4: Isotopic range of parchment skins by stationer and collection. Number of samples in parentheses. Source of parchment, Lee Collection: G.F Gaubert, L. Houghton Messrs. Boyle. Tye Collection: Witherbys. Wills Collection: Somerset. Lord Collection: Winskill.

Arguably, the most important source of imported skins was Ireland, with tens of thousands entering Britain's western ports annually. In 1438, *The George* of Youghal, Co. Cork, alone brought 4,100 sheepskins, 2,800 lamb skins, and hundreds of goat, deer, hare and squirrel skins to the port of Bristol (Bush 1824). Such quantities were not unusual, and the trade continued well into the later post-medieval period (Lough 1916; Armour 1956; Clarkson 1966). Distinguishing between British and Irish livestock isotopically is very challenging, with considerable overlap in observed values (Piasentier *et al.* 2003; Schmidt *et al.* 2004; Camin *et al.* 2007; von Holstein e*t al.* 2014; Zazzo *et al.* 2015), though Irish sheep typically have lower $\delta^{15}N$ values, perhaps due to lower stocking densities and intensity of farming. Plants and sheep from both regions frequently display more depleted $\delta^{13}C$ values than in other parts of Europe, as the higher humidity results in greater carbon isotope fractionation during plant growth, which is transferred to the animal's tissue during biosynthesis (Madhaven *et al.* 1991; van Klinken *et al.* 1994; Camin *et al.* 2007). Fifteen skins from a collection in Chester, a city known to have imported Irish skin (Armour 1956), have a mean $\delta^{13}C$ value of -22.8‰ (13^{th}-20^{th} century mean = -22.5‰) and a mean $\delta^{15}N$ value of 8.6‰ (13^{th}-20^{th} century mean = 9.2‰). It is tempting to suggest that these lower nitrogen values indicate the use of Irish skins, but the variation between the two countries is of insufficient magnitude to make a confidence determination. The stationer through which these skins were sold is unknown, and as highlighted in Chapter 4, the eventual location of the deed or the area the legal agreement concerns most likely bears no relation to the origin of the sheep. Genetic analysis of an 18^{th}-century parchment deed indicated affinity with British midland sheep breeds such as the Leicester Longwool and Border Leicester, but also affinity with the modern Galway sheep (Teasdale e*t al.* 2015). This may however, be due to New Leicester sheep being brought to the west coast of Ireland in the 18^{th} century to improve the local stock (British Galway Sheep Society 2018), but does highlight the connectivity of these two countries. Greater understanding of supply routes used by parchment makers would aid the identification of Irish skins.

After Ireland, France was the next largest exporter of skins to Britain. Along with wine and brandy, skins were considered one of the principal exports from France in the 19^{th} century (McCulloch 1847, 15), although the trade stretched back centuries (Kowaleski 1990). Due to the comparable temperature, humidity, precipitation and farming practices between much of northern and central France and the UK, there is considerable overlap in the isotopic values observed in sheep. Modern and archaeological collagen samples have produced $\delta^{13}C$ values from –23.5‰ to –18‰ and $\delta^{15}N$ values from 2.4‰ to 10.1‰ (Camin e*t al.* 2007; Goude & Fontugne 2017), although compared to the UK, carbon values are typically more enriched and nitrogen values more depleted. Most parchment samples fall within this range, and it is again difficult to discount the presence of French skins.

One way of assessing the importance of French skins is to examine isotope values contemporary with the Napoleonic Wars (1803-1815), as Mortimer (1810) suggests that fresh skins and finished parchment were only imported "in times of peace". Hostilities between the two counties had long impeded trading relations (Crouzet 1985), but the volume of imports and exports was disrupted by the French Revolutionary Wars of the 1790s and again, severely, at the outbreak of war in 1803, particularly with the imposition of the Continental Blockage which restricted British access to European goods (Ashton 1975, 120-1; Broadberry *et al.* 2015, 212). During the war, the range of $\delta^{15}N$ values in parchment constricts to 2.5‰, compared to 4.5‰ in the preceding decade (1792-1802), and 5.2‰ in the decade after Waterloo (1816-1826) (Figure 8.5). A Levene's test of homogeneity of variance indicates that only the increase in variation after the war is significant (A to B: p=0.061; B to C: p=0.028[13]). The range of $\delta^{13}C$ values, increases from 1.8‰ before the war, to 2.5‰ during and 2.8‰ after, although these changes in variance are not statistically significant (A to B: p=0.067; B to C: p=0.103). but there is a statistically significant difference between $\delta^{13}C$ values from during and after the war (Mann-Whitney U test, U=879.5, p=0.030). Despite these variations, mean values stayed relatively static across these episodes ($\delta^{13}C$: –22.65‰ ± 0.1; $\delta^{15}N$: 8.78‰ ± 0.03), and it is likely that this variability if affected by the greater number of samples in Period C.

Smuggling was rife (Daly 2007), but the restricted access to European goods can be seen in the doubling of tallow prices, which prior to 1803 had been imported in large quantities from eastern Europe (Copus 1989), and the rising price of port wine from Portugal to its primary market in Britain (Maxwell 1995, 42-3). Yet, it cannot be said with any confidence that this isotopic variation reflects a disruption in the supply of French skins and an increased use of domestic animals, particularly as there is little change in mean values. An alternative theory is that existing domestic supply was affected by the war, with skins diverted into the booming leather industry driven by the demand for clothing and equipment for soldiers (Clarkson 1974, 143; Riello 2006).

Following Britain's enforced protectionism at the turn of the 19th century, the range of $\delta^{13}C$ values in parchment significantly increases, with the presence of more enriched skins, potentially more typical of a French origin (Camin *et al.* 2007; Goude & Fontugne 2017). French parchment in the 19th century was considered superior to that of any other country (Mortimer 1820; Willement 1845), and with extensive production in Flanders, it was ideally suited for importing into Britain. Post-medieval sheep from northern France and the Low Countries have produced $\delta^{13}C$ values between –22 and –15‰ and $\delta^{15}N$ between 6 and 10‰ (Ervynck *et al.* 2014; Colleter *et al.* 2017), and therefore may suggest the presence of skins from this region in British parchment. If this is the case, a potential

[13] A = 1792-1802 (n=23); B = 1803-1815 (n=33); C = 1816-1826 (n=90)

motivation may have been the improvement of sheep in the late-18th and 19th century, with a drive for meatier and fatter sheep. Fat makes the processing of skin difficult, inhibiting the penetration of aqueous reagent, and also reduces the tensile strength. Potentially, skin processors were seeking leaner skins from France. Despite this, the values still fall within the range expected for British sheep. Sheep bone collagen presented in Chapter 7 had a mean $\delta^{13}C$ value of –22.0‰, and ranged from –23 to –20.8‰, while parchment over the same period has a mean $\delta^{13}C$ value of –22.5, and ranges from –24.6 to –16.4‰, with a mean $\Delta^{13}C_{bone\text{-}parchment}$ spacing of 0.6‰, this suggests a likely domestic origin for the majority of skins.

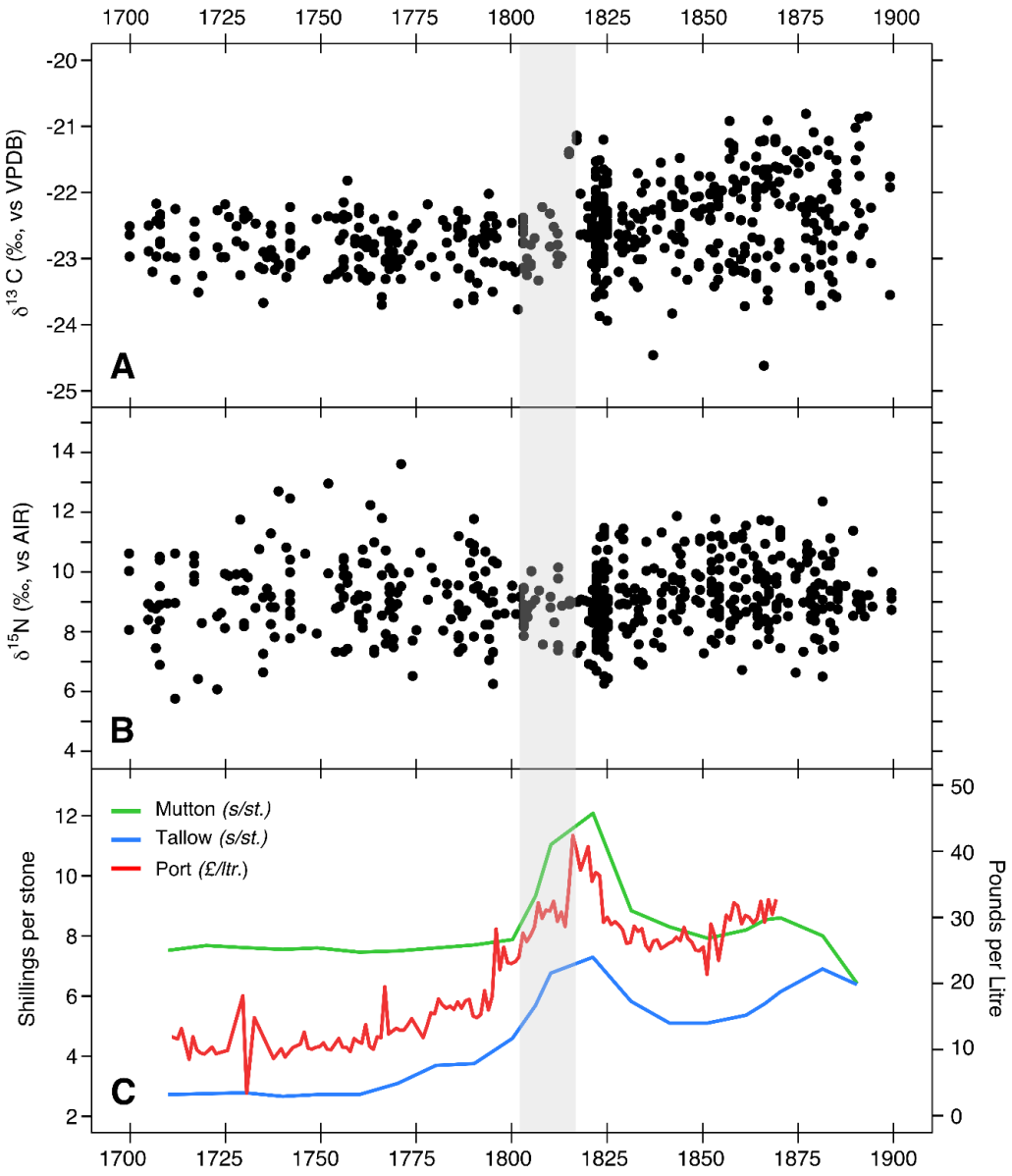


Figure 8.5: The impact of the Napoleonic Wars and the Continental Blockade on A) $\delta^{13}C$ values, B) $\delta^{15}N$ values, C) and the price of mutton, tallow and port wine in Britain (prices from Clark 2004; 2007)

With most $\delta^{13}C$ between 1800-1900 falling within the range typically expected British sheep, an alternative interpretation of this increasing variation is that it reflects the expanding range sheep were transported internally in the 19th century. As with agriculture and industry, historians have asserted that the transport developments of the period amounted to a 'revolution' (Deane 1965, 72-86; Bagwell 1974; Bogart 2005). The ease and distance with which people, livestock, raw material and goods could move increased dramatically, facilitated by improvements in roads, inland waterways, the development of steamships, and the arrival of the railways. Agricultural 'improvement', markets and transport interacted on each other, particularly in the decades following the Napoleonic Wars when there were improvements in all three (Blackman 1975, 48). The surging urban population created greater concentrated demand, which shaped farming and production decisions, and were connected over long-distances by increasing faster and cheaper forms of transport.

Livestock had been driven to towns and markets 'on the hoof' for millennia, navigating an extensive network of ancient roads and paths that by the 16th century formed the 'King's Highway' (Webb & Webb 1963). The Crown invested few resources in road maintenance, although improvements were made between 1500 and 1700 (Overton 1996, 142), supporting the movement of sheep towards large towns, and off the Welsh hills into England (Trow-Smith 1959, 7-10). In many areas the poor condition of roads hindered the progress of livestock, with sheep often unable to travel in winter, increasing the price of mutton (1959, 16). Starting in the 1660s, Turnpike Acts transferred temporary control and the right to collect tolls to private bodies, who maintained the principal roads of the country (Bogart 2005). Money raised was used to pave and widen roads, and lower gradients, increasing their capacity and speed with which they could be travelled (2005, 488). Between 1750 and 1770 the turnpike system diffused across much of the country, peaking in the 1830s.

A long-distance walk to market was often detrimental to the animal, losing considerable weight and condition, and with it the farmer's profits. Over the 100-mile journey from Norfolk to London, a yearling sheep could lose 6 lb of mutton and 4 lb of fat, as much as one-fifth of its weight (Perren 2000, 974; Turner *et al.* 2001, 190). The emergence of steam powered ships in the early-19th century cut journey times from weeks to days, and were used to bring animals from areas such as Aberdeen, Carlisle, Tyneside and Lincoln down to the rapacious London meat markets (Trow-Smith 1959, 323-4; Hill 1974, 100; Perren 2000, 968-70). Sheep were also shipped from north Wales to the hungry industrial towns of Liverpool and Manchester (Scola 1992, 45; Armstrong & Williams 2011, 268-9). The steamers not only increased domestic trade, but also from across the Irish Sea, with 400,000 live lambs imported between 1815 and 1825 alone (Armstrong & Bagwell, 1983, 159; Bagwell & Lyth 2006, 28). With the advent of the railways in the 1830s the range and speed increased further (Armstrong, 1991, 81-2). Moving freight by rail took one-tenth of the time it did by horse drawn

wagon, at only one-twentieth of the cost (Bogart 2013, 2). The rapidly expanding rail network revolutionised the droving industry, bringing an end to the long-distance drive 'on the hood', and enabling large quantities of animals to be transported at short notice across the length and breadth of the country (Overton 1996, 142; Perren 2000; Trow-Smith 1959, 232-5).

London's population surged from 960,000 in 1801 to over 2.3 million in 1851 (Great Britain Census Office 1854, 15), requiring a dramatic increase in the range from which livestock were drawn. By the mid-19th century, livestock entering the capital's main meat market at Smithfield came not only from across the British Isles, but from France, Belgium, Denmark, Holland, Norway, Spain, Portugal, Sweden, Brazil, Argentina and the United States (Metcalf 2012, 17; Bridger 2013, 64). In 1830, a designated skin market at Leadenhall was opened to deal with the sale of these animal's hides. Writing a few years after its opening, Charles Knight notes that sheepskins were often bought here in bulk by fellmongers who prepared the skin to be later sold to parchment makers (Knight 2014, 27). The location of the market was perfect for the parchment makers, who by the late-19th and 20th century, were increasingly located around the Greater London area (Kelly 1936).

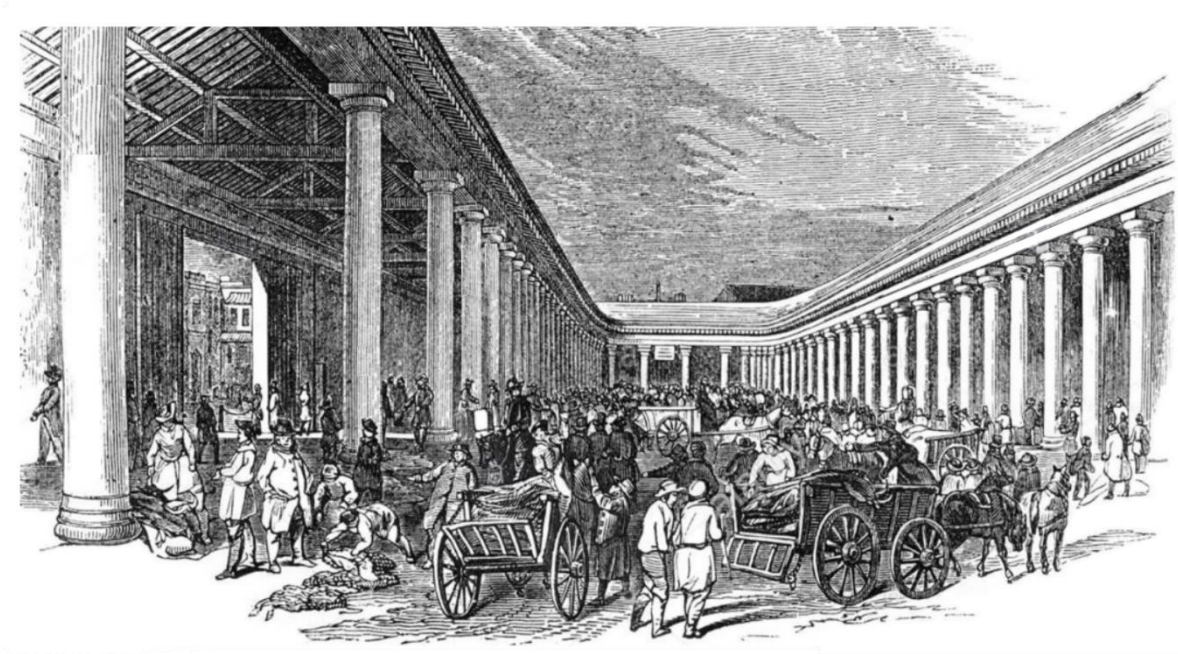


Figure 8.6: Skins being unloaded from wagons at the Leadenhall Skin Market, 1850 (Exploring Southwark 2018)

A potential farming practice driving this enrichment in $\delta^{13}C$ values but limited change in $\delta^{15}N$ values, could be the increased use of seaweed as a fertiliser. Seaweeds from the British coast display $\delta^{13}C$ values between –18.5 to –13.1‰ (Balasse *et al.* 2006), but unlike fishmeal, can have relatively low

δ^{15}N values between 1.7 and 3.1‰ (Bateman & Kelly 2007), comparable with livestock manure. The use of seaweed to improve soil fertility is documented in early farming manuals (i.e. Markham 1676, 68), although its use increased in the mid- to late-19th century (Turner e*t al.* 2001, 84). However, Turner's e*t al.* (2001, 84) analysis of 979 farm records suggests that even at the height of its use only 5% of applied it, so is unlikely to be responsible for the significant change we see. Alternatively, the increased consumption of C_4 plant protein, particularly maize and millet which have δ^{13}C values between –14.0 and –11.0‰ (Jahren & Kraft 2008), could have had an impact, although it was not until the late-19th and 20th century that they were imported in large quantities and were more likely to be fed to cattle (Mitchell 1971, 98-99; Brassley 2000, 577; Turner *et al.* 2001). Oil cakes too are an unlikely source, as the sunflower and rapeseed oils commonly used for their production, typically have depleted values between –35.6 to –26.2‰ (Jahren & Kraft 2008), not dissimilar from pasture.

The clearest evidence from enrichment due to manuring comes from the statistically significant increases in δ^{15}N values, contemporary with 'High Farming' of the mid-19th century. There was a major intensification in manuring during the 1830s and 1840s (Turner *et al.* 2001) as livestock numbers climbed, and use the use of pigeon manure, bonemeal and human excrement became more widespread (Turner *et al.* 2001, 81-8; Broadberry *et al.* 2015, 106). The quantity of fertilisers retained in the country increased from 26,000 tons between 1810-14 to 781,000 in 1872-76 (Turner *et al.* 2001, 86). Assuming, naively, a uniform distribution, this would have facilitated an increase in manuring rates on arable land from 0.23 kg/acre to 56.6 kg/acre[14]. The quantity and variety available is epitomised by the practices of a farmer in Saltmarsh, East Yorkshire, who in the early-19th century used cow manure, horse manure, bone dust, rape dust, wheat dust, whale blubber, guano (Turner *et al.* 2001, 87). In the 1840, the highly nitrogenous guano excrement from Peruvian seabirds was first imported into Britain (Nesbit 1852) and the first investigations into synthetic fertilisers were made (Jones 2012, 1). This increasing use of organic fertilisers perhaps explains the statistically significant increase in δ^{15}N values from 9‰ (6.3-11.9‰) between 1800-49 to 9.4‰ (6.5-12.4‰) between 1850-99. This potential manuring signal is supported by the statistically significant increase from 8.8% (6.3-11.5‰) in 1800-24, to 9.2% (6.4-11.9%) between 1825-49, contemporary with this period of intensification.

The most isotopically distinct samples are seven skins from the late-19th and 20th century, which present δ^{13}C values greater than –20‰ (–19.68‰ to –16.27‰). In modern studies, –20‰ has been used as a discriminator between livestock raised with or without the addition of C_4 plant protein in the diet (Boner & Förstel 2004). Two samples with values of –19.6%, are on the limit of those seen

[14] Calculated with 11.35 million acres of arable land in the 1810s, and 13.83 million acres in the 1870s (Broadberry *et al.* 2015, Table 2.10)

in modern UK samples and therefore could be assumed to British, but those from –18.7‰ to –16.2‰ are more enriched than modern livestock from Europe, Turkey or north-west Africa and show the greatest comparability with livestock raised in the US on maize (Piasentier *et al.* 2003; Schmidt *et al.* 2004; Hedges *et al.* 2005; Jahren & Kraft 2008; Camin *et al.* 2007; Perini *et al.* 2009; von Holstein *et al.* 2013; Zazzo *et al.* 2015; Mekki *et al.* 2016). Maize was imported by the late-19th century, and used as a livestock fodder (Mitchell 1971, 98-99; Turner *et al.* 2001), so these animals could potentially have been raised in the UK.

In the 20th century, British skin processors increasingly turned to using imported skins of hair-sheep breeds from parts of East Africa (Covington 2009, 67; Redwood 2016, 22-3). The sheep of Egypt and Ethiopia had long been known for their lack of wool fibres and limited fat content (Youatt 1840, 115-6), and possessed strong but soft skins, with a tight dermal fibre network and limited dermal lipocytes (Briggs 1981, 35; Covington 2009, 67). Although C_4 plants prevail in the warmer and drier lowlands of East Africa, C_3 plants thrive in the cooler and wetter highlands (Tieszen *et al.* 1979; Smith *et al.* 2002), where in Ethiopia at least, extensive sheep farming is often located (Wint & Robinson 2009; Gizaw *et al.* 2010). Predominantly C_3 eating terrestrial herbivores from East Africa have presented comparable $\delta^{13}C$ values (between –21.2 and –17.6‰) and $\delta^{15}N$ values (between 5.6 and 10‰) (Ambrose & DeNiro 1986), although due to the aridity of the area, $\delta^{15}N$ values can often be even higher (Ambrose & DeNiro 1986). The $\delta^{13}C$ values of these samples is considerably different to British sheep bone from the 20th century, supporting a non-domestic origin. Modern sheepskins display highly depleted in ^{13}C, likely due to variations in atmospheric CO_2 and increasing humidity. Mean $\delta^{15}N$ values from the 20th century are lower than any previous century, and may reflect the increasing use of synthetic fertilisers. Synthetic fertilisers are produced by the oxidation of NH_3, which in turn is produced by the reduction of atmospheric nitrogen (N_2) (Michalski *et al.* 2015). As such, synthetic fertilisers have $\delta^{15}N$ values (relative to AIR) of around 0%, resulting in lower $\delta^{15}N$ values in crops fertilised by synthetics than those receiving manure (Bateman & Kelly 2007).

The continuity and restricted range of $\delta^{13}C$ values in 13th to early-19th century parchment was also seen in bone collagen from Chapter 7, supporting the domestic origin of the vast majority of these skins. It is highly likely that similar supply routes were used, with sheep processed in town making use of their meat, bones and skin. The trend for increasing higher $\delta^{13}C$ values in the 19th century, however, is not seen in bone. Interpretation is hampered by the small and broadly-dated dataset for bone collagen, however, this may lend weight to the increasing use of non-domestic skins. Alternatively, this may reflect a divergence in existing supply routes, although as most of the 18th-19th century material also came from animal craft-waste, this seems unlikely (Scott 1985; O'Connor 1995; Reilley 2011). Values in excess of -20‰ seen in late-19th and 20th century parchment were not

seen in bone, supporting the conclusion that this parchment was manufactured utilising skins which had been imported from considerable distances. The significant variation in parchment $\delta^{15}N$ values is also seen in bone, confirming the high degree of variability in land management strategies in post-medieval Britain, particularly with regards to the manuring and the grazing of sheep on stubble.

8.4.2 Case study - Lower Winskill Farm

The majority of the deeds analysed in this study come from artificial collections, put together by archives and private collectors, but bear no relation to each other. The exception of this are 48 deeds relating to Lower Winskill Farm, Settle, North Yorkshire (Figure 8.7), which has been in the ownership of the Foster-Lord family since the late-16th century. These manuscripts offer the opportunity to explore changes in the provisioning of skin through isotopic analysis, as well as integrating the results with the history of the farm which has been compiled by its current owner Tom Lord (2009; 2010).

Following the dissolution of Sawley Abbey in 1537, its lands and property were transferred to the barony of Darcy de Darcy (Bush 1996, 218), and Lower Winskill Farm was sold to the sitting tenant Thomas Foster in 1591 (Lord 2009, 1). The farm consisted of stone-walled fields, with some area devoted to arable cultivation, probably oats (Kerridge 1967, 164), but particularly focussed on raising cattle for milk and sheep for wool (Lord 2009, 1). The Foster's also had the right to graze a number of livestock on nearby communal pastures. Although an exposed location, the Craven District was a profitable area for sheep farming, with its 'sweet and healthy grass' (*The Farmer's Magazine* 1874, 308) producing animals of an excellent constitution (Kerridge 1967, 164) and over twelve tonnes of wool a year (Trow-Smith 1957, 119). Typical of the area, the cattle ran on the grain stubble during winter, and were provided with hay indoors, but the same 'luxury' was not afforded to sheep (Lord 2009, 2). Between February and May all the livestock were taken off the pasture, allowing the early grasses and plants to grow, so that when the sheep lambed in May there was enough forage for them to produce milk (2009, 2). The farming practice stayed much the same until 1789 when an Act of Parliament enclosed the communal pastures, some of which were incorporated into the Foster's farm. Technological advances came in the 19th century with an improved system of manure collection in the cowshed, which was pumped into a spreader on a horse-drawn cart and applied to the 15 acres of meadow (Lord 2009, 3). Lord (2009, 2-3) estimates that in the 16th and 17th century stocking densities were around 1 sheep per 0.75 acres, but would have increased with enclosure, and again in the late-19th century as the farm focussed on sheep farming.

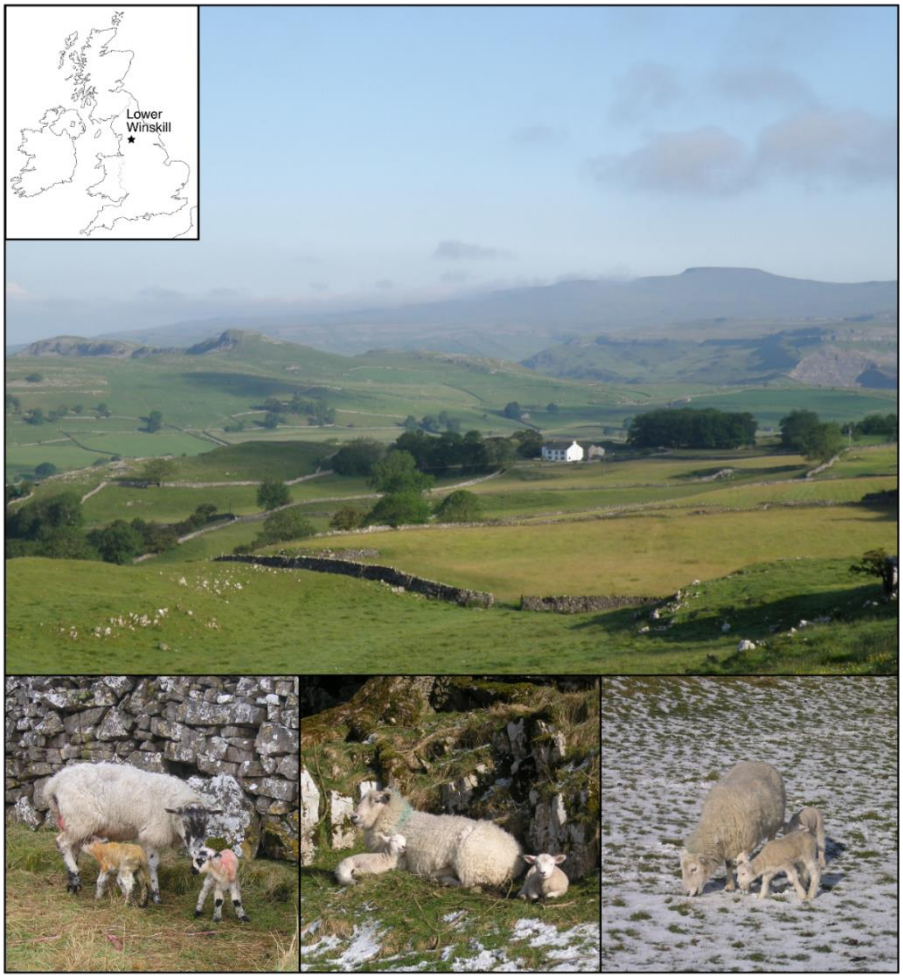


Figure 8.7: Lower Winskill Farm, Settle, North Yorkshire. The lower photos show the prolific Swaledale mules and Texel crosses currently raised on the farm; suckling minutes after birth, bonding, and seeking out grass amongst the snow (Photos by T. Lord)

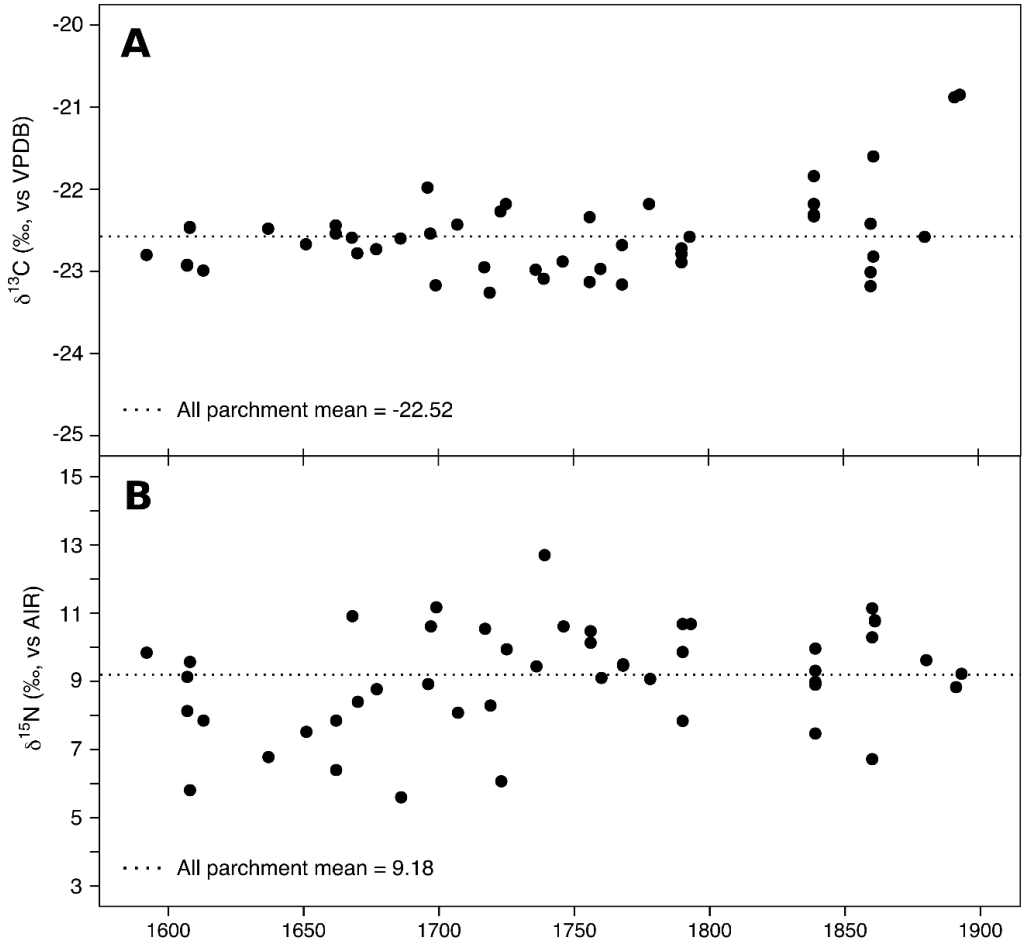


Figure 8.8: Isotope results from the Winskill Collection. A) $\delta^{13}C$ and B) $\delta^{15}N$

The Winskill deeds do not present any stationer's marks until the 18th century, and with the more localised markets of the 16th and 17th centuries, one might speculate that the parchment used for the earlier documents was produced locally, utilising sheepskins from the Craven District. The earliest thirteen skins (1592-1686) show comparatively lower $\delta^{15}N$ values (5.6 to 10.9‰, mean = 8‰), and largely fall below the mean value for all parchment samples (9.18‰) (Figure 8.8). Relating this to the historical information complied by Lord (2009), this could reflect the regions focus on wool production, resulting in a greater availability of older skins not exhibiting an elevated nitrogen value from the ingestion of milk; lower stocking densities; and the consumption of non-systematically manured pasture alone. These earliest skins have a range in $\delta^{13}C$ values of 0.5‰ (–22.9 to –22.4‰, mean = 22.6‰), while across the next three skins from 1696 to 1699 this range more than doubles to 1.2‰ (–23.1 to –21.9‰, mean 22.5‰). This dramatic increase in the range of values occurs shortly after the enactment of the 1694 Stamp Act (5 & 6 Wm. & Mar. c.21) which required a revenue stamp to be fixed to every individual sheet of a legal documents prior to engrossing; an action which could only be carried out at the Stamp Office, Lincoln's Inn, London, or one of the forty regional stamp

distributors (Hughes 1941; Dagnall 1994). The location of distributors is unknown, but in 1826 their distribution was considered "*arbitrary and capricious* … [with] *none at Bristol, and as many as seven in York*" (Hallam 1826, 11). Wherever located, the supply of parchment for deeds became concentrated in the hand of a few suppliers, and it appears that by the early-18th century regional isotopic signatures were lost. From 1694, the carbon isotope values from Winskill show greater variability, and nitrogen values become more enriched, likely indicating that we can no longer be confident of their origin.

8.5 Summary

The results of this study highlight the time-sensitive analysis in trade, craft and animal husbandry that can be revealed by the isotopic analysis of parchment. The majority of deeds display $\delta^{13}C$ and $\delta^{15}N$ values comparable with those of archaeological bone collagen, and fall within the range expected for modern British sheep. Historical records indicate the skins were imported in vast quantities from Ireland and France, although carbon and nitrogen isotope analysis was not discriminating enough to confirm their presence. A constriction in the range of $\delta^{15}N$ values is seen contemporary with the Napoleonic Wars, although this may reflect a change in domestic supply, rather than a restriction on imports. A number of 20th century skins have $\delta^{13}C$ values considerably more enriched than those of 20th and 21st century British sheep, and are likely to have been imported from some distance.

$\delta^{15}N$ values display considerable variability across the centuries, likely reflecting the use of both young and mature skins, as well as the diversity of geographical origin and farming practices. Parchment makers like Noah Crook, who supplied Witherbys & Co., and whose hands may have produced a number of early-19th century deeds analysed in this study, undoubtedly sourced their skins from a wide range. Located on the main road from Oxford to London, Crook, for example, may have utilised animals being driven to the capital from across the West Country and Wales. Analysis of a collection of deeds associated with Lower Winskill Farm, North Yorkshire, suggest that regional isotopic signatures in parchment may be lost as early as the 1690s as the supply of deeds was restricted to a number of approved regional distributors. The range in nitrogen values potentially indicates variability in the consumption of crops from manured fields, as well in the additional feeding of grains and root vegetables to ewes to improve their condition during lambing.

Chapter 9

Conclusions

9.1 Outcomes of the study

This thesis aimed to explore the potential of using parchment as an isotopic resource. For this, I undertook experimental parchment production, the analysis of paired bone and skin samples, and conducted a free-ranging diet study. I then applied this information to historic sheepskin parchment deeds, identified by peptide mass fingerprinting, to investigate sheep husbandry in post-medieval Britain. The results from this studies enable the research aims to be addressed:

1. *To identify the species used for parchment legal deeds*

Prior to this study, only 8 deeds had been identified using modern scientific techniques; this total now stands at 671. Identifying the species used for parchment via visual features can be challenging, due to the removal or distortion of follicle patterns during manufacture. Peptide mass fingerprinting of 663 legal deeds from the 12th to 20th century using MALDI-ToF MS **(Chapter 4)** indicates that they were predominantly written on sheepskin. 640 (96.5%) were identified as sheep, and the remaining 23 (3.5%) classified as sheep/goat. $\delta^{13}C$ and $\delta^{15}N$ values from these two groups showed no statistically significant difference, likely suggesting that many of these 23 skins are also those of sheep.

The root of this preferential use may lie in the unique characteristics of sheepskin, with its high potential to delaminate due to excessive fat deposits at the *papillary-reticular* junction. As such, ink erasure and attempts to alter the document may have been more visible than if they had been written on goat or calfskin. However, a more parsimonious explanation is their increased availability and cheaper cost. Between 2-5 million sheepskins were yielded annually (Broadberry *et al.* 2015, 100-5), and the finest quality parchment was cheaper than the poorest quality vellum (Gullick 1991). The result from this analysis aided later isotopic interpretations, as we can be confident that potential isotopic variation in historic parchment does not come from the presence of multiple species.

2. *To explore the use of parchment as an isotopic resource*

Campana *et al.* (2010) and Pollard & Brock (2011) have previous published the stable isotope analysis of parchment, yet both have speculated that values in finished parchment may not be the same as the fresh skin. In particular, both studies have noted $\delta^{15}N$ values considerably higher than would be expected from terrestrial herbivores. Through the experimental manufacture of 47 parchment skins, and the further analysis of parchment supplied by a commercial producer, this study confirmed that parchment production impacts on measured values in **(Chapter 5)**. Using traditional methods (straight-lime) a mean enrichment in $\delta^{13}C$ values of $0.15 \pm 0.68‰$ was observed, and a mean enrichment in in $\delta^{15}N$ values of $0.23 \pm 0.84‰$. Using more industrialised methods (additional sulfides) a mean enrichment in values of $0.23 \pm 0.47‰$ was observed, and a mean enrichment in in $\delta^{15}N$ values of $0.57 \pm 0.29‰$. There was no statistically significant difference in the skin to parchment offset between these two methods, suggesting that the technological change of the Victorian period is unlikely to have a significant impact on isotope values in historic parchment.

Four potential factors were identified as the main drivers behind this isotopic variation: amide sidechain hydrolysis; the removal of lipids and non-collagenous proteins; and the removal of keratin. The main chemical modification during liming is the conversion of some amide function groups of asparagine and glutamine to carboxyl groups (deamidation). Side chain hydrolysis favours bonds containing lighter isotopes, and results in an enrichment in the remaining residues (Bada *et al.* 1989; Silfer *et al.* 1992; McClelland & Montoya 2002; Chikaraishi *et al.* 2007; 2009). More than half of available side chains are likely to have been hydrolysed during liming (Menderes *et al.* 1999), contributing to the mean enrichment in parchment. Further enrichment may have resulted from the removal of ^{13}C-depleted lipids and non-collagenous proteins during liming and fleshing, resulting in a proportional increase in collagen and the relatively enriched amino acid glycine (Koppenhoefer 1939; Pollard & Brock 2011; McMahon *et al.* 2015). Finally, the structural modification of removing the keratinous wool and epidermis, which is typically isotopically depleted relative to collagen (Tieszen & Fagre 1993; O'Connell *et al.* 2012; von Holstein *et al.* 2013), may too have contributed to this mean enrichment.

To further aid the interpretation of isotope values from historic parchment, a free-ranging diet study was undertaken to explore the relationship between diet and skin **(Chapter 6**). As expected, skin and parchment was enriched over diet. The isotopic spacing between diet and parchment was influenced by production, but displayed an enrichment in $\delta^{13}C$ values between 3.0 to 5.7‰ over diet, and enrichment in $\delta^{15}N$ values of 2.3 to 6.8‰. This was not a controlled feeding study, and therefore there was some uncertainty over the 'true' isotopic composition of each sheep's diet, but the level of

enrichment was consistent with the findings of other studies (Hedges *et al.* 2006). These results indicate that some parchment can possess high $\delta^{15}N$ values due to individual metabolism, and not solely due to farming practices such as high levels of manuring.

There is currently no widely accepted method for checking the integrity of collagen from skin or parchment (Lamb 2016, 6), although C/N ratios (a standard for bone collagen and dentine; DeNiro 1985; Ambrose 1990; van Klinken 1999) were found to be useful. C/N ratios in modern skin ranged from 3.1 to 3.4, 3.2 to 3.4 in modern parchment, and 3.1 to 3.6 in historic parchment **(Chapter 8)**. Parchment production had a variable impact on C/N ratios in skin, although may prove useful in identifying skins with has undergone significant deamidation and the loss of nitrogenous compounds.

3. *To enable $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ isotope data from parchment to be integrated with those from bone collagen*

Due to variations in tissue composition, rate of turnover and the routing of amino acids, skin and bone collagen within the same individual has been seen to display different isotopic signatures (Lyon & Baxter 1978; Finucane 2007; Corr *et al.* 2009). Prior to this study, the offset between bone and parchment had not been calculated, therefore to address this, the isotopic spacing was explored through the paired analysis of 30 samples from modern sheep **(Chapter 6**). As with skin, parchment $\delta^{13}C$ values were seen to be depleted relative to those in bone (0.6 ± 0.8‰), while $\delta^{15}N$ values were enriched (1.3 ± 1.8‰).

This pattern has been seen in the paired analysis of archaeological human remains, and previously explained through the different temporal resolution of these tissues, with the skin indicating an abrupt change in diet, location or health prior to death (Iacumin *et al.* 1996; 1998; Corr *et al.* 2009). However, this explanation is not feasible for these modern sheep. Bone and skin are both dominated by Type 1 collagen, and thought to have analogous dietary/biosynthetic origins of amino acids (Ambrose 1993; Collins *et al.* 2002; Covington 2009; Corr *et al.* 2009). This research suggests that the small variation the amino acid composition of these tissue may influence their isotopic difference, as well as the routing of amino acids. The different collagen synthesis rates of these tissue undoubtedly plays a part, but this isotopic difference was also seen in the skins of stillborn lambs who could not have developed a turnover related variation.

The calculation of this offset aided the interpretation of values from historic parchment by permitting the integration with those from contemporary sheep bone collagen **(Chapter 8)**. 15th to 20th century bone collagen presented a mean $\delta^{15}N$ value of 6.1‰, ranging from 3.9 to 9.2‰ **(Chapter 7)**.

Parchment across the same period had a mean value of 9.2‰, and ranged from 5.6 to 13.6‰. Considering a mean bone to parchment spacing of 1.3‰, this supports an interpretation that young lambskins with minimal fat and visual flaws were used, as well as those from more mature sheep. Similarly, bone had a mean $\delta^{13}C$ value of –22.0‰, ranging from –23 to –20.8‰, while parchment had a mean $\delta^{13}C$ value of –22.5‰, ranging from –24.6 to –16.3‰. With a mean bone to parchment spacing of 0.6‰, this supports the domestic origin of the majority of skins, but also suggests that a number of 20th century skins may have been imported.

4. *To apply these methods to parchment and bone from post-medieval Britain to examine sheep husbandry and land management*

The insight gained from the analysis of modern specimens aided the interpretation of $\delta^{13}C$ and $\delta^{15}N$ isotope results from 47 sheep bones from the 15th to 20th century York and London **(Chapter 7)**, and 663 British parchment deeds from the 13th to 20th century **(Chapter 8)**. This analysis facilitated an examination of the age of animals used, their geographical origin, and farming practices such as manuring.

With the exception of seven skins from the 10th century, 638 deeds from the 13th to late-19th century displayed $\delta^{13}C$ values between –24.6‰ and –20.8‰, indicating that the overwhelming majority of sheep were grazing on C3 plants. Despite the importation of C4 plants such as millet and maize in the 19th century (Seemann 1866; Mitchell 1971), they do not appear to have been used as sheep feed, supporting historical accounts that they were more likely to be fed to more profitable cattle (Brassley 2000). As noted above, these values are comparable with those from archaeological bone collagen and the range observed in modern British terrestrial herbivores, suggesting the use of domestic sheepskins (FERA 2015; Camin e*t al.* 2007). Documentary records indicate that sheepskins were imported in large quantities from Ireland and mainland Europe throughout this period, unfortunately due to the comparable climate and farming practices across north-west Europe $\delta^{13}C$ and $\delta^{15}N$ isotope analysis did not prove sufficiently discriminating to confidently identify skins from these countries. $\delta^{15}N$ values fluctuated contemporary with the Napoleonic Wars, however this may be due to disruption in domestic supply, rather than an embargo on French skins and parchment. With the lifting of the Continental Blockade, an increasing presence of parchment with elevated $\delta^{13}C$ values was observed, perhaps more similar to those of a French origin (Goude & Fontugne 2017), therefore the presence of imported skins cannot be discounted.

18th and 19th century stationer's accounts **(Chapter 4)** indicate that parchment for legal deeds was sourced from considerable distances. For example, although located in London, Witherbys & Co. procured their skins from parchment makers in the south-west of England. The fresh skins were also likely to have been obtained from a wide geographical range, with parchment makers such as Samuel Bishop of Bristol potentially utilising skins that came up the Bristol Channel, and Charles Franklin of London potentially selecting from sheep driven to the capital from across Britain. Contemporary with transport developments of the 19th century, such as turnpikes, steamships and steam locomotives (Bagwell 1974), $\delta^{13}C$ values shown increasing variation, likely reflecting the greater range which livestock, skins, feed and fertiliser could move. Seven skins from the late-19th and 20th century displayed $\delta^{13}C$ values from –19.7‰ to –16.3‰, suggesting C4 plants may have formed part of the diet. The use of maize was more widespread by this period, but skins were also obtained from an even greater range, and may potentially have come from East Africa (Covington 2009, 67; Redwood 2016).

The potential use of imported skins, and those from young lambs, makes examination of sheep husbandry and land management challenging, but it is clear that British agriculture underwent significant change in the early-19th century. $\delta^{13}C$ values in bone and parchment remain remarkably static across the 13th to late-18th century, suggesting a rather restricted diet, with sheep primarily grazing on pasture, but occasionally receiving supplementary forage and fodder crops. Contemporary $\delta^{15}N$ values, however, highlight some variability in management strategies, particularly over access to crops from well manured fields, such as grain, stubble and root vegetables. When integrated with zooarchaeological evidence, it appears that the size increase seen in the 16th and 17th centuries was not accompanied by any significant, or isotopically distinguishable, change in diet. These results are at odds with some historical interpretations of 'improvement', particularly Eric Kerridge, whose *Agricultural Revolution* announced that "the agricultural revolution took place in England in the 16th and 17th centuries and not in the 18th and 19th" (1967, 4).

The isotopic data presented in this thesis instead indicates a change in animal management during the 19th century, the period traditionally ascribed to the Agricultural Revolution (Chambers & Mingay 1966; Overton 1986; 1996; Turner e*t al.* 2001). From this period onwards, $\delta^{13}C$ values become increasingly variable, with many skins exhibiting increasingly higher values; in contrast, $\delta^{15}N$ values become less variable, but mean values increase. The major technological developments of this period were clover and turnips, which were gradually integrated into arable rotations, removing the need for bare fallows (Turner *et al.* 2001, 71-3). The importance of these humble crops lay in their ability to increase livestock carrying capacity, and therefore manure, the main fertiliser of arable land. These new crops were often cultivated in rotation with wheat and barley as part of the

'Norfolk four-course', with their restorative function contributing to an almost 50% increase in grain yields between 1750 and 1850 (Broadberry *et al.* 2015). The increased use of manure and other organic fertilisers is visible in the parchment, with a significant increase $\delta^{15}N$ in the early-mid 19th century contemporary a major intensification in their use (Turner *et al.* 2001, 86). Other late-18th and 19th century improvements included selective breeding, which changed the size and shape of animals, and improved the rate at which feed was converted into meat (O'Connor 1982; 1995; Overton 1986; Thomas 2005b).

These changes in animal and land management were intimately associated with enclosure. The shift from collective farming to private ownership facilitated far greater control, and the opportunity for farmers to experiment with new feeds, fertilisers, rotations, and may well be why we see greater isotopic variability in carbon values. New 'scientific' approaches to farming diffused across the country, propagated through societies, journals and handbooks. In 1800 there were 35 English agricultural societies, but more than 360 by 1845 (Tarlow 2006, 36). Chamber & Mingay (1966) regarded these post-1750 changes as 'revolutionary' as they estimated British agriculture fed an additional 6.5 million people in 1850 than it did in 1750 (Overton 1986, 2).

Despite these results, the limitation of using isotope evidence to explore animal husbandry must be noted. A major issue is the variability that livestock can exhibit over small geographical areas. As seen in Chapter 6, sheep from Seaton Ross, East Yorkshire, were raised across a 4.5-acre farm and consumed near identical diets. Despite this, bone collagen $\delta^{13}C$ and $\delta^{15}N$ values showed a range of 2.1‰ and 2.5‰, respectively. Due to the rapid turnover of skin collagen, it exhibited a greater range of 2.7‰ and 4.3‰. This high degree of individual variability makes identifying small changes in diet very challenging, but may also lead to the erroneous interpretation of results. For example, two of these sheep (JSK24 and JSK25), had much lower $\delta^{15}N$ than the rest of the flock, which in an archaeological population could be incorrectly interpreted as the result of consuming a different diet. Moreover, due to the high degree of similarity in the isotopic composition of livestock feeds (Jahren & Kraft 2008) it is possible that changes in diet may not be detectable isotopically. It is therefore possible that the diet of sheep during the 13th and early-19th century did change, but it was insufficiently distinct to alter the isotopic values of the tissue.

Although this study identified variation in isotope values which are likely the consequence of changes in farming practice (i.e. increased manuring), they may not be the result of 'improvement'. Agricultural developments had different trajectories, and the size increase of the 16th and 17th centuries, and the shape change of the 18th and 19th, may not have been associated with nutritional development. They could have been brought about by changes in flock composition or through

selective breeding – management decisions which would not be identifiable isotopically. As such, isotope analysis provides evidence which must be integrated with zooarchaeological and historical enquiry to fully examine the post-medieval 'improvement' of sheep.

9.2 Future research

The results of this study highlight a number of areas where further historic, biomolecular and zooarchaeological research may be beneficial, and enable interpretation of the data presented here in greater confidence. Although a range of techniques were employed, a number wee beyond the scope of this thesis due to financial, time and technical constraints, but could be applied to further our understanding of parchment as a molecular archive and sheep husbandry in the post-medieval period.

Historical

- The examination of unpublished stationer's accounts may provide further insight into the provisioning of skins and the distribution of deeds. The accounts examined in Chapter 4 dated from between 1705 and 1806, therefore information prior to the introduction of the 1694 Stamp Act and again after the advent of steam transport would be useful in examining their impact. Unfortunately, surviving stationer's accounts are rare, and not detail the location of where skins were purchased from. For this study the accounts of stationers in Nottingham, Derby and Birmingham were examined, but did not list any parchment suppliers.

- The examination of import records from along the southern coast may provide useful information on the importing of French skins and parchment.

Biomolecular

- Analysis of the amino acid composition of skin before and after processing may provide further insight into the impact of chemical and structural modifications to collagen. This may potentially measure deamidation levels, and the proportional increase in glycine, which could be examined alongside the extent of $\delta^{13}C$ and $\delta^{15}N$ enrichment.

- Analysis of the amino acid composition of bone and skin from the same individual may aid the interpretation of the isotopic difference between these tissue. Compound specific isotope analysis of paired samples from stillborn lambs could be used to examine variation in the routing of amino acids, any potentially help explain the isotopic variation seen in these young animals.

- To aid the identification of skin from Ireland and France, post-medieval parchment deeds through to have been manufactured in these regions could provide a useful dataset. It is not known how beneficial isotopic analysis may be, as sheep from these countries have a similar range of $\delta^{13}C$ and $\delta^{15}N$ values and it is possible that finished parchment was exported from Britain. Genetic analysis of parchment from these area may therefore provide greater evidence on the use of imported skins. At present aDNA analysis has only been conducted on a small number of post-medieval parchment samples (Teasdale *et al.* 2015; 2017). A more comprehensive analysis of these deeds may highlight genetic affinity with breeds from these regions.

- $\delta^{18}O$ and δD isotope analysis may prove useful in identifying the geographical origin of skins. Animals derive oxygen and hydrogen isotopes from their diet, which are closely associated with precipitation and local drinking water. Both have been use for provenancing on a regional scale, and therefore may provide information on the distribution of skins across Britain. The impact of production on both isotopes would need to be examined first, as it is highly likely that isotopic fractionation or exchange may occur when the skins are submerged in water and calcium hydroxide.

<u>Zooarchaeology</u>

- Direct ^{14}C dating of bones would provide greater confidence over phasing. This would be particularly beneficial at sites which present evidence for size and shape change to better identify when this is occurring.

- Greater emphasis should be placed on publishing dental measurement data, as well as depth measurements from long bones. These would provide the opportunity to better examine the role of environmental and genetic factors on shape change.

- While the 'slenderness' and 'conformation' indices examined by O'Connor (1982) provide a great deal of information about the changing shape of sheep in the post-medieval period, geometric morphometrics (GMM) may be useful in examining these changes at a finer scale. GMM could be used to examine the refinement of the carcass in the 19th century, and examine how the skeleton responded to breeder's desires to reduce the bone-to-muscle ratio.

9.3 Concluding remarks

This thesis demonstrates that parchment is a valuable high-resolution and isotopic resource, through which centuries of craft, trade and animal husbandry can be explored. When discarded by the Sun Fire Office Archives, deed RT215, for example, was just another contract concerning the sale of land in London on the 3rd September 2011. However, it is now a piece of sheepskin parchment which contributes to our understanding of the disruption in the domestic and international supply of raw materials during the Napoleonic Wars. Samples EB04 was just a mortgage from Essex in 1927, but is now the skin of a sheep that may have travelled from East Africa, and offers insight into the movement of skins over considerable distances. This research makes a significant contribution to archival research, demonstrating the value of post-medieval deed. For historians and archaeological scientists this thesis contributes to our knowledge on the provisioning of skins and trends in animal and land management.

While the text of post-medieval legal deeds may be of limited historical significance, this does not negate the physical value of dated mammalian collagen. Through proteomic and stable isotope analysis, information can be gathered on the species used, their geographical origin, and the conditions under which the animal was raised. This is, however, only a fraction of techniques that can be applied to this extraordinary archive: genetic analysis can provide further information on provenance and the development of breeds in this period; palaeopathological analysis can be applied to the dermatological issues preserved in the parchment; microscopic and imaging analysis can examine changes in fibre colour and diameter; and further imaging can be used to examine technological changes in the shaving, smoothing or splitting of skins.

For agricultural historians and zooarchaeologists, this research provides new information into sheep husbandry in post-medieval Britain. This time-sensitive analysis suggests significant agricultural developments in the 19th century, the period traditionally ascribed to the Agricultural Revolution. While recognising the use of young and imported lambskins, the isotopic data indicates that farming practices were diverse, with considerable variation in manuring practices and management strategies. Significant variation in $\delta^{13}C$ and $\delta^{15}N$ values did not occur until post-1800, which most likely reflects the impact of enclosure, new cropping rotation, as well as the increased distance skins and feed were transported. For the wider field of archaeological science, this data provides a baseline for sheep throughout the post-medieval period, as well as new data on the isotopic spacing between, diet, bone, skin and parchment. It is hoped that this research will enable a much more extensive proteomic and isotopic analysis of parchment from across the UK and Europe, as parchment reveals a history not only written in ink, but in the protein itself.

Appendix

Table A.1: $\delta^{13}C$ and $\delta^{15}N$ values and elemental composition data for all modern skin, bone and parchment samples. Species: S = Sheep, C = Calf, G = Goat, P = Pig. Season: SP = Spring (MAM), S = Summer (JJA), A = Autumn (SON), W = Winter (DJF).

Table A.2: Species identification results (peptide mass fingerprinting), $\delta^{13}C$ and $\delta^{15}N$ values and elemental composition data for historic parchment samples

Table A.3: $\delta^{13}C$ and $\delta^{15}N$ values and elemental composition data for post-medieval sheep bone collagen from York and London

References

Primary Sources

London Metropolitan Archives – Witherbys & Co. Ltd. LMA/4682:

C/04/001 – Ledger 'D', 1795-1798
C/06/002 - Journal No. 7, 1767-1769
I/01/001 – Percival & Anthony Witherby Research Files
I/01/006 – Firm History
I/01/002 – Ledger, 1798-1806

National Archives – Records of the Prerogative Court of Canterbury. NA/PROB:

/11/1698/337 – Will of Noah Crook, Fell Monger, Parchment Maker and Tanner of Wheatley, Oxfordshire, 2nd May 1825
11/1666/104 - Will of Isaac Abel Kevitt, Parchment Maker of Coventry, Warwickshire, 6th February 1823

Northamptonshire Archives. NRO:

ML/010 - Bookseller & Stationers Account Book, 1779-1780
ML/088 - Bookseller Account Book, 1777
ML/089 – John Clay of Daventry – Bookseller Account Book, 1774-1775
ML/691 – Bookseller & Stationers a/c Book, 1764-1773
ML/692 – Bookseller & Stationers a/c Book, 1763-1766
ML/692 - Bookseller & Stationers a/c Book, 1770-1777

Acts of Parliament

An Act for granting to Their Majesties several duties on Vellum, Parchment and Paper for 10 years, towards carrying on the war against France.
[Stamp Act 1694] 28th June 1694. 6 & 6 William & Mary. c.21

An Act to repeal so much of an Act, passed in the 2nd year of King James I, as prohibits the use of Horse Hides in making boots and shoes; and an Act for the better preventing the damaging of Raw Hides and Sins in the flaying thereof.
[The Flaying Act 1800] 30th June 1800. 39 & 40 George III. c.66

Online Resources

ABMAP 2003. *Animal Bone Metrical Archive Project.* Dataset: University of Southampton; Distributor: ADS, York. [Online] [Accessed 01.02.16] Available at: http://archaeologydataservice.ac.uk/archives/view/abmap/

British Galway Sheep Society 2018. *Our history.* [Online] [Accessed 02.04.17] Available at: http://www.britishgalwaysheepsociety.co.uk/our-history/

British Library. n.d. 'Charter of King John of England (1199-1216), granting liberties to the English people ('Magna Carta'): Runnymede, 15th June 1215. *Cotton MS Augustus ii.106: 1215.* Collection Item description [No date]. [Online] [Accessed 22.01.18] Available at: https://www.bl.uk/collection-items/magna-carta-1215

British Pathé 1939. *The Very Idea* [Film]. Pathé Studios London [Online] [Accessed 19.03.2018] Available at: https://www.britishpathe.com/video/making-parchment-issue-title-is-the-very-idea/query/parchment

Crowley, D.A. 1983. 'The Borough of Marlborough', In *A history of the County of Wiltshire: Volume 12, Ramsbury and Selkley Hundreds; the Borough of Marlborough.* Victoria County History - Wiltshire. London. *British History Online.* [Online] [Accessed 15.06.17] Available at: https://www.british-history.ac.uk/vch/wilts/vol12/pp199-229

Debenham's & Co. 1932. *The romance of leather* [Film]. Debenham's & Co. Studios Beverley [Online] [Accessed 19.03.2018] Available at: http://www.yorkshirefilmarchive.com/film/romance-leather

Exploring Southwark. 2018. *The leather market.* [Online] [Accessed 15.01.18] Available at: http://www.exploringsouthwark.co.uk/the-leather-market/

FAO, Food Organisation of the United Nations. 2014. Agriculture & Consumer Protection Department. *Gridded Livestock of the World (GLW)* (Updated Version) [Online] [Accessed 04.06.17] Available at: http://www.fao.org/ag/againfo/resources/en/glw/home.html

Hampshire County Council. 2008. *Parchment making in Havant.* [Online] [Accessed 28.12.14] Available at: http://www3.hants.gov.uk/museum/havant-museum/havant-local-studies/parchment.html

Lévêque, E. 2013. 'An Irish Sheep Tale' *National Library of Ireland.* [Online] [Accessed 25.07.17] Available at: https://blog.nli.ie/index.php/2013/05/24/an-irish-sheep-tale-700-year-old-parchments-conserved.

Lobel, M. 1957. 'Parishes: Cuddesdon', In *A history of Oxford: Volume 5, Bullingdon Hundred.* Victoria County History - Oxfordshire. London. *British History Online.* [Online] [Accessed 15.06.17] Available at: https://www.british-history.ac.uk/vch/oxon/vol5/pp96-116

Maxted, I. 2014. Exeter Working Papers in Book History: Biographical and bibliographical information on the book trade [Online] [Accessed 07.11.2017] Available at: http://bookhistory.blogspot.co.uk/2014/10/homepage

Oklahoma State, Department of Animal Science. *Sheep.* [Online] [Accessed 04.02.18] Available at: http://www.ansi.okstate.edu/breeds/sheep

Pergamena/Makers Row 2018. *Pergamena leather suppliers: vegetable tanned leather and handmade parchment.* [Online] [Accessed 29.03.2018] Available at: https://makersrow.com/apparel/new-york/pergamena

Southdown Sheep Registry. 2018. *About the breed.* [Online] [Accessed 23.03.17] Available at: http://www.oldeenglishbabydollregistry.com/about-the-breed/

St. Kilda Soay Sheep Project [Online] [Accessed 05.08.17] Available at: http://soaysheep.biology.ed.ac.uk/

Veale, E.M. 2003. 'VIII. The trade in skins in the Fifteenth and Sixteenth Century', In *The English Fur Trade in the Later Middle Ages. British History Online.* [Online] [Accessed 16.06.17] Available at: https://www.british-history.ac.uk/london-record-soc/vol38/156-180

Secondary Sources

A'Hern, B. 2004. 'The British industrial revolution in a European mirror', In Floud, R., Humphries, J, and Johnson, P. (eds.) *The Cambridge economic history of modern Britain Vol. 1: 1700-1870.* Cambridge: Cambridge University Press, pp. 1-52.

Abend, A.G. and Smith, T.D. 1995. 'Differences in ratios of stable isotopes of nitrogen in long-finned pilot whales *(Globiecphala melas) in the western and eastern North Atlantic', ICES Journal of Marine Science* **52**(5): 837-841.

Adam, C.L. and Findlay, P.A. 1997. 'Effect of nutrition on testicular growth and plasma concentrations of gonadotropins, testosterone and insulin-like growth factor I (IGF-I) in pubertal male Soay sheep', *Journal of Reproduction and Fertility* **111**:121-125.

Albarella, U. 1997. 'Size, power, wool and veal: zooarchaeological evidence for late Medieval innovations', In De Boe, G. and Verhaeghe, F. (eds.) *Environment and subsistence in Medieval Europe – Papers of the 'Medieval Europe Bruges 1997' Conference* **9**:19-30.

Albarella, U. 2003. Tanners, tawyers, horn working and the mystery of the missing goat, In Murphy, P. and Wiltshire, P. (eds.) *The Environmental Archaeology of Industry*. Oxford: Oxbow Books, pp. 71-86.

Albarella, U. and Davis, S.J.M. 1994. *Medieval and post-medieval mammal and bird bones from Launceston Castle, Cornwall: 1961-1982 excavations.* AML Report 18/94

Albarella, U. and Davis, S.J.M. 1996. 'Mammals and birds from Launceston Castle, Cornwall: decline in status and the rise of agriculture', *Environmental Archaeology* **12**(1):1-156.

Albarella, U. Beech, M., Curl, J., Locker, A., Moreno-Garcia, M and Mulville, J. 2009. *Norwich Castle: excavations and historical survey, 1987-98. Part III: A zooarchaeological study.* East Anglian Archaeology, Occasional Paper **22**.

Alexander, J.G. 1978. *Insular manuscripts 6th to the 9th Century. A survey of manuscripts illuminated in Britain in the British Isles.* London: Harvey Miller.

Alexander, M.M., Gerrard, C.M., Gutierrez, A. and Millard, A.R. 2015. 'Diet, society, and economy in late Medieval Spain: stable isotope evidence from Muslim and Christians from Gandia, Valencia', *American Journal of Physical Anthropology* **156**:263-272.

Allen, R. 1992. *Enclosure and the yeoman.* Oxford: Clarendon Press.

Allen, R. 2000. 'Economic structure, and agricultural productivity in Europe, 1300-1800', *European Review of Economic History* **3**:1-25.

Allen, R. 2008. 'The nitrogen hypothesis and the English agricultural revolution': A biological analysis', *Journal of Economic History* **68**(1):182-210.

Ambrose, S.H. 1990. 'Preparation and characterisation of bone and tooth collagen for isotopic analysis', *Journal of Archaeological Science* **17**(4):431-451.

Ambrose, S.H. 1993. 'Isotopic analysis of paleodiets: methodological and interpretative considerations', In Sandford, M.K. (ed.) *Investigations of ancient human tissue.* Langhorne, PA: Gordon & Breach Science Publishers, pp. 59-130.

Ambrose, S.H. 2000. 'Controlled diet and climate experiments on nitrogen isotope ratios of rats', In Ambrose, S.H. and Katzenberg, M.A. (eds.) *Biogeochemical approaches to palaeodietary analysis.* New York, NY: Kluwer Academic, pp.243-259.

Ambrose, S.H. and DeNiro, M.J. 1986. 'The isotopic ecology of East African mammals', *Oecologia* **69**: 395-406.

Ambrose, S.H. and Norr, L. 1993. 'Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate', In Lambert, J. and Grupe, G. (eds.) *Prehistoric human bone – archaeology at the molecular level.* Berlin: Springer-Verlag, pp.1-37.

Anonymous. 1806. *The flaying Act considered: as, without benefiting the public, most severely oppressing the butchers of London. By an impartial observer.* London: E. Thomas.

Anonymous. 1947. 'Applied biology in the leather industry', *Nature* **159**(4048):750-751.

Anonymous.1833. *The Bristol poll book, being a list of the householders, freeholders and freemen, who voted at the general Election for members to serve in Parliament for the City and County of Bristol.* Bristol: J. Wansbrough.

Aranibar, J.N., Anderson, I.C., Epstein, H.E., Feral, C.J., Swap, R. and Ramontsho, J. 2008. 'Nitrogen isotope composition of soil C_3 and C_4 plants along land use gradients in southern Africa', *Journal of Arid Environments* **72**:326-337.

Armitage, P. 1983. 'Faunal remains', In Thompson, F., Green, F. and Schofield, J. *Excavations at Aldgate 1974.* Transactions of the London and Middlesex Archaeological Society, 125-33.

Armitage, P.L. 1977. *The mammalian remains from the Tudor site of Baynard's Castle, London: A biometrical and historical analysis.* PhD Thesis, Royal Holloway College, London.

Armitage, P.L. 1980. *A preliminary description of British cattle from the late-12th to the early-16th century.* AML Report 3131.

Armour, C. 1956. *The trade of Chester and the state of the Dee navigation 1600-1800.* PhD Thesis, University College London.

Armstrong, J. 1991. 'Railways and costal shipping in Britain in the later Nineteenth century', In Wrigley, C. and Shepherd, J. (eds.) *On the Move: Essays in Labour and Transport History Presented to Philip Bagwell.* London: The Hambledon Press, pp. 76-103.

Armstrong, J. and Bagwell, P.S. 1983. 'Costal shipping', In Aldcroft, D.G. and Freeman, M.J. (eds.) *Transport in the Industrial Revolution.* Manchester: Manchester University Press, pp. 142-176.

Armstrong, J. and Williams, D.M. 2011. *The impact of technological change: the early steamship in Britain.* Research in Maritime History No. 47. Newfoundland, St. Johns: International Maritime Economic History Association.

Ashton, T.S. 1975. *Economic fluctuations in England 1700-1800* (2nd Edition). Oxford: Clarendon Press.

Atkinson, D. and Sibley, R.M. 1997. 'Why are organisms usually bigger in colder environments? Making sense of a life history puzzle', *Trends in Ecology and Evolution* **12**:235-239.

Ault, W.O. 1972. *The open-field farming in Medieval England: A study of village by-laws.* New York, NY: Barnes & Noble.

Ayliffe, L.K., Cerling, T.E., Robinson, T. and Ehleringer, J.R. 2004. 'Turnover in tail and hair breadth CO_2 of horses fed on isotopically varied diet', *Oecologia* **139**(1):11-22.

Babraj, J.A., Cuthbertson, D.J.R., Smith, K., *et al.* 2005. 'Collagen synthesis in human musculoskeletal tissues and skin', *American Journal of Endocrinology and Metabolism* **289**:864-869.

Bada, J.L., Schoeninger, M.J. and Schimmelmann, A. 1989. 'Isotopic fractionation during peptide bond hydrolysis', *Geochimica et cosmochimica acta* **53**(12):3337-3341.

Badae, E., Miu, E., Budrugeac, P., Giurginca, M., Masic, A., Badea, N., Della-Gatta, G. 2012. 'Damage ranking of historic parchment: from microscopic studies of fibre structure to collagen denaturation assessment by Micro DSC', *Preservation Science* **9**:97-109.

Bagwell, P.S. 1974. *The Transport Revolution 1770-1985.* London: Routledge

Bagwell, P.S. and Lyth, P. 2006. *Transport in Britain: From Canal Lock to Gridlock.* London: A&C Black

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**:1299-1305.

Bailey, W. 1784. *Bailey's British directory, or, merchant's and trader's useful companion, for the year 1784, in four volumes.* London: J. Andrews.

Baker, A.R.H. and Butlin, R.A. 1973. *Studies of field systems in the British Isles.* Cambridge: Cambridge University Press.

Balasse, M. and Ambrose, S.H. 2005. 'Distinguishing sheep and goats using dental morphology and stable carbon isotopes in C_4 grassland environments', *Journal of Archaeological Science* **32**(5):691-702.

Balasse, M., Tresset, A., Dobney, K. and Ambrose, S.H. 2006. 'The use of isotope ratios to test for seaweed eating in sheep', *Journal of Zoology* **266**(5):283-291.

Balssse, M., Tresset, A. and Ambrose, S.H. 2006. 'Stable isotope evidence ($\delta^{13}C$, $\delta^{18}O$) for winter feeding on seaweed by Neolithic sheep of Scotland', *Journal of Zoology* **270**(1):170-176.

Bar-Gal, G., Greenblatt, C., Woodward, S., Broshi, M. and Smith, P. 1999. 'The genetic signature of the Dead Sea Scrolls', In Goodblatt, A., Pinnick, A and Schwartz, D. (eds.) *Historic perspectives: from the Hasmoneans to Bar Kokhba in the light of the Dead Sea Scrolls. Proceeding of 4th International Symposium of the Orion Centre for the Study of the Dead Sea Scrolls and Associated Literature, 27-31 January 1999.* Leiden: Brill, pp. 165-171.

Barrett, J.H., Orton, D. Johnstone, C. *et al.* 2011. 'Interpreting the expansion of sea fishing in medieval Europe using stable isotope analysis of archaeological cod remains', *Journal of Archaeological Science* **38**(7):1516-1524

Basha, W.A., Chamberlain, A.T., Zaki, M.E., Kandeel, W.A., Fares, N.H. 2016. 'Diet reconstruction through stable isotope analysis of ancient mummified soft tissues from Kulubnarti (Sudanese Nubia)', *Journal of Archaeological Science: Reports* **5**(1):71-79.

Bateman, A.S. and Kelly, A.S. 2007. 'Fertiliser nitrogen isotope signatures', *Isotopes in Environmental and Health Studies* **43**(3):237-247.

Bateman, A.S., Kelly, S.D., Jickells, T.D. 2005. 'Nitrogen isotope relationships between crops and fertilises: implications for using nitrogen isotope analysis as an indicator of agricultural regime', *Journal of Agricultural and Food Chemistry* **53**:5760-5765.

Bates, P. 2012. *External parasites of small ruminants: A practical guide to their prevention and control.* Oxford: CAB International.

Beeton, I. 1861. *Mrs Beeton's Cookery Book.* London: S.O. Beeton Publishing.

Bergamo, T.F., Botta, S. and Copertino, M. 2016 'Lipid extraction in stable isotope analyses of juvenile sea turtle skin and muscle', *Aquatic Biology* **25**:1-6.

Bieńkiewicz, J.K. 1983. *Physical chemistry of leather making.* Malabar, FL: Krieger.

Billingsley, J. 1795. *General view of agriculture of the Country of Somerset, with observations on the means of its improvement. Drawn up for the consideration of the Board of Agriculture and Internal Improvement.* Bath: R. Cruttwell.

Bingley, W. 1809. *Memoirs of British quadrupeds, illustrative principally of their habits of life, instincts, sagacity, and use to mankind. Arranged according to the system of Linnaeus.* London: Darton & Harvey.

Black, M. 1984. *Cambridge University press, 1584-1984.* Cambridge: Cambridge University Press.

Blackman, J. 1975. 'The cattle trade and agrarian change on the eve of the railway age', *The Agricultural History Review* **23**(1):48062.

Bleasdale, M. 2016. *Stable isotope analysis of 19th century human and faunal remains from Queens Chapel of the Savoy London.* MSc Thesis, University of York.

Blith, W. 1649. *The English Improver Improved.* London: John Wright.

Blomley, N. 2007. 'Marking private property: Enclosure, common right and the work of hedges', *Rural History* **18**(1):1-21.

Bocherens, H. and Drucker, D. 2003.'Trophic level isotope enrichment of carbon and nitrogen in bone collagen: case studies from recent and ancient terrestrial ecosystems', *International Journal of Osteoarchaeology* **13**:46-51.

Bogaard A., Heaton, T.H.E., Poulton, P. and Merbach, I. 2007. 'The impact of manuring on nitrogen isotope ratios in cereals: archaeological implications for reconstruction of diet and crop management practices', *Journal of Archaeological Science* **34**(3):335-343.

Bogaard, A., Fraser, R., Heaton, T.H. *et al.* 2013. 'Crop manuring and intensive land management by Europe's first farmers', *Proceeding of the National Academy of Sciences* **110**(31):12589-12594.

Bogart, D. 2005. 'Turnpike trusts and the transportation revolution in 18th century England', *Explorations in Economic History* **42**: 479-508.

Bogart, D. 2013. 'The turnpike roads of England and Wales'. Report for: Cambridge Group of the History of Population and Social Structure.

Bond, J.M. and O'Connor, T.P. 1999. *Bones from Medieval deposits at 16022 Coppergate and other sites in York.* The Archaeology of York 15/5.

Boner, M. and Förstel, H. 'Stable isotope variation as a tool to trace the authenticity of beef', *Annals of Bioanalytical Chemistry* **378**: 301-310

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

Bowden, P.J. 1962. *The wool trade in Tudor and Stuart England.* London: Routledge.

Bower, M.A., Campana, M.G., Checkley-Scott, C., Knight, B., Howe, C.J. 2010. 'The potential for extraction and exploitation of DNA from parchment: a review of the opportunities and hurdles', *Journal of the American Institute of Conservation* **33**:1-11

Bowes, J.H. and Elliot, R.G. 1957. 'The composition of the epidermis', *Journal of the Society of Leather Trades' Chemists* **41**(2):87-93.

Bowly, T. 2012. 'Herring of Sligo and Salmon of Bann': Bristol's maritime trade with Ireland in the Fifteenth Century', In Gorski, R. (ed.) *Roles of the Sea in Medieval England.* Woodbridge: The Boydell Press.

Bradley, R. 1727 (ed.) *Husbandry and trade improv'd.* Houghton, J. 1694. *Friday 7th 1694. Num. CXXIII.* London: Wooman and Lyon, pp. 323-330.

Brassley, P. 2000. 'Farming techniques I: Animal nutrition', In Collins, E.J.T. (ed.) *The Agrarian History of England and Wales Vol.7: 1850-1914.* Cambridge: Cambridge University Press.

Bridger, G. 2013. *Britain and the making of Argentina.* Southampton: WIT Press.

Briggs, P.S. 1981. *Gloving, clothing and special leather.* London: Tropical Products Institute

Britton, K. 2017. 'A stable relationship: isotopes and bioarchaeology are in it for the long haul', *Antiquity* **91**(358):853-864.

Broadberry, S., Campbell, B.M.S., Klein, A., Overton, M., van Leeuwen, B. 2015. *British economic growth, 1270-1870.* Cambridge: Cambridge University Press.

Brock, F. 2013. 'Radiocarbon dating of historical parchments', *Radiocarbon* **55**(2):353-363

Brodal, P. 2010. *The central nervous system.* Oxford: Oxford University Press.

Broderick, L. 2014. 'Commercial zooarchaeology of the 'modern' era: a survey of attitudes and practices', *Anthropozoologica* **49**(1):19-32.

Brown, D.J., Wolcott, M.L. and Crook, B.J. 2010 'The measurement of skin thickness in Merino sheep using real time ultrasound', *Wool Technology and Sheep* **48**(4): 1-12.

Brown, M. 2014. *North & South: the isotopic analysis of four body tissues assessed alongside historical sources to reconstruct a comparative palaeodietary analysis of two post-medieval sites.* MSc Thesis, University of York

Brown, M.P. 2003. *The Lindisfarne Gospels: society, spirituality and the scribe.* London: The British Library.

Brown, R. 1793. *General view of agriculture of the West Riding of Yorkshire. Drawn up for the consideration of the Board of Agriculture and Internal Improvement.* Edinburgh: James Watson.

Browning, N.E, Dold, C. I-Fan, J. and Worthy, G.A. 2014. 'Isotope turnover rates and diet-tissue discrimination in skin of ex situ bottlenose dolphins *(Tursiops truncates)*', Journal of Experimental Biology **217**(2):214-221.

Buckley, M., Collins, M.J., Thomas-Oates, J., Wilson, J.C. 2009. 'Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry', *Rapid Communications in Mass Spectrometry* **23**(23): 3843-3854.

Buckley, M., Whitcher Kansa, S., Howard, S., Campbell, S., Thomas-Oates, J., Collins, M.J. 2010. 'Distinguishing between archaeological sheep and goat bones using a single collagen peptide', *Journal of Archaeological Science* **37**:13-20.

Burger, J., Hummel, S. and Hermann, B. 2000. 'Paleogenetics and cultural heritage. Species determination and STR-genotyping from ancient DNA in art and artefacts', *Thermochimica Acta* **365**:141-146.

Burger, J., Pfeiffer, I., Hummel, S., Fuchs, R., Brening, B. and Hermann, B. 2001. 'Mitochondrial and nuclear DNA from (pre) historic hide-derived material', *Ancient Biomolecules* **3**:227-238.

Bush, H. 1824. *Bristol town duties: a collection of original and interesting documents.* Bristol.

Bush, M. 1996. *The Pilgrimage of Grace: A study of the rebel armies of October 1536.* Manchester: Manchester University Press.

Camin, F., Bontempo, K. Heinrich, K. *et al.* 2007. 'Multi-element (H,C,N,S) stable isotope characteristics of lamb meat from different European regions', *Annals of Bioanalytical Chemistry* **389**:309-320.

Camin, F., Bontempo, K., Perini, M. and Piasentier, E. 2016. 'Stable isotope ratio analysis for assessing the authenticity of food of animal origin', *Comprehensive Reviews in Food Science and Food Safety* **93**(3):1-10.

Campagnolo, A., Giacometti, A., MacDonald, L., Mahony, S., Terras, M. and Gibson, A. 2016. Cultural heritage destruction: experiments with parchment and multispectral imaging, In Bodard, G. and Romanello (eds.) *Digital classics outside the echo-chamber: teaching, knowledge exchange & public engagement.* London: Ubiquity Press, pp. 121-146.

Campana, M.G., Bower, M.A., Bailey, M.J., Stock, F., O'Connell, T.C., Edwards, C.J., Checkley-Scott, C., Knight, B., Spencer, M. and Howe, C.J. 2010. 'A flock of sheep, goats and cattle: ancient DNA analysis reveals complexities of historic parchment manufacture', *Journal of Archaeological Science* **37**:1317-1325.

Campbell, A.W., Bain, W.E., McRae, A.E., et al. 2004 'Bone density in sheep: genetic variation and quantitative trait loci localisation', *Bone* **33**(4):540-548.

Campbell, B.M.S. 1983. 'Agricultural progress in medieval England: some evidence from eastern Norfolk', *The Economic History Review* **36**(1):26-46.

Campbell, B.M.S. 2006. *English Seigniorial Agriculture, 1250-1450.* Cambridge: Cambridge University Press.

Campbell, B.M.S. 2008. 'England: land and people', In Rigby, S.H. (ed.) *A companion to Britain in the later middle ages.* London: John Wiley & Sons.

Carrott, J., Dobney, K., Irving, B. *et al.* 1995. *Assessment of biological remains and sediments from excavations at the Magistrates' Court site, Hull (HMC94).* EAU Report 95/17.

Carus-Wilson, E.M. and Coleman, O. 1963. *England's export trade, 1275-1547.* Oxford: Clarendon Press.

Castellanos, J.Z. and Pratt, P.F. 1981. 'Mineralisation of manure nitrogen – correlation with laboratory indexes', *Soil Science Society of America Journal* **45**:354-357.

Chalmber, G. 1782. *The propriety of allowing a qualified exploration of wool discussed historically.* London: BT Batsford.

Chambers, J.D. and Mingay, G.E. 1996. *The agricultural revolution, 1750-1850.* London: B.T. Batsford.

Chance, E., Colvin, C., Cooper, J., Day, C., Hassall, T., Jessup, M. and Selwyn, N. 1979. 'Early Modern Oxford', In, Crossley, A. and Elrington, C.R. (eds.) *A History of the County of Oxford Vol 4: The City of Oxford.* London: Victoria County History.

Charlton, S., Alexander, M., Collins, M.J., Milner, N. *et al.* 2016. 'Finding Britain's last hunter-gatherers: a new biomolecular approach to 'unidentifiable' bone fragments utilising bone collagen', *Journal of Archaeological Science* **73**:55-61.

Chikaraishi, Y., Kashiyama, Y., Ogawa, N.O., Kitazato, H. and Ohkouchi, N. 2007. 'Metabolic control of nitrogen isotope composition of amino acids in macroalgae and gastropods: implications for aquatic food web studies', *Marine Ecology Progress Series* **342**(1):85-89.

Chikaraishi, Y., Ogawa, N.O., Kashiyama, Y. and Takano, Y. 2009. 'Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids', *Limnology and Oceanography Methods* **7**:740-50.

Choi, W.J., Lee, S.M. Ro, H.M., Kim, K.C. and Yoo, S.H. 2002. 'Natural ^{15}N abundance of maize and soil amended with urea and composted pig manure', *Plant and Soil* **245**: 223-232.

Choi, W.J., Ro, H.M. and Lee, S.M. 2003. 'Natural ^{15}N abundances of inorganic nitrogen in soil treated with fertilisers and compost under changing soil moisture regimes', *Soil Biology and Biochemistry* **35**:1289-1298.

Clark, G. 1991. 'Labour productivity in English agriculture, 1300-1860', In Campbell, B. and Overton, M. (eds.) *Land, labour and livestock: historical studies in European agricultural productivity.* Manchester: Manchester University Press.

Clark, G. 1993. 'Agriculture and the industrial revolution, 1700-1850', In Moykr, J. (ed.) *The British industrial revolution: an economic assessment.* Boulder, CO: Westview Press, pp.227-266.

Clark, G. 2004. 'The price history of English agriculture, 1209-1914', *Research in Economic History* **22**: 41-123.

Clark, G. 2007.'The long march of history: farm wages, population and economic growth, England, 1209-1869', *The Economic History Review* **60**(1):97-135.

Clark, J. 1986. *Influence of diet on wool and leather characteristics of ewe and wether Southdown cross Romney lambs*. Palmerston North, NZ: New Zealand Leather and Shoe Research Association.

Clark, R.T. 1934. 'Studies on the physiology of reproduction in the sheep: I. The ovulation rate of the ewe effected by the plan of nutrition', *The Anatomical Record* **60**(2):125-134.

Clarkson, C. 1992. 'Rediscovering parchment: the nature of the beast', *The Paper Conservator* **16:** 5-26.

Clarkson, L.A. 1960. *The English leather industry in the sixteenth and seventeenth centuries (1563-1700).* PhD Thesis, University of Nottingham.

Clarkson, L.A. 1965. 'The leather crafts in Tudor and Stuart England', *British Agricultural History Review* **38**:25-37

Clarkson, L.A. 1966. 'The leather crafts in Tudor and Stuart England', *The Agricultural History Review* **14**(1): 25-39.

Clarkson, L.A. 1974. 'The English Bark Trade, 1660-1830', *Agricultural History Review* **22**: 136-152.

Clarkson, L.A. 1989. 'The manufacture of leather', In Mingay, G.E. (ed.) *The agrarian history of England and Wales, Vol VI, 1750-1850.* Cambridge: Cambridge University Press.

Clutton-Brock, T., Pemberton, J., Coulson, T., Stevenson, I. MacColl, A. 2004. The sheep of St Kilda, In Clutton-Brock, T.H. and Pemberton, J.M. (eds.) *Soay sheep: dynamics and selection in an island population.* Cambridge: Cambridge University Press, pp. 17-36.

Coates, G. 1822. *Coates' herd book: containing the pedigrees of improved short-horned cattle.* Vol. I. London: H. Strafford.

Codron, D., Sponheimer, M., Codron, J., Newton, I., Lanham, J.L. and Clauss, M. 2012. 'The confounding effects of source isotopic heterogeneity on consumer-diet and tissue-tissue stable isotope relationships', *Oecologia* **169**(4):939-953.

Colleter, R., Clavel, B., Pietrzak, A., Ducheseme, S., Schmidt, L., Richards, M.P., Telemon, N., Crubezy, E. and Jaouen, K. 2017. 'Social status in late medieval and early modern Brittany: insights from stable isotope analysis', *Archaeological and Anthropological Science* **10**:1-15.

Collins, E.J.T. 1995. 'Did mid-Victorian agriculture fail? Output, productivity and technological change in 19th century farming', *ReFRESH: Recent findings of research into economic and social history* **21**:1-4.

Collins, M.J., Nielsen-Marsh, C.M., Hiller, J., Smith, C.I., Roberts, J.P., Prigodich, R.V., Wess, T.J., Csapo, J., Millard, A.R. and Turner-Walker, G. 2002. 'The survival of organic matter in bone: a review', *Archaeometry* **44**(3):383-394.

Collins, M.J., Waite, E.R. and van Duin, A.C.T. 1999. 'Predicting protein decomposition: the case of aspartic acid racemisation kinetics', *Philosophical Transactions of the Royal Society of London. Series B – Biological Sciences* **354**: 51-64.

Colonese, A.C., Farrell, T., Lucquin, A., Firth, D., Charlton, S., Robson, H.K., Alexander, M.M. and Craig, O.E. 2015. 'Archaeological bone lipids as palaeodietary markers', *Rapid Communications in Mass Spectrometry* **29**(7):6111-618.

Commisso, R.G. and Nelson, D.E. 2006. 'Modern plant δ^{15}N values reflect ancient human activity', *Journal of Archaeological Science* **33**(8): 1167-1176.

Commisso, R.G. and Nelson, D.E. 2008 'Correlation between modern plant δ^{15}N values and activity areas of medieval Norse farms', *Journal of Archaeological Science* **35**(2):492-504.

Connell, B., Davis, S.J.M. and Locker, A. 1997. *Animal Bones from Camber Castle, East Sussex, 1963-83.* AML Report 107/97.

Cooper, A. and Green, C. 2017. 'Big Questions for Large Complex Dataset: approaching time and space using composite object assemblages', *Internet Archaeology* **45**

Cooper, J. 2011. *Stable isotope investigations of sheep mandibles from Swinegate, York.* BSc Dissertation, University of York.

Copus, A.K. 1989. 'Changing markets and the development of sheep breeds in southern England, 1750-1900', *Agricultural History Review* **37**(1):36-51.

Corr, L.T, Richards, M.P., Grier, C., Mackie, A., Beattie, O. and Evershed, R.P. 2009. 'Probing dietary change of the Kwädąy Dän Ts'ìnchį individual, an ancient glacier body from British

Columbia: II. Deconvoluting whole skin and bone collagen δ13C values via carbon isotope analysis of individual amino acids', *Journal of Archaeological Science* **36**(1):12-18.

Cousins, R. 2017. *Parchment and glove making in Havant.* The Spring Arts & Heritage Centre, Havant.

Covington, A.D. 2009. *Tanning chemistry: the science of leather.* London: Royal Society of Chemistry.

Covington, A.D. and Alexander, K.T.W. 1993. 'The production of soft leather Part I. The mechanism of chemical softening by lubrication', *Journal of the American Leather Chemists Association* **88**:241-253.

Craik, H. (ed.) *English Prose. Vol. I. Fourteenth to Sixteenth Century.* London: Bartleby.

Crouzet, F. 1985. *Britain Ascendant: Comparative Studies in Franco-British Economic History*. Cambridge: Cambridge University Press.

Culley, G. 1788. 'Reply to queries III, In Young, A. *Annals of agriculture and other useful arts.* Vol. XIV. Bury St Edmunds: J. Rackham, pp. 470-5.

Curtis-Summers, S., Montgomery, J. and Carver, M. 2014. 'Stable isotope evidence for dietary contrast between pictish and medieval populations at Portmahomack, Scotland', *Medieval Archaeology* **58**:21-43.

D'Arcy, J.B. 1990. *Sheep management and wool technology.* Kensington, NSW: New South Wales University Press.

Da Rold, O. 2011. 'Materials', In Gillespie, A. and Wakelin, D. (eds.) *The production of books in England, c.1350-1530.* Cambridge: Cambridge University Press, pp. 12-33

Dagnall, H. 1994. *Creating a good impression: three hundred years of The Stamp Office and stamp duties.* London: Her Majesties Stationery Office.

Daly, G. 2007. 'English smugglers, the Channel, and the Napoleonic Wars, 1800-1814', *Journal of British Studies* **46**(1): 30-46.

Davis, S.J.M. 1981. 'The effect of temperature change and domestication on the body size of late Pleistocene to Holocene mammals of Israel', *Paleobiology* **7**(1):101-114.

Davis, S.J.M. 1991a. *Faunal remains from Closegate I & II, Newcastle, Tyne & Wear.* AML Report 81/91.

Davis, S.J.M. 1991b. *Faunal remains from the late Saxon – Medieval farmstead at Eckweek in Avon, 1988-1989 excavations.* AML Report 35/91.

Davis, S.J.M. 1996. 'Measurements of a group of adult female Shetland sheep skeletons from a single flock: a baseline for zooarchaeologists', *Journal of Archaeological Science* **23**(4):593-612.

Davis, S.J.M. 1997. 'The agricultural revolution in England: some zooarchaeological evidence', *Anthropozoologia* **25**/**26**:413-428.

Davis, S.J.M. 2000. 'The effect of castration and age on the development of the Shetland sheep skeleton and metric comparison between bones of males, females and castrates', *Journal of Archaeological Science* **27**(5):373-390.

Davis, S.J.M. 2008.'Zooarchaeological evidence for Moslem and Christian improvements of sheep and cattle in Portugal', *Journal of Archaeological Science* **35**:991-1010.

Davis, S.J.M. and Beckett, J.V. 1999. 'Animal husbandry and agricultural improvement: the archaeological evidence from animal bones and teeth', *Rural History* **10**(1):1-17.

Dawson, T.E., Mamsbelli, S., Plamboeck, A.H. and Templer, P.H. 2002. 'Stable isotopes in plant ecology', *Annual Review of Ecological Systems* **33**:507-559.

de Hamel, C. 1992. *Medieval craftsmen: scribes and illuminators.* London: British Museum.

de Hamel, C. 2017. *Meetings with remarkable manuscripts.* London: Penguin.

de Hoffmann, E. and Stroobant, V. 2007. *Mass spectrometry: principles and applications* (3rd Edition). New York, NY: Wiley.

de Luca, A., Boisseau, N., Tea, N., Louvet, I, Robins, R., Forham, A., Charles, M and Hankard, R. 2012. 'δ^{15}N and δ^{13}C in hair from newborn infants and their mothers: a cohort study', *Paediatric Research* **71**:598-604

Deane, P.M. 1965. *The first industrial revolution.* Cambridge: Cambridge University Press.

Dempsey, M. 1955. 'Some seasonal variations in lambskins', *Journal of the Royal Microscopical Society* **75**(2): 103-110.

DeNiro, M.J. 1985. 'Post-mortem preservation and alteration of *in vivo* bone collagen isotope ratios in relation to palaeodietary reconstruction', *Nature* **317**:806-809.

DeNiro, M.J. and Epstein, S. 1978. 'Influence of diet on the distribution of carbon isotopes in animals', *Geochimica et Cosmochimica Acta* **42**(5):495-506.

DeNiro, M.J. and Epstein, S. 1981. 'Influence of diet on the distribution of nitrogen isotopes in animals', *Geochimica et Cosmochimica Acta* **45**(3):341-351.

Dent, B., Forbes, S., Stuart, B. 2004. 'Review of human decomposition processes in soil', *Environmental Geology'* **45**:576-585.

Diderot, D. and d'Alembert, J.B. 1776. *Encyclopédie, ou dictionnaire raisonné des sciences, des arts et des métiers.*

Dobberstein, R.C., Collins, M.J., Craig, O.E., Taylor, G., Penkman, K. and Ritz-Timme, S. 2009. 'Archaeological collagen: why worry about collagen diagenesis?', *Archaeological and Anthropological Sciences* **1**(1):31-42.

Dobney, K., Fitter, R., Irving, B., Jaques, D., Johnstone, C., Kenward, H., Milles, A. and Shaw, T. 1994. *Technical report: biological remains from the Medieval moat at Hall Garth, Beverley, North Humberside.* Report 94/60. Environmental Archaeology Unit, York.

Dobney, K., Jaques, D. and Irving, B. 1995. *Of butchers & breeds: report on vertebrate remains from various sites in the City of London.* Lincoln: Lincoln Archaeological Studies **5**.

Donahue, D.J., Olin, J.S., Harbottle, G. 2002. 'Determination of the radiocarbon age of parchment of the Vinland Map', *Radiocarbon* **44**(1):45-52.

Dover, R. and White, N.A. 1991. 'The cell proliferation kinetics of the epidermis', In Goldsmith, L.A. (ed.) *Physiology, biochemistry, and molecular biology of the skin* (2nd Edition). Oxford: Oxford University Press, pp. 239-265.

Dowling, D.F. 1955. 'The thickness of cattle skin', *Australian Journal of Agricultural Research* **6**(5):776-785.

Dowling, D.F. 1964. 'The significance of the thickness of cattle skin', *The Journal of Agricultural Science* **62**(3):307-311.

Driver, J. 1990. 'Faunal remains', In Driver, J., Rady, J. and Sparks, M. *Excavations in the cathedral precincts, 2 Linacre Gardens, 'meister ormers' and St. Gabriel's Chapel. The Archaeology of Canterbury Vol. IV.* Maidstone: Kent Archaeological Society, pp.228-257.

Dumville, D. 2001. 'English libraries before 1066: use and abuse of manuscript evidence', In Richards, M.P. (ed.) *Anglo-Saxon Manuscripts: Basic Readings.* London: Routledge, pp. 169-200.

Dyer, C. 2004. 'Alternative agriculture: goats in medieval England', In Hoyle, R.W. (ed.) *People, landscape and alternative agriculture: essays for Joan Thirsk.* Agricultural History Review Supplement Series **3**, pp. 20-38

Eghball, B., Wienhold, B.J. Gilley, J.E. and Eigenberg, R.A.2002. 'Mineralisation of manure nutrients', *Journal of Soil and Water Conservation* **57**:470-473.

el-Harake, W.A., Furman, M.A, Cook, B., Sreekumaran, K., Kukowski, J. and Brodsky, I.G. 1998. 'Measurement of dermal collagen synthesis rate in vivo in humans', *American Physiological Society* **274**(4):586-591.

Elliot, K, H., Roth, J.D. and Crook, K. 2017. 'Lipid extraction techniques for stable isotope analysis and ecological assays', *Methods in Molecular Biology* **1609**:1609-1624

Ellman, J. 1831. 'On folding sheep', *The Farmers Magazine* **10**:110.

Ernle, Lord. 1912. *English Farming Past and Present.* Oxford: Oxford University Press.

Ervynck, A., Boudin, M., Brande, T.V.D. and Strydonck, M.V. 2014. 'Dating human remains from the historic period in Belgium: diet changes and the impact of marine and freshwater reservoir effects', *Radiocarbon* **56**(2):779-788.

Evans, J.A., Chenery, C.A., and Montgomery, J. 2012. 'A summary of strontium and oxygen isotope variation in archaeological human tooth enamel excavated from Britain', *Journal of Analytical Atomic Spectrometry* **27**: 754-764.

Feather, J. 1984. 'John Clay of Daventry: the business of an eighteenth- and nineteenth-century stationer', *Studies in Bibliography* **37**:198-209.

FERA Science 2015. Beef Origin Project 2: Improvements of the British beef isotope landscape map (Isoscape) York: FERA; London: DEFRA.

Ferraz, T.P.L., Fiuza, M.C., dos Santos, M.L.A., Pontes de Carvalho, L. and Soares, N.M. 2004. 'Comparing of six methods for the extraction of lipids from serum in terms of effectiveness and protein preservation', *Journal of Biochemical and Biophysical Methods* **58**:187-193.

Fernandes, R., Nadeau, M.J. and Grootes, P.M. 2012. 'Macronutrient-based model for dietary carbon routing in bone collagen and bioapatite', *Archaeological and Anthropological Sciences* **4**(4):291-301.

Fiddyment, S., Holsinger, B., Ruzzier, C., Devine, A., Binois, A., Albarella, U., Fischer, R., Nichols, E., Curtis, A., Cheese, E., Teasdale, M.D., Checkley-Scott, C., Milner, S.J., Rudy, K.M., Johnson, E.J., Vnoucek, J., Garrison, M., McGrory, S., Bradley, D.G., Collins, M.J. 2015. 'Animal origin of 13th century uterine vellum revealed using noninvasive peptide fingerprinting', *Proceedings of the National Academy of Sciences* **112** (49): 15066-15071.

Fiddyment, S., Vnouček, J., Collins, M.J., and Sykes, N. *forthcoming*. 'Biomolecular and visual examinations', In Zachs, B. (ed.) *Lvcas Glos. Cvum A 360^{o}: A 12th Century manuscript from St. Augustine's Abbey Canterbury.*

Finucane, B.C. 2007. 'Mummies, maize and manure: multi-tissue stable isotope analysis of late prehistoric human remains from the Ayacucho Valley, Peru', *Journal of Archaeological Science* **34**(12): 2115-2124.

Fisher, A. and Thomas, R. 2012. 'Isotopic and zooarchaeological investigation of later medieval and post-medieval cattle husbandry at Dudley Castle, West Midlands', *Environmental Archaeology* **17**(2): 151-167.

Fitzherbert, A. 1534. *Boke of husbandry.*

Flores, P., Fenoli, J. and Helin, P. 2007. 'The feasibility of using $\delta^{13}C$ and $\delta^{15}N$ values for discrimination between conventionally and organically fertilised pepper (*Capsicum annum* L.)', *Journal of Agricultural Food Chemistry* **55**:5740-5745.

Fogel, M.L., Tuross, N. and Owsley, D 1989. 'Nitrogen isotope tracers of human lactation in modern and archaeological populations', *Carnegie Institute Year Book* **88**: 111-117.

Fothergill, B.T., Thomas, R.M. and Morris, J. 2012. 'Avian tibial dyschondroplasia in 19th century turkey (*Meleagris gallopavo* L. 1758) remains from the Royal London Hospital', *International Journal of Palaeopathology* **2**(4):240-45.

Fox, J. 1997. *Tanning barn to church: the dissenting congregation of Wheatley over two hundred years.* Wheatley United Reform Church.

Fraser, R., Bogaard, A., Heaton T.H.E. *et al.* 2011. 'Manuring and stable nitrogen isotope ratios in cereal and pulses: towards a new archaeobotanical approach to the inference of land and dietary practices', *Journal of Archaeological Science* **38**(10):2790-2804.

Friedli, H., Lotscher, H., Oeschger, H., Siegenthaler, U. and Stauffer, B. 1986. 'Ice core record of the $^{13}C/^{12}C$ ratio of atmospheric CO_2 in the past two centuries;, *Nature* **324**:237-238.

Fry, B. 2006. *Stable isotope ecology.* New York, NY: Springer.

Fuchs, E. 1983. 'Evolution and complexity of the genes encoding the keratins of human epidermal cells', *Journal of Investigative Dermatology* **81**:141-145.

Fuller, B.T., Fuller, J.L., Harris, D.A. and Hedges, R.E. 2006. 'Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope ratios', *American Journal of Physical Anthropology* **129**(2): 279-293.

Fussell, G.E. and Goodman, C. 1930. 'Eighteenth century estimates of British sheep and wool production', *Agricultural History* **4**(4): 131-151.

Gerber, G., Gerber, G. and Altman, K.I. 1960. 'Studies of the metabolism of tissue proteins', *The Journal of Biological Chemistry* **233**(9):2653-2656.

Ghioni, C., Hiller, J.C., Kennedy, C.J., Aliev, Odlyha, M., Boulton, M. and Wess, T.J. 2005. *Journal of Lipid Research* **46**(12):2726-2734.

Giacometti, A., Campagnolo, A. MacDonald, L., Mahony, S., Robson, S., Weyrich, T., Terras, M. and Gibson, A. 'The value of critical destruction: Evaluating multispectral image processing methods for the analysis of primary historical texts', *Digital Scholarship in the Humanities* **32**(1): 101-122.

Gidney, L.J. 1989. 'The mammal and bird bones from the 1986 excavation at Crown Court, Newcastle-upon-Tyne, Tyne & Wear. Parts I & II.

Gilchrist, R. 1996. 'The animal bones', In Foreman, M. *Further excavations at the Dominican Priory, Beverley, 1986-89.* Sheffield Excavation Reports **4**. Sheffield: Sheffield Academic Press, pp.213-225.

Gizaw, S., Tegegne, A., Gebremedhin, B. and Hoekstra, D. 2010. *Sheep and goat production and marketing systems in Ethiopia: Characteristics and strategies for improvement.* Addis Ababa, Ethiopia: International Livestock Research Institute.

González-Martin, I., González-Martin, C., Hernandez-Mendez, J., Marques-Macias, E. and Poveda, F.S. 1999. 'Use of isotope analysis to characterise meat from Iberian-bread swine', *Meat Science* **52**:437-441.

Gordon, R. 2015. *Feeding the city: zooarchaeological perspectives on urban provisioning and consumption behaviours in post-medieval England (AD 1500-2900).* PhD Thesis, University of Leicester.

Gordon, R., Thomas, R. and Foster, A. 2015. 'The health impact of selective breeding in poultry: a probable case of 'creeper' chicken *(Gallus gallus)* from 16th century Chester, England', *International Journal of Paleopathology* **9**:1-17.

Goude, G. and Fontugne, M. 2017. 'Carbon and nitrogen isotopic variability in bone collagen during the Neolithic period: Influences of environmental factors and diet', *Journal of Archaeological Science* **70**: 117-131.

Gransden, A. 2013. *Historical Writing in England: 550-1307 and 1307 to the early-16th century.* London: Routledge.

Great Britain Census Office. 1854. *The Census of Great Britain on 1851.* London: Longman, Brown, Green and Longmans.

Grigg, D.B. 1980. *Population growth and agrarian change.* Cambridge: Cambridge University Press.

Guintard, C. and Lallemand, M. 2003. 'Osteometric study of metapodial bones in sheep (*Ovis aries*, L. 1758), *Annals of Anatomy* **185**:573-583.

Guiry, E., Harpley, B., Jones, Z. and Smith, C. 2014. 'Integrating stable isotope and zooarchaeological analyses in historical archaeology: a case study from the urban 19th century commonwealth block site, Melbourne, Australia', *International Journal of Historical Archaeology* **18**(3):4150440.

Guiry, E., Noel, S. and Tourigny, E.J. 2012. 'Stable isotope bone chemistry and human/animal interactions in historical archaeology', *Northeast Historical Archaeology* **41**:126-143.

Guiry, E., Szpak, P. and Richards, M.P. 2017. 'Isotopic analyses reveal geographical and socioeconomic patterns in historical domestic animal trade between predominantly wheat- and maize-growing agricultural regions in eastern North America', *American Antiquity* **82**(2):341-352.

Guiry, E.J., Szpak, P. and Richards, M.P. 2016. 'Effect of lipid extraction and ultrafiltration on stable carbon nitrogen isotopic compositions of fish bone collagen', *Rapid Communications in Mass Spectrometry* **30**: 1591-1600

Gullick, M. 1991. 'From Parchmenter to Scribe: Some observations on the manufacture and preparation of medieval parchment based on a review of the literary evidence', In Rück, P. (ed.) *Pergement. Geschichte, Struktur, Restaurierung, Herstellung.* Sigmaringen, Germany, pp. 143-157.

Hagadorn, A. 2012. 'Parchment making in eighteenth-century France: historical practices and the written record', *Journal of the Institute of Conservation* **35**(2):165-188.

Haines, B.M. 1999. *Parchment: the physical and chemical characteristics of parchment and the materials used in its conservation.* Northampton: Leather Conservation Centre.

Hall, D. 2014. *The open fields of England.* Oxford: Oxford University Press.

Hallam, H. 1826. 'Board of Stamps: Letter from Henry Hallam, Esq.', In *Accounts and Papers, Four Volumes. (1) Relating to Assessed Taxes; Stamps; Rates of Duties; Beer, Hops, and Malt; Spirits; The Distillers.* Vol XVII, pp. 3-13.

Halprin, K.M. 1972. 'Epidermal 'turnover time' – a re-examination', *The British Journal of Dermatology* **86**(1): 14-19.

Hammond, J. 1932. *Growth and development of mutton qualities in the sheep.* Biological Monographs and Manuals. London: Oliver and Boyd.

Handle, I., Watt, K.A., Pilkington, J.G., Pemberton, J.M., Macrae, A., Scott, P., McNeilly, T.N., Berry, J.L., Clements, D.N., Nussey, D.H., Mellanby, R.J. 2016. 'Vitamin D status predicts reproductive fitness in a wild sheep population', *Scientific Reports* **6(**18986): 1-11.

Hare, E.P., Fogel, M.L., Stafford, T.W., Mitchell, A.D. and Hoering, T.C. 1991. 'The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins', *Journal of Archaeological Science* **18**(3):277-292.

Harrison, L. 2015. 'Creating the slum: representations of poverty in Hungate and Walmgate districts of York, 1875-1914', *Ex Historia* **7**:61-89.

Hartlib, S. 1652. *Legacie; or an enlargement of the discourse of husbandry.* London: H. Hills.

Hatch, K.A., Crawford, M.A, Kunz, A., Thomsen, S.R., Eggett, D.L., Nelson, S.T. and Roeder, B.L. 2006. 'An objective means of diagnosing anorexia nervosa and bulimia nervosa using $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios in hair', *Rapid Communications in Mass Spectrometry* **20**:2267-3373.

Havinden, M. 1961. 'Agricultural progress in open-field Oxfordshire', *Agricultural History Review* **9**(2);73-83.

Heaton, K., Kelly, S.D., Hoogeweff, J. and Woolfe, M. 2008. 'Verifying the geographic origin of beef: The application of multi-element isotope and trace element analysis', *Food Chemistry* **107**: 506-515.

Heaton, T.H.E. 1999. 'Spatial, species and temporal variations in the $^{13}C/^{12}C$ rations of C_3 plants: implications for palaeodietary studies', *Journal of Archaeological Science* **26**:637-649.

Heaton, T.H.E., Jones, H.P. and Tsipropoulus, T. 2009. 'Variations in the $^{13}C/^{12}C$ ratios of modern wheat grain, and implication for interpreting data from Bronze Age Assiros Toumba, Greece', *Journal of Archaeological Science* **36**:2224-2233.

Heaton, T.H.E., Vogel, J.C., von le Chevallerie, G. and Collett, G. 1986. 'Climate influence on the isotopic composition of bone nitrogen', *Nature* **322**(28):822-823.

Hedges, R.E.M. and Reynard, L.M. 2007. 'Nitrogen isotopes and the trophic level of humans in archaeology', *Journal of Archaeological Science* **34**:1240-1251.

Hedges, R.E.M. Stevens, R.E. and Koch, R.L. 2006. 'Isotopes in bones and teeth', In Leng, M.J (ed.) *Isotopes in paleoenvironmental research.* Developments in Paleoenvironmental Research **10**. Gordrech: Springer, pp.117-145.

Hedges, R.E.M., Clement, J.G., Thomas, C.D. and O'Connell, T.C. 2007. 'Collagen turnover in the femoral mid-shaft: modelled from anthropogenic radiocarbon tracer measurements', *American Journal of Physical Anthropology* **133**(2):808-816.

Hedges, R.E.M., Law, I.A., Bronk, C.R., Housley, R.A. 1989. 'The Oxford accelerator mass spectrometry facility: technical developments in routine dating', *Archaeometry* **31**(2):99-113.

Hedges, R.E.M., Thompson, J.M.A. and Hull, B.D. 2005. 'Stable isotope variation in wool as a means to establish Turkish carpet provenance', *Rapid Communications in Mass Spectrometry* **19**(22):3187-3191.

Hellevang, H. and Aagaard, P. 2015. 'Constrains on natural global atmospheric CO_2 fluxes from 1860 to 2010 using a simplified explicit forward modern', *Scientific Reports* **5**:1-12.

Henderson, A.E. 1959. 'The relationship between feed and quantity and kind of wool produced', *New Zealand Grassland Association* **18**:68-79

Henderson, E.F. 1912. *Select historical documents of the middle ages: translated and edited by Ernest F. Henderson.* London: Bell & Sons.

Hendy, J., Welker, F., Demarchi, B., Speller, C., Warinner, C. and Collins, M.J. 2018. 'A guide to ancient protein studies', *Nature Ecology & Evolution* **2**(4).

Henrickson, R.L., Ranganayaki, M.D. and Asghar, A. and Bailey, D. 1984. 'Age, species, breed, sex, and nutrition effect on hide collagen', *Critical Reviews in Food Science and Nutrition* **20**(3):159-172.

Henzel, W.J., Wantanabe, C. and Stults, J.T. 2003. 'Protein identification: the origins of peptide mass fingerprinting', *Journal of the American Society for Mass Spectrometry* **14**(9):931-942.

Hill, F. 1974. *Victorian Lincoln.* Cambridge: Cambridge University Press.

Hillson, S. 2005. *Teeth* (2nd Edition). Cambridge: Cambridge University Press.

Hobbie, E.A., Macko, S.A. and Williams, M. 2000. 'Correlations between foliar ^{15}N and nitrogen concentration may indicate plant-mycorrhizal interactions', *Oecologia* **122**:273-283.

Hobson, K.A. 1999. 'Tracing origins and migration of wildlife using stable isotopes: a review', *Oecologia* **120**(3):3140326.

Hobson, K.A. and Wassenaar, L.I. 2008. *Tracking animal migration with stable isotopes.* London: Elsevier.

Hobson, K.A., Alisauskas, R.T. and Clark, R.G. 1993. 'Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet', *Condor* **95**:388-394.

Home, T.H. 1830. *The complete grazier; or farmer's and cattle breeder's and dealer's assistant. Together with an introductory view of the different breeds of neat cattle, sheep, horses and swine: the present state of the wool trade, and the improvement of British wool* (5th Edition). London: Baldwin & Cradock.

Hossner, K.L. 2005. *Hormonal regulations of farm animal growth.* Wallingford: CABI Publishing.

House of Commons. 1837. *Accounts & Papers. Vol. 52 (II). Session 31st January to 17th Jul7 1837.*

Hughes, E. 1941. 'The English stamp duties, 1664-1764', *The English Historical Review* **56**(222): 234-264.

Iacumin, P., Bocherens, H., Chaix, L. and Mariotti, A. 1998. 'Stable carbon and nitrogen isotopes as dietary indicators of ancient Nubian populations (Northern Sudan)', *Journal of Archaeological Science* **25**: 293-301.

Iacumin, P., Bocherens, H., Mariotti, A. and Longinelli, A. 1996. 'An isotopic paleoenvironmental study of human skeletal remains in the Nile Valley', *Paleogeography Paleoclimatology Paleoecology* **126**: 15-30.

IBM 2013. IBM SPSS Statistics for Windows, Version 22.0. IBM Corporation, Armonk, NY, USA.

Jahren, A.H. and Kraft, R.A. 2008. 'Carbon and nitrogen stable isotopes in fast food: signatures of corn and confinement', *Proceedings of the National Academy of Sciences* **105**(46):17855-17860.

Jay, M. 2009. 'Breastfeeding and weaning behaviour in archaeological populations: evidence from the isotopic analysis of skeletal materials', *Childhood in the Past* **2**(1):163-178.

Jefferies, J. 2005. *The UK population: past, present and future.* Focus ON: People and Migration. London: Office for National Statistics.

Jolly, M. and Lyne, A.G. 'The response of skin and wool growth to local subdermal temperature change in a sheep', *The Journal of Agricultural Science* **75**(3): 501-515.

Jones, B.E. 2004. 'Gelatin: manufacture and physico-chemical properties', In Podczeck, F. and Jones, B.E. (eds.) *Pharmaceutical Capsules.* London: Pharmaceutical Press, pp. 23-60.

Jones, E.T. 1998. *The Bristol shipping industry in the 16th Century,* PhD Thesis, University of Bristol.

Jones, R. 2012. *Manure matters: historical, archaeological and ethnographic.* Farnham: Ashgate.

Journal of the House of Commons 1801. Volume 56, 1801-1801. London: H.M. Stationery Office.

Journal of the House of Commons 1803. Volume 19, 1718-1721. London: H.M. Stationery Office.

Juchauld, F., Bonnenberger, P. and Komenda, A. 2010. 'Identification de l'eséce animales des parchemins', In Zerdoun, M., Bar-Yehouda, C. and Bourlet, C. (eds.) *Matériaux du livre médiéval: actes du colloque du groupement de recherce (GDR) 2836, Paris, CNRS, 7-8,* pp. 13-28.

Keeling, C.D., Piper, S.C. Bacastow, R.B., Wahlen, M., Whorf, T.P., Heinmann, M. and Meijer, H.A. 2005. 'Atmospheric CO_2 and $^{13}CO_2$ exchange with the terrestrial biosphere and oceans from 1978 to 2000: observations and carbon cycle implications', In Baldwin, I (ed.) *A history of atmospheric CO_2 and its effect on plants, animals, and ecosystems.* Ecological Studies Vol. 1**17**. New York: Springer, pp.83-113.

Kelly, S. 2010. The British Beef Origin Project (BBOP). UK Food Standards Agency

Kelly's Directory of stationers, printers booksellers, paper makers and parchment makers of England, Scotland & Wales. 1936. London: Kelly & Co.

Kennedy, C.J. and Wess, T.J. 2003. 'The structure of collagen within parchment – a review', *Restaurator* **24**(2): 61-80.

Kerley, S.J. and Jarvis, S.C. 1996. 'Preliminary studies of the impact of excreted N on cycling and uptake of N in pasture systems using natural abundance stable isotopic discrimination', *Plant and Soil* **178**: 287-294.

Kerridge, E. 1967. *The agricultural revolution.* London: Routledge.

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H. and Jones, R.I. 2006. 'A revised model for lipid-normalising $\delta^{13}C$ values from aquatic organisms, implications for isotope mixing models', *Journal of Applied Ecology* **43**(6):1213-1222.

Kirby, D.P., Buckley, M., Promise, E., Trauger, S.A., Holdcraft, T.R. 2013. 'Identification of collagen-based materials in cultural heritage', *Analyst* **138**(17): 4849-4858.

Knight, C. 2014. *London: Volume 5* (Reprint, 1842 Original) Cambridge Library Collection – British and Irish History, 19th Century. Cambridge: Cambridge University Press.

Kobbe, N., Vogel, J., Pritzkow, W., Panne, U., Fry, H., Lochotzke, H.M. and Preiss-Weigert, A. 2006. 'C and N isotope variation in urine and milk of cattle depending on the diet', *Annals of Bioanalytical Chemistry* **386**(1):104-108.

Kohn, M.J. 2010. 'Carbon isotope composition of terrestrial C_3 plants as indicators of (paleo)ecology and (paleo)climate', *Proceedings of the National Academy of Sciences* **107**(46):19661-19695.

Koopmans, C.J., Dam, D., Tietema, A. and Verstraten, J.M. 1997. 'Natural ^{15}N abundance in two nitrogen saturated forest ecosystems', *Oecologia* **111**:470-480.

Koppenhoefer, R.M. 1938. 'The lipid of sheepskin. I. Lipid of fresh sheepskin', *Journal of the American Leather Chemists Association* **33**(4): 203-215.

Koppenhoefer, R.M. 1939. 'The Lipids of Sheep Skins. II. The effect of pullery processes on the lipids of sheep skin', *Journal of the American Leather Chemists Association* **34**(5): 100-112.

Körner, C., Farquhar, G.D. and Wong, S.C. 1988. 'Carbon isotope discrimination by plants follows latitudinal and altitudinal trends', *Oecologia* **88**(1):30-40.

Koster, M.L. 2009. 'Making an epidermis', *Annals of the New York Academy of Sciences* **1170**(1): 7-10.

Kowaleski, M. 1990. 'Town and country in late-medieval England: the hide and leather trade', In Cornfield, P. and Keene, D.J. (eds.) *Work in towns 850-1850.* Leicester, pp.57-73.

Lalande, J.J. 1762. 'Art de Faire le parchemin', Academic Royale Des Sciences Description Des Arts et Metiers, 3. Paris.

Lamb, AL. 2016. 'Stable isotope analysis of soft tissues from mummified human remains', *Environmental Archaeology* **21**(3): 1-14.

Larsen, C.S. *Bioarchaeology: Interpreting behaviour from the human skeleton.* Cambridge Studies in Biology and Evolutionary Anthropology. Cambridge: Cambridge University Press.

Laslett, P. (ed.) *The earliest classics: John Graunt and Gregory King.* New Jersey: Gregg Press.

Laurent, G.J. 1982. 'Rates of collagen synthesis in lung, skin and muscle obtained *in vivo* by a simplified method using [^3H]proline', *Biochemical Journal* **206**:535-544.

Lawrence, J. 1809. *A general treatise on cattle, the ox, the sheep, and the swine: comprehending their breeding, management, improvement and diseases* (2nd Edition). London: Sherwood, Neely & Jones.

Lawson, A. 1825. *The modern farrier; or the art of preserving the health and curing the diseases of horses, dogs, oxen, cows, sheep and swine.* London: Mackenzie & Dent.

Leach, I. 1995. *Hides and skins for the tanning industry.* FAO Agricultural Services Bulletin 123. Food & Agricultural Organisation of the United Nations.

Ledgard, S.F., Freney, J.R. and Simpson, J.R. 1984. 'Variations in natural enrichment of ^{15}N in the profiles of some Australian pasture soils', *Australian Journal of Soil Research* **22**:155-164.

Lee-Thorp, J.A., Sealy, J.C., van der Merwe, N.J. 1989. 'Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet', *Journal of Archaeological Science* **16**(6):585-599.

Leigh, P. 1759. *A copy of the poll for a citizen for the City and liberty of Westminster.* London: J. Osborn.

Lewis, A. 1986. *Plants and Nitrogen.* Institute of Biology's Studies in Biology 1**66**.

Li, S.M., Liu, L., Oddy, V.H. and Nolan, J.V. 2007. 'Protein synthesis rates in the skin components and skeletal muscle of sheep selected for divergent clean fleece weight in response to below- and above-maintenance nutrition', *Australian Journal of Agricultural Research* **58**: 1031-1039.

Li, S.M., Mata, G., O'Donoghue, H. and Masters, 1998. 'The influence of live weight, live weight change and diet on the protein synthesis in the skin and skeletal muscle in young Merino sheep', *British Journal of Nutrition* **79**:267-274.

Liden, K., Takanhashi, C. and Nelson, D.E. 1995. 'The effects of lipids in stable carbon isotope analysis and the effect of NaOH Treatment of the composition of extracted bone collagen', *Journal of Archaeological Science* **22**(2):321-326.

Lim, S.S., Choi, W.J., Kwak, J.H., Jung, J.W. and Chang, S. 2007. 'Nitrogen and carbon isotope responses of Chinese cabbage and chrysanthemum to application of liquid pig manure', *Plant and Soil* **295**:67-77.

Lord, T. 2009. 'A millennium of farming at Lower Winskill', *North Craven Heritage Trust Journal*, 1-4.

Lord, T. 2010. Guide to the Medieval dry stone walled landscape at Winskill in the Yorkshire Dales National Park. XII International Dry Stone Walling Conference, University of Cumbria, September 2010.

Louden, J.C. 1831. *An encyclopaedia of agriculture.* London: Longman, Rees, Orme, Brown & Green.

Lough, S.M. 1916. 'Trade and industry in Ireland in the Sixteenth Century', *Journal of Political Economy* **24**(7):713-730.

Lyall, R.J. 1989. Materials: the paper revolution, In, Griffiths, J. and Pearsall, D. (eds.) *Book Production and Publishing in Britain, 1375-1475.* Cambridge: Cambridge University Press, pp. 11-30.

Lyne, A.G. 1957. 'The development of the epidermis and hair canals in the Merino sheep foetus', *Australian Journal of Biological Science* **10**(2):390-397.

Lyne, A.G. 1961. 'The postnatal development of wool follicles, shedding, and skin thickness in inbred Merino and Southdown-Merino crossbred sheep', *Australian Journal of Biological Science* **14**(1):141-156.

Lyne, A.G. 1964. 'Effect of adverse nutrition on the skin and wool of follicles in Merino sheep', *Australian Journal of Agricultural Research* **15**(5):788-801.

Lyne, A.G. and Hollis, D.E. 1968. 'The skin of sheep: a comparison of body regions', *Australian Journal of Biological Science* **21**(3):499-528.

Lyon, T.D. and Baxter, M.S. 1978. 'Stable carbo isotopes in human tissues', *Nature* **273**:750-751.

Ma, B. and Dwyer, I.M. 1998. 'Nitrogen uptake and use of two contrasting maize hybrids differing in leaf senescence', *Plant and Soil* **1999**:283-291.

Macko, S.A., Fogel-Estep, M.L. and Hare, P.E. 1987. 'Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms' *Chemical Geography (Isotope Geoscience Section)* **65**:79-92.

Macko, S.A., Fogel-Estep, M.L., Engel, M.H. and Hare, P.E. 1986. 'Kinetic fractionation of stable isotopes during amino acid transamination', *Geochimica et Cosmochimica Acta* **50**:2143-2146.

Madgwick, R., Mulville, J. and Stevens, R.E. 2012. 'Diversity in foddering strategy and herd management in late Bronze Age Britain: An isotopic investigation of pigs and other fauna from two midden sites', *Environmental Archaeology* **17**(2): 126-140.

Madhavan, S., Treichel, I. and O'Leary, M.H. 1991. 'Effects of relative humidity on carbon isotope fractionation in plants', *Plant Biology* **104**(4): 292-294.

Makarewicz, C. and Pederzani, S. 2017. 'Oxygen (δ^{18}O) isotope distinction between co-localised wild and domestic goats in the Gobi Desert, Mongolia: Complications to tracking mobility and seasonality in herbivores', *Paleogeography, Paleoclimatology, Paleoecology* **10**:1-15.

Maltby, M. 1979. *The animal bones from Exeter, 1971-1975.* Exeter: Exeter Archaeological Reports Vol. 2.

Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A. and Tardieux, P. 1981. 'Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification process', *Plant and Soil* **63**(3):413-430.

Markham, G. 1631. *The whole art of husbandry.* London.

Markham, G. 1648. *A way to get wealth: by approved rules of practice in good husbandry and huswiferie.* London: Roger Jackson.

Markham, G. 1676. *Farewell to husbandry.* London

Marshall, W. 1790. *The rural economy of the midland counties; including the management of livestock in Leicestershire and its environs, Together with minutes on agriculture and planting in the district of the midland station.* London: C. Nichol.

Mascall, L. 1627. *The government of cattle.* London.

Maxwell, C.A. 2007. *Animal hide processing: impact on collagen structure*. PhD Thesis, Cardiff University.

Maxwell, C.A., Wess, T.J. and Kennedy, C.J. 2006. 'X-ray diffraction study into the effects of liming on the structure of collagen', *Biomacromolecules* **7**(8): 2321-2326.

Maxwell, K. 1995. *Pombal, Paradox of the Enlightenment.* Cambridge: Cambridge University Press.

Mays, P.K., McAnulty, R.J., Campa, J.S. and Laurent, G.J. 1991. 'Age-related changes in collagen synthesis and degradation in rat tissues', *Biochemical Journal* **276**:307-313.

McAnulty, R.J. and Laurent, G.J. 1987. 'Collagen synthesis and degradation In Vivo. Evidence for rapid rates of collagen turnover with extensive degradation of newly synthesized collagen in tissues of the adult rat', *Collagen and Related Research* **7**:93-104.

McClelland, J.W. and Montoya, J.P. 2002. 'Trophic relationships and the nitrogen isotopic composition of amino acids in plankton', *Ecology* **83**(3):2173-2180.

McCulloch, J.R. 1847. *A descriptive and statistical accounts of the British Empire. Exhibiting its extent, physical capabilities, population, industry and civil and religious institutions.* Vol. II. London: Longman, Brown, Green & Longmans.

McLaughlin, G.D. and Theis, E.R. 1945. *The Chemistry of Leather Manufacture.* New York, N.Y: Reinhold Publishing Corporation.

McMahon, K.W., Polito, M.J., Abel, S., McCarthy, M.D. and Thorrid, S.R. 2015. 'Carbon and nitrogen isotope fractionation of amino acids in an avian marine predator, the Gentoo penguin *(Pygoscelis papua)', Ecology and Evolution* **5**(6):1278-1290.

Medeiros, L., da Silveira-Monteiro, D., Petitet, R. and Bugoni, L. 2015. 'Effect of lipid extraction on the isotope values of sea turtle bone collage', *Aquatic Biology* **23**:191-199.

Mekki, I., Camin, F., Perini, M., Smeti, M., Hajji, H., Mahouachi, M., Piasentier, E. and Atti, N. 2016. 'Differentiating the geographical origin of Tunisian indigenous lamb using stable isotope ratio and fatty acid content', *Journal of Food Composition and Analysis* **53**(1): 40-48.

Menderes, O. 2002. *Fundamental aspects of the chrome tanning reaction.* PhD Thesis, University of Leicester.

Menderes, O., Covington, A.D., Waite, E.R., and Collins, M.J. 1999. 'The mechanisms and effect of collagen amide group hydrolysis during liming', *Journal of the Society of Leather Technology Chemists* **83**(2):107-110.

Metcalf, R.S. 2012. *Meat, Commerce and the City: The London Food Market, 1800-1855.* London: Routledge.

Michalski, G., Kolanowski, M. and Riha, K.M. 2015. 'Oxygen and nitrogen composition of nitrate in commercial fertilisers, nitric acid, and reagent salts', *Isotopes in Environmental and Health Studies* **51**(3):382-391.

Michel, A. 2014. 'Skin deep: An outline of the structure of different skins and how it influences behaviour in use. A practitioners guide', In Harris, S. and Veldmeijer, A.J. (eds.) *Why Leather? The Material and Cultural Dimensions of Leather*. Leiden: Sidestone Press, pp. 23-40.

Millard, A., Blesch, J., Scollan, M., Rex, J., Ulewicz, K., Grocke, D. 2015. *Isotopic studies: Appendix: Carbon and nitrogen isotopes in animals from Durham City.* Palace Green Library Excavations 2013 (PGL13).

Millington, P.F. and Wilkinson, R. 1983. *Skin.* Cambridge: Cambridge University Press.

Mingay, G.E. 1997. *Parliamentary Enclosure in England.* London: Routledge.

Mitchell, B.R. 1971. *Abstract of British historical statistics.* University of Cambridge, Department of Applied Economic Monograph **17**. Cambridge: Cambridge University Press.

Miura, K. and Goto, A.S. 2012. 'Stable nitrogen isotopic fractionation associated with transamination of glutamic acid to aspartic acid: implications for understanding ^{15}N enrichment in ecological food webs', *Organic Geochemistry* **28**:13-17.

Montagna, W. and Parakkal, P.F. 2012. *The structure and function of skin* (3rd Edition). London: Elsevier.

Moore, D.R., Phillips, S.M., Babraj, J.A., Smith, K. and Rennie, M.J. 2005. 'Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions', *American Journal of Endocrinology and Metabolism* **288**:1153-1159.

Morris, J. 2014. 'Explorations in anatomy: the remains from the Royal London hospital', *Anthropozoologica* **49**(1):109-120.

Mortimer, T. 1810. *A General Dictionary of Commerce, Trade, and Manufactures; Exhibiting Their Present State in every Part of The World; And carefully compiled from the Latest and Best Authorities.* London: Richard Phillips.

Mosbacher, J.B., Michelsen, Stelvig, M., Hendrichsen, D.K., and Schmidt, N. 2016. 'Show me your rump hair and I will tell you what you ate – the dietary history of Muskoxen *(Ovibos moschatus)* revealed by sequential stable isotope analysis of guard hairs', *PLoS One* **11**(4).

Moykr, J. 1985. *The economics of the industrial revolution.* Lanham, MA: Rowman & Littlefield.

Možir, A., Cigić, I. L., Marinšek, M. and Strlič, M. 2014. 'Material properties of historic parchment: A reference collection system', *Studies in Conservation* **59**(3):136-149.

Müldner, G. and Richards, M.P. 2005. 'Fast or feast: reconstructing diet in later medieval England by stable isotope analysis', *Journal of Archaeological Science* **32**(2):39-48.

Müldner, G. and Richards, M.P. 2007. 'Stable isotope evidence for 1500 years of human diet at the city of York, UK', *American Journal of Physical Anthropology* **133**(1):682-697.

Mulville, J., Madgwick, R., Stevens, R., O'Connell, T., Craig, O., Powell, A., Sharples, N. and Parker-Pearson, M. 2009. 'Isotope analysis of faunal material from South Uist, Western Isles, Scotland', *Journal of the North Atlantic* **2**(1):51-59.

National Academy of Science (NAS) 1975. *Nutrient Requirements of Sheep* (5th Edition). Board of Agriculture and Renewable Resources, Committee on Animal Nutrition, Subcommittee on Sheep Nutrition. Washington, DC: National Academy of Sciences.

National Academy of Sciences. 1975. *Nutrient Requirements of Sheep* (5th Edition). Washington, DC: National Academy of Sciences.

National Archives 2015. *Deaccessioning and disposal: guidance for archive services.* February 2015.

Nesbit, J.C. 1852. *On Peruvian guano; its history, composition and fertilising qualities; with the best mode of its application on the soil.* London: Longman & Co.

NIIR Board of Consultants & Engineers 2011. *Leather processing & tanning technology handbook.* New Delhi, India: NIIR Projects,

Noddle, B. 1978. 'Some minor skeletal differences in sheep', In Brothwell, D.R., Thomas, K.D. and Clutton-Brock, J. (eds.) *Research problems in zooarchaeology*. UCL Institute of Archaeology Publications, Vol. 20. Occasional Publication No, **3**, pp.133-141.

Noddle, B. 1986. 'Mammalian bone report', In Bateman, J. and Redknap, M. *Coventry: excavations on the town wall 1976-78.* Coventry: Coventry County Council, pp. 100-119.

Noddle, B. 2002. 'The mammal bones', In Thomas, A. and Boucher, A. *Hereford City Excavations Vol. 4: 1976-1990 – Further Sites and Evolving Interpretations.* Hereford County Archaeological Trust, pp. 103-114.

Norris, A.L. 2009. *Age as a function in inter-tissue spacing of stable carbon isotope values in juvenile human remains from the Dakhleh Oasis, Egypt.* MA Thesis, University of Central Florida.

North, D.C. 1981. *Structure and change in economic history.* London: W.W. Norton & Co.

O'Connell, T.C., Hedges, R.E., Healey, M.A. and Simpson, A.H. 2001. 'Isotopic comparisons of hair, nail and bone', *Journal of Archaeological Science* **28**(11):1247-1255.

O'Connell, T.C., Kneale, C.J., Tasevska, N. and Kuhnle, G.G. 2012. 'The diet-body offset in human nitrogen isotopic values', *American Journal of Physical Anthropology* **149**(3):426-434.

O'Connell, T.C. 2017. ''Trophic' and 'source' amino acids in trophic estimation: a likely metabolic explanation', *Oecologia* **184**(2):317-326.

O'Connor, T.P. 1982. *The archaeological interpretation of morphological variations in British sheep limb bones.* PhD Thesis, University of London.

O'Connor, T.P. 1984a. *Bones from Aldwark, York.* AML Report 4391.

O'Connor, T.P. 1984b. *Select groups of bones from Skeldergate and Walmgate.* The Archaeology of York 15/1.

O'Connor, T.P. 1988. *Bones from the General Accident Site, Tanner Row*. The Archaeology of York 15/2.

O'Connor, T.P. 1995. 'Size increase in post-medieval English sheep: the osteological evidence', *Archaeofauna* **4**:81-91.

O'Leary, M.H. 1988. 'Carbon isotopes in photosynthesis', *BioScience* **38**(5):328-336.

Ockerman, H.W. and Hansen, C.L. 2000. *Animal by-product processing & utilization.* Washington, DC: CRC Press.

Odland, G.F. 1991. 'Structure of Skin', In, Goldsmith, L.A. (ed.) *Physiology, biochemistry, and molecular biology of the skin* (2nd Edition). Oxford: Oxford University Press, pp. 3-62.

Olalde, I., Brace, S., Reich, D. *et al.* 2018. 'The Beaker Phenomenon and the genomic transformation of northwest Europe', *Nature* **555**:190-196

Oosthuizen, S. 2005. 'New light on the origin of open-field farming?', *Medieval Archaeology* **49**: 165-193.

Ortea, I., O'Connor, G. and Maquet, A. 2016. 'Review of proteomics for food authentication', *Journal of Proteomics* 1**47**(2):212-225.

Orton, D. and Morris, J. 2016. 'Met-analyses in zooarchaeology', Introduction to special collection, *Open Quaternary*

Orton, D. Gaastra, J. and Vander-Linden, M. 2016. 'Between the Danube and deep blue seas: zooarchaeological meta-analysis reveals variability in the spread and development of Neolithic farming across the Western Balkans', *Open Quaternary* **2**(6):1-26.

Orton, D., Morris, J., Locker, A. and Barrett. J. 2014. 'Fish for the city: meta-analysis of archaeological cod remains and the growth of London's northern trade', *Antiquity* **88**:516-530.

Overton, M. 1996. *Agricultural revolution in England: the transformation of the agrarian economy 1500-1850.* Cambridge: Cambridge University Press.

Overton, M. 1986. 'England, 1540-1850', *ReFRESH 3: Recent Findings of Research in Economic & Social History* **3**:1-4.

Palop, R. 2016. *Sheepskin and cattle hide degreasing.* Cromogenia Linits. Barcelona August 2016.

Pálsson, H. and Vergés J. 1952a. 'Effect of plant of nutrition on growth and development of carcass quality in lambs. Part I: The effect of high and low planes of nutrition at different ages', *The Journal of Agricultural Science* **42**:1-41

Pálsson, H. and Vergés J. 1952a.; Effect of plant of nutrition on growth and development of carcass quality in lambs. Part II: Effect on lamb of 30 lb carcass weight', *The Journal of Agricultural Science* **42**:42-93

Parkinson, R. 1810. *Treaties on the breeding and management of livestock.* London: Cadell & Davis.

Pauli, J.N., Newsome, S.D., Hayden, B. *et al.* 2017. 'Why we need a centralised repository for isotopic data', *Proceeding of the National Academy of Sciences* **114**(12): 2997-3001.

Peek, H. and Hall, C. 1962. *Archives of the University of Cambridge: An historical introduction.* Cambridge: Cambridge University Press.

Pellegrini, M., Pouncett, J., Jay, M., Parker-Pearson, M., and Richards, M.P. 2016. 'Tooth enamel oxygen 'isoscapes' show a high degree of human mobility in prehistoric Britain', *Scientific Reports* **6:** 1-9.

Perini, A., Camin, F., Bontempo, L., Rossmann, A. and Piasentier, E. 2009. 'Multielement (H, C, N, O, S) stable isotope characteristics of lamb meat from different Italian regions', *Rapid Communications in Mass Spectrometry* **23**:2573-2585.

Perren, R. 1989. 'Markets and Marketing', In Mingay, G.E. (ed.) *The agrarian history of England and Wales. Vol VI 1750-1850.* Cambridge: Cambridge University Press, pp. 190-274.

Perren, R. 2000. 'The marketing of agricultural products: farm gate to retain store', In Collins, E.J.T. and Thirsk, J. (eds.) *The Agrarian History of England and Wales: Volume VII 1850-1914 (Part II).* Cambridge: Cambridge University Press, pp. 953-998.

Perry, P.J. 1973. *British agriculture, 1875-1914.* Abingdon: Routledge.

Petrie, O.J. 1995. *Harvesting of textile animal fibres.* FAO Agricultural Services Bulletin 122. Food & Agricultural Organisation of the United Nations.

Philips, R. 1808. *Dishley Ram.* [Painting] Printed 1808.

Phillpotts, R. 1863. 'Notes on the market-gardening of Cornwall', *Journal of the Bath and West of England Society for the Encouragement of Agriculture, Arts, Manufacture and Commerce* **11**(1):22-28.

Piasentier, E., Valusso, R., Camin, F. and Versini, G. 2003. 'Stable isotope ratio analysis for authentication of lamb meat', *Meat Science* **64**:239-247.

Pitt, W. 1809. *A general view of agriculture of the County of Leicester; with observations on the means of its improvement. Published by order of the Board of Agriculture and Internal Improvement.* London: Richard Philips.

Pitt, W. 1810.*A general view of agriculture of the County of Worcester; with observations on the means of its improvement. Published by order of the Board of Agriculture and Internal Improvement.* London: Richard Philips.

Pollard, A.M. and Brock, F. 2011. 'Provenancing Parchment, Leather and Paper using Stable Isotopes', In Neate, S., Howell, D., Ovenden, R. and Pollard, A.M. (eds.) *The Technological study of books and manuscripts as artefacts.* London: BAR International Series, pp. 85-90.

Poole, J. and Reed, R. 1962.' The preparation of leather and parchment by the Dead Sea Scroll community', *Technology and Culture* **3**:1-26

Popkin, P.R., Baker, P., Worley, F., Payne, S. and Hammon, A. 2012. 'The sheep project (I): determining skeletal growth, timing of epiphyseal fusion and morphometric variation in unimproved Shetland sheep of known age, sex, castration status and nutrition', *Journal of Archaeological Science* **39**(6):1775-1792.

Popp, B.N., Graham, B.S., Olson, R.J., Hannides, C.C.S., Lott, M.J., Lopez-Ibarra, G.A., Galvan-Magana, F. and Fry, B. 2007. 'Insight into the trophic ecology of Yellowfin Tune, *Thunnus albacares,* from compound-specific Nitrogen isotope analysis of proteinaceous amino aids', In Dawson, T.E. and Siegwolf, R.T.W. (eds.) *Terrestrial Ecology.* Amsterdam: Elsevier, pp. 173-190.

Porter, V. 1991. *The Southdown sheep.* Chichester: The Weald & Downland Open Air Museum.

Potten, C.S., Safhill, R. and Maibach, H.I. 1987. 'Measurement of the transit time for cells through the epidermis and stratum corneum of the mouse and guinea-pig', *Cell and Tissue Kinetics* **20**: 461-472.

Poulakakis, N., Tselikas, A., Bitsakis, I., Mylonas, M. and Lymberakis, P. 2007. 'Ancient DNA and the genetic signature of ancient Greek manuscripts', *Journal of Archaeological Science* **34**: 675-680.

Price, D. 1809. *A system of sheep-grazing and management as practices in Romney Marsh.* London: Richard Phillips.

Price, J. 1939. *North American packer hides.* New York: Pratt Brothers

Purchas, R.W. Davies, A.S. and Abdullah, A.Y. 1991. 'An objective measure of muscularity: changes with animal growth and differences between genetic lines of Southdown sheep', *Meat Science* **30**(1):81-94.

Pythian-Adams, C. 1979. *Desolation of a City: Coventry and the urban crisis of the late middle ages.* Cambridge: Cambridge University Press.

Rackham, D.J. 1977. *Animal remains from a 17th century pit at Blackgate, Newcastle.* AML Report 2969.

Rackham, D.J. 1978. *Corner Tower, Newcastle. Identification of the animal bones.* AML Report 2972.

Rackham, D.J. 1988. *A report on selected groups of 16th-18th century bone and shell from excavations at Blackfriars, Newcastle.* AML Report 4216.

Rackham, D.J. n.d. *A report on the vertebrate remains and mollusc shells* [No date]. AML Report 2412.

Rackham, O. 1986. *The history of the countryside.* London: Dent.

Rainsford, C. 2012. *Block H1 and H2: The faunal remains: 1550-1939.* Report for YAT.

Rainsford, C., O'Connor, T.P and Connell, P. 2016. 'The embarrassment of riches: rationalising faunal assemblages from large urban sites', *International Journal of Osteoarchaeology* **26**(2):221-231.

Rapisarda, P., Camin, F., Fabroni, S., Perini, M., Torrisi,B., and Intrigliolo, F. 2010. 'Influence of different organic fertilisers on quality parameters and the N, C, H, S and O values of orange fruit (*Citrus sinensis* L. Osbeck)', *Journal of Agricultural Food Chemistry* **58**:3502-3506.

Raven, J. 2014. *Bookscape: geographies of printing and publishing in London before 1800.* London: British Library.

Redwood, M. 2016. *Gloves and Glove-making.* Oxford: Bloomsbury Publishing.

Reed, R. 1973. *Ancient skins, parchments and leather.* London: Seminar Press.

Reitz, E.J. and Wing, E.S. 2008. *Zooarchaeology* (2nd Edition). Cambridge Manuals in Archaeology. Cambridge: Cambridge University Press.

Resing, K.A. and Dale, B.A. 1991. 'Proteins of keratohyalin', In, Goldsmith, L.A. (ed.) *Physiology, biochemistry, and molecular biology of the skin* (2nd Edition). Oxford: Oxford University Press, pp. 148-167.

Rich, A. and Crick, F.H. 1961. 'The molecular structure of collagen', *Journal of Molecular Biology* **3**(5):483-506.

Richards, J. 1993. *The Bedern Foundry*. The Archaeology of York 10/3.

Riello, G. 2006. *A Foot in the Past: Consumers, Producers and footwear in the Long Eighteenth Century.* Oxford: Oxford University Press.

Robbins, C.R. 2012. *Chemical and physical behaviour of human hair* (5th Edition). New York: Springer.

Robertson, J. 1792. *General view of agriculture in the county of Perth. With observations on the means of its improvement drawn up for consideration of The Board of Agriculture and Internal Improvement.* London: William Morison.

Robinson, J.J., Rooke, J.A. and McEvoy, T.G. 2002. 'Nutrition for conception and pregnancy', In Freer, M. and Dove, H. (ed.) *Sheep Nutrition.* Wallingford: CABI Publishing, pp.189-212.

Rogers, J.E.T. 1866. *A history of agricultural prices in England: from the year after the Oxford Parliament (1259) to the commencement of the Continental War (1793). Vol I. 1259-1400.* Oxford: The Clarendon Press.

Rogers, J.E.T. 1881. *A history of agricultural prices in England: from the year after the Oxford Parliament (1259) to the commencement of the Continental War (1793). Vol III. 1401-1582.* Oxford: The Clarendon Press.

Rogers, J.E.T. 1887. *A history of agricultural prices in England: from the year after the Oxford Parliament (1259) to the commencement of the Continental War (1793). Vol V. 1582-1702.* Oxford: The Clarendon Press.

Rogers, K.M. 2008. 'Nitrogen isotopes as a screening tool to determine the growing regimes of some organic and nonorganic supermarket produce from New Zealand', *Journal of Agricultural and Food Chemistry* **56**:4078-4083.

Rowntree, S. 1901. *Poverty: a study of town life.* London: MacMillan.

Rushmer, R.F., Buettner, K.J.K., Short, J.M., and Odland, G.F. 1966. 'The skin', *Science* **154**:343-348.

Ryan, K. 1987. 'Holes and flaws in medieval Irish manuscripts', *Peritia* **3**(7): 243-264.

Ryder, M.L. 1958. 'Follicle arrangement in skin from wild sheep, primitive domestic sheep and in parchment'. *Nature* **182**:1-6

Ryder, M.L. 1960a. 'Follicle remains in some British parchments', *Nature* **187**:130-132.

Ryder, M.L. 1960b. 'The arrangement of wool fibres in the skin of sheep', *New Scientist* **206**(8): 1133-1135.

Ryder, M.L. 1962. 'The histological examination of skin in the study of the domestication of sheep', *Zeitschrift für Tierzüchtung und Züchtungsbiologie* **77**: 168-171.

Ryder, M.L. 1964. 'Parchment – its history, manufacture and composition', *Journal of the Society of Archivists* **2**(9): 391-399.

Ryder, M.L. 1966. 'Coat structure in Soay sheep', *Nature* **211**:1092-1093

Ryder, M.L. 1968. 'Fleece structure in some native and unimproved breeds of sheep', *Journal of Animal Breeding and Genetics* **85**(1): 143-170.

Ryder, M.L. 1971. 'Wool growth cycles in soay sheep', *The Journal of Agricultural Science* **76**(1): 183-197.

Ryder, M.L. 1973. *Hair*. The Institute of Biology's studies in biology No. **41**. London: Edward Arnold.

Ryder, M.L. 1983. *Sheep & man.* London: Duckworth.

Ryder, M.L., Padfield, D.G. and Bell, R.M. 1957. 'A survey of the follicle populations in a range of British sheep breeds', *Journal of Agricultural Science* **49**(3):275-284.

Salvagno, L. and Albarella, U. 2017. 'A morphometric system to distinguish sheep and goat post-cranial bones', *PLoS One* **12**(6).

Santos, F.J., Gomez-Martinez, I. and Garcia-Leon, M. 2010. 'Radiocarbon dating of medieval manuscripts from the University of Seville', *Nuclear Instruments and Methods in Physics Research B* **268**(7):1038-1040.

Sargent, C.P. 1938. 'Physical factors affecting the localisation of the booth and shoe trade in England', *Geography* **23**:250-258.

Saunders, A. and Phillips, G. 1993. 'The mammal and bird bones from Mytongate', In Evans, D. *Excavations in Hull 1975-76. Hull Old Town Report Series 2.* East Riding Archaeologist **4**:191-192.

Saxl, H. 1954. *An investigation of the qualities, the methods of manufacture and the preservation of historic parchment and vellum with a view to identifying the animal species used.* MSc Thesis. University of Leeds.

Schmidt, O., Quilter, J.M., Bahar, B., Moloney, A.P., Scrimgeour, C.M., Begley, I.S. and Monahan, F.J. 2004. 'Inferring the origin and dietary history of beef from C, N and S stable isotope ratio analysis', *Food Chemistry* **91**: 545-549.

Schoeninger, M.J. 1989. 'Reconstructing prehistoric human diet', In Price, T.D. (ed.) *The chemistry of prehistoric human bone.* Cambridge: Cambridge University Press, pp. 38-67.

Schoeninger, M.J. and DeNiro, M.J. 1984. 'Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals', *Geochimica et cosmochimica acta* **48**(4):625-639.

Schummer, A., Wilkens, H., Vollmerhaus, B. and Habermehl, K.H. 1981. *The Circulatory System, the skin, and the Cutaneous Organ of the Domestic Mammals.* Berlin: Springer-Verlag GmBH.

Schwartz, H.P. and Schoeninger, M.J. 1991. 'Stable isotope analyses in human nutritional ecology', *American Journal of Physical Anthropology* **34**(13):283-321.

Schwertl, M., Matthew, C., Auerswald, K., Schnyder, H. and Crook, K. 2003. 'Isotopic composition of cow tail switch hair as an information archive of the animal environment', *Agriculture, Ecosystems & Environment* **109**:153-165.

Scola, R. 1992. *Feeding the Victorian City: food supply of Manchester, 1770-1870.* Manchester: Manchester University Press.

Scola, R. 1992. *Feeding the Victorian City: The food supply of Manchester 1770-1870.* Manchester: Manchester University Press.

Scott, D.W. 2018. *Colour Atlas of Farm Animal Dermatology.* Hoboken, NJ: John Wiley & Sons.

Scott, S.A. 1985. *Bones from The Bedern.* AML Report 4821 85/33

Scott, S.A. 1988. *The animal bones from St. Mary's Manor, Beverley.* Prepared for the Humberside Archaeological Unit 88/27.

Scott, S.A. 1989. 'The animal bones from Beverley Gate', In Evans, D. *Excavations at the Beverley Gate, and other parts of the town defences of Kingston-Upon-Hull.* HAP Report.

Scott, S.A. 1991. 'The animal bones', In Armstrong, P., Tomlinson, D. and Evans, D. *Excavations at Lurk Lane, Beverley, 1979-82.* Sheffield Excavation Reports 1. Dorchester: The Dorset Press, pp.216-233.

Scott, S.A. 1993. 'The animal bone from Queen Street', In Evans, D. *Excavations in Hull 1975-76. Hull Old Town Report Series 2.* East Riding Archaeologist **4**:192-194.

Sealy, J., van der Merwe, N.J., Lee-Thorpe, J. and Lanham, J.L. 1987. 'Nitrogen isotopic ecology in Southern Africa: implication for environmental and dietary tracing', *Geochimica et Cosmochimica Acta* **51**(10):2707-2717.

Seemann, B.. 1866. *Journal of botany, British and foreign.* London: Robert Hardwicke.

Shakespeare, W. 2005. *Hamlet.* Penguin Shakespeare. London: Penguin Group.

Sharphouse, J.H. 1971. *Leather technician's handbook.* London: Leather Producers' Association.

Silfer, J.A., Engel, M.H. and Macko, S.A. 1992 'Kinetic fractionation of stable carbon and nitrogen isotopes during peptide bone hydrolysis: Experimental evidence and geochemical implications', *Chemical Geology: Isotope Geosciences Section* **101**(3):211-221.

Sinclair, J. 1802. 'Hints regarding cattle', *Famers Magazine* **3**:149-150.

Slatkin, M. and Racimo, F. 2016. 'Ancient DNA and human history', *Proceedings of the National Academy of Sciences* **113**(23):6380-6387

Smedley, M.P., Dawson, T.E., Comstock, J.P., Donovan, L.A., Sherill, D.E. and Cook, C.S. 1991. 'Seasonal carbon isotope discrimination in a grassland community', *Oecologia* **85**(3):314-320.

Smil, V. 2005. 'Nitrogen in modern European agriculture', In Scholliers, P., van Molle, L. and Sarasua, C. (eds.) *Land, shops and kitchens: technology and the food chain in 20th century Europe.* Comparative Rural History Network (CORN) **7**. Belgium: Brepols, pp.1-10.

Smith, J.M., Lee-Thorp, J.A. and Sealy, J. 2002. 'Stable carbon and oxygen isotope evidence for late Pleistocene to middle Holocene climate fluctuation in the interior of southern Africa', *Journal of Quaternary Science* **17**(7): 683-695.

Smith, T.M. 2012. *Enclosure and agricultural improvement in north-west Lincolnshire from c.1600 to 1850.* PhD Thesis, University of Nottingham.

Sponheimer M., Robinson, T., Ayliffe, L., Passey, B., Roeder, B., Shipley, L., Lopez, E., Cerling, T., Dearing, D. and Ehleringer, J. 2003a. 'An experimental study of carbon-isotope fractionation between diet, hair, and faeces of mammalian herbivores', *Canadian Journal of Zoology* **81**:871-876.

Sponheimer M., Robinson, T., Ayliffe, L., Passey, B., Roeder, B., Shipley, L., Lopez, E., Cerling, T., Dearing, D. and Ehleringer, J. 2003b. 'Nitrogen isotopes in mammalian herbivores: hair ^{15}N values from a controlled feeding study', *International Journal of Osteoarchaeology* **13**:80-87.

Spooner, W.C. 1844. *The sheep: its history, structure, economy and diseases* (1st Edition). London: Cradock & Co.

Stephenson, M.J. 1988. 'Wool yields in the medieval economy', *Economic History Review* **61**:369-391.

Stinson, T. 2011. 'Counting sheep: potential applications of DNA analysis to the study of medieval parchment production', In Franz, F., Fritze, C., Vogeler, G. (eds.) *Kodikologie und paläographie im digitalenzeitalter 2, schriften des institutsfür dokumentologie und editorik, Band 3.* Norderstedt, Germany: Books on Demand, pp. 191-207.

Stone, R. 1997. *Some British empiricists in the social science.* Cambridge: Cambridge University Press.

Strlič, M., Cigić, I.K., Rabin, I., Kolar, J., Pihlar, B. and Cassar, M. 2009. 'Autoxidation of lipids in parchment', *Polymer Degradation and Stability* **94**(6):886-890.

Strohalm, M., Kavan, D., Novák, P., Volny, M., Havilek, V. 2010. 'mMass 3: A cross-platform software environment for precise analysis of mass spectrometric data', *Analytical Chemistry* **82**(11):4648-4651.

Styring, A.K., Sealy, J.C., Evershed, R.P. 2010. 'Resolving the bulk δ^{15}N values of ancient human and animal bone collagen via compound-specific nitrogen isotope analysis of constituent amino acids', *Geochimica et Cosmochimica Acta* **74**: 241-251.

Sutoh, M., Koyama, T. and Yoneyma, T. 1987. 'Variations of natural ^{15}N abundances in the tissues of digesta of domestic animals', *Radioisotopes* **36**:74-77.

Sykes, N.J. 2014. *Beastly questions: animal answers to archaeological questions.* London: Bloomsbury.

Sykes, N.J., Wan, V., Robinson, J., Timney, B. and Reynolds, R. n.d. *Horncores, horses and hides; the animal remains from City Road, Chester* [No date]. Unpublished report to LP Archaeology.

Szpak, P. 2014. 'Complexities of nitrogen isotope biogeochemistry in plant-soil systems: implications for the study of ancient agricultural and animal management practices', *Frontiers in Plant Science* **5**.

Szpak, P., Longstaffe, J.F., Millaire, J.J. and White, C.D. 2009. 'Stable isotope biogeochemistry of seabird guano fertilisation: results from growth chamber studies with maize *(Zea Mays)*', *PLoS One* **7**(8).

Tanner, H. 1859. 'The sheep of the west of England', *Journal of the Bath and West of England Society for the Encouragement of Agriculture, Arts, Manufacture and Commerce* **7**:57-107.

Tarlow, S. 2007. *The archaeology of improvement in Britain, 1750-1850.* Cambridge Studies in Archaeology. Cambridge: Cambridge University Press.

Teasdale, M.D., Fiddyment, S., Vnoucek, J., Mattiangeli, V., Speller, C., Binois, A., Carver, M., Dand, C., Newfield, T.P., Webb, C.C., Bradley, D.G., Collins, M.J. 2017. 'The York Gospels: a 1000-year biological palimpsest', *Royal Society Open Science* **4**: 1-11.

Teasdale, M.D., van Dorn, N.L., Fiddyment, S., Webb, C., O'Connor, T.P., Hofreiter, M., Collins, M.J., Bradley, D.G. 2015. 'Paging through history: parchment as a reservoir of ancient DNA for next generation sequencing', *Philosophical Transactions of the Royal Society* B **370**(1660):1-7.

The Famers Magazine. 1814. *Volume 15.* London.

The Farmer's Magazine. 1874. *Volume 76.* London.

The Jurist. 1845. *Volume 8.* London.

The Society for The Diffusion of Useful Knowledge. 1842. *The penny magazine, new series.* London: Charles Knight & Co.

Thirsk, J. 1967a. 'The farming regions of England', In Thirsk, J. (ed.) *The agrarian history of England and Wales. Vol. IV. 1500-1640.* Cambridge: Cambridge University Press, pp.1-112.

Thirsk, J. 1967b. Farming Techniques, In Thirsk, J. (ed.) *The Agrarian History of England and Wales. Volume IV: 1500-1640.* Cambridge: Cambridge University Press, pp.161-199

Thirsk, J. 1984. *The rural economy of England: collected essays.* New York, NY: Hambledon-Continuum.

Thomas, R. 2005a. *'Animals, economy and status: the integration of zooarchaeological and historical evidence in the study of Dudley castle, West Midlands (c.1100-1750).* Oxford: British Archaeological Reports British Series **392**.

Thomas, R. 2005b. 'Zooarchaeology, improvement and the British agricultural revolution', *International Journal of Historical Archaeology* **9**(2):71-88.

Thomas, R. 2005c. 'Perceptions versus reality: changing attitudes towards pets in medieval and post-medieval England', In Plukowski, A. (ed.) *Just skin and bone? new perspectives on human-animal relations in the historic past.* Oxford: British Archaeological Reports International Series **1401,** pp. 95-105.

Thomas, R. 2009. 'Bones of contention: why later post-medieval assemblages of animal bones matter', In Horning, A. and Palmer, M. (eds.) *Crossing paths or sharing tracks: future directions in the archaeological study of post-1550 Britain and Ireland.* Woodbridge: Boydell and Brewer, pp.133-148.

Thomas, R. 2014. 'Tortoises and the exotic animal trade in Britain from medieval to 'modern', *Testudo* **8**(1):56-68.

Thomas, R., Holmes, M. and Morris, J. 2013. '"So bigge as bigge may be": tracking size and shape change in domestic livestock in London (AD 1220-1900)', *Journal of Archaeological Science* **40**(8):3309-3325.

Thomas, S. 1912. 'A commentation on goat skins of the world', *Shoe and Leather Reporter* **108**(1): 19-33.

Thomas, S. 1983. 'Leathermaking in the Middle Ages', In Thomson, R. and Beswick *(eds.) Leather manufacture through the ages. proceedings of the 27th East Midlands industrial archaeology conference, Northampton,* pp. 1-10.

Thomson, R. 2006. 'The manufacture of leather', In Kite, M. and Thomson, R. *Conservation of leather and related materials.* Oxford: Butterworth-Heinemann; Elsevier, pp. 66-82.

Tieszen, L.L. 1991. 'Natural variations in the carbon isotope values of plants: implications for archaeology, ecology and paleoecology', *Journal of Archaeological Science* **18**(3):227-248.

Tieszen, L.L. and Fagre, T. 1993. 'Effect of diet quality and composition on the isotopic composition of Respiratory CO_2, bone collagen, bioapatite and soft tissues', In Lambert, J.B. and Grupe, G. (eds.) *Prehistoric human bone.* Berlin: Springer, pp. 121-151.

Tieszen, L.T., Senyimba, M.M., Imbamba, S.K. and Troughton, J.H. 1979. 'The distribution of C_3 and C_4 grasses and carbon isotope discrimination along an altitudinal and moisture gradient in Kenya', *Oecologia* **37**: 337-350.

Timmer, C.P. 1969. 'The turnip, the new husbandry, and the English agricultural revolution', *Quarterly Journal of Economics* **83**(3):375-395.

Toniolo, L., D'Amato, A., Saccenti, R., Gulotta, D., Righetti, P.G. 2012. 'The silk road, Marco Polo, a bible and its proteome: A detective story', *Journal of Proteomics* **75**(11): 3365-3373.

Touraine, B., Daniel-Vedele, F. and Forde, B.G. 2001. 'Nitrate uptake and its regulation', In Lea, P.J. and Morot-Gaudry, J.J. (eds.) *Plant Nitrogen.* Berlin: Springer-Verlag, pp.1-36.

Towers, J, Gledhill, A. and Bond, J. 2015. 'An investigation of cattle birth seasonality using $\delta^{13}C$ and $\delta^{18}O$ profiles within first molar enamel', *Archaeometry* **56**(1):208-236.

Toynbee, A. 1884. *Lectures on the industrial revolution in England: popular addresses, notes and other fragments.* London: Rivingtons.

Treasure, E.R., Church, M. and Gröcke, D.R. 2016. 'The influence of manuring on stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in Celtic bean (*Vica faba* L.): archaeobotanical and palaeodietary implications', *Archaeological and Anthropological Science* **8**:555-562.

Tregear, R.T. 1966. *Physical functions of skin.* Cambridge, MA: Academic Press.

Trow-Smith, R. 1957. *A history of British livestock husbandry to 1700.* London: Kegan & Paul.

Trow-Smith, R. 1959. *A history of British livestock husbandry, 1700-1900.* London: Kegan & Paul.

Tuke, J. 1800. *General view of the agriculture of the North Riding of Yorkshire: drawn up for the consideration of the Board of Agriculture and Internal Improvement.* London: McMillan.

Turner, M.E. 1998. 'Counting sheep: waking up to new estimates on livestock numbers in England c. 1800', *Agricultural History Review* **47**(2):142-161.

Turner. M.E., Beckett, J.V. and Afton, B. 2001. *Farm Production in England, 1700-1900.* Oxford: Oxford University Press.

Turner-Walker, G. 2008. 'The chemical and microbial degradation of bones and teeth', *Advances in Human Paleopathology* **4**:3-29.

Tusser, T. 1580. *Five hundred points of good husbandry*

van Dorn, N. 2012. *The application and limitations of a minimally destructive approach to archaeological proteomics.* PhD Thesis, University of York.

van Dorn, N. 2014. ‘Zooarchaeology by mass spectrometry (ZooMS)’, In Smith, C. (ed.) *Encyclopaedia of Global Archaeology.* New York, NY: Springer, pp. 7998-8000.

van Duin, A.C.T. and Collins, M.J. 1998. ‘The effects of conformational constraints on aspartic acid racemisation’, *Organic Geochemistry* **29**(7):1227-1232.

van Klinken, G. J., Richards, M.P. and Hedges, R.E.M. 2000. ‘An overview of causes for stable isotopic variation in past European human populations: environmental, ecophysiological and cultural effects’, In, Ambrose, S.H. and Katzenberg, M.A. (ed.) *Biogeochemical Approaches to Paleodietary Analysis.* Advances in Archaeological and Museum Science Vol. **5**. Boston, MA: Springer, pp. 39-63.

van Klinken, G.J. 1999. ‘Bone collagen quality indicators for palaeodietary and radiocarbon measurements’, *Journal of Archaeo*logical Science **26**(6):687-695.

van Klinken, G.J., van der Plicht, H and Hedges, R.E. 1994. ‘Bone $^{13}C/^{12}C$ ratios reflect (paleo-) climatic variation’, *Geophysical Research Letters* **21**(6):445-448.

Vann, S. and Grimm, J. 2010. ‘Post-medieval sheep *(Ovis aires)* metapodia from southern Britain’, *Journal of Archaeological Science* **37**(7):1532-1542.

Verenitch, S. and Mazumder, A. 2012. ‘Carbon and nitrogen isotopic signatures and nitrogen profile to identify adulteration in organic fertilisers’, *Journal of Agricultural Food Chemistry* **60**(34): 8278-8285.

Vincent, N. 2012. *Magna Carta: A Very Short Introduction.* Oxford: Oxford University Press.

Vitoária, L., Otero, N., Soler, A., and Canals, A. 2004. ‘Fertiliser characterisation: isotopic data (N, S, O, C and Sr)’, *Environmental Science Technology* **38**(12):3254-62.

Vnouček *forthcoming.* ‘Codicological assessment of insular manuscript parchment: *The Ceolfrith Leaves* and *Codex Amiatinus*’. *Journal of the Institute of Conservation.*

Voight, C.C., Rex, K., Michener, R., and Speakman, J. 2008. ‘Nutrient routing in omnivorous animals tracked by stable carbon isotopes in tissues and exhaled breath’, *Oecologia* **157**(1):31-40.

Vogel, J.C. 1978. ‘Isotopic assessment of the dietary habits of ungulates’, *South African Journal of Science* **74**(8):298-301.

von den Driesch, A, 1976. *A guide to the measurements of animal bones from archaeological sites.* Peabody Museum Bulletin 1. Cambridge, MA: Harvard University Press.

von Holstein, I.C.C, von Tersch, M., Coutu, A.N., Penkman, K.E.H., Makarewicz, C.A. and Collins, M.J. 2018. ‘Collagen proteins exchange O with demineralisation and gelatinisation reagents and also with atmospheric moisture’, *Rapid Communications in Mass Spectrometry* **32**(6): 523-534

von Holstein, I.C.C., Hamilton, J., Craig, O.E., Newton, J. and Collins, M.J. 2013. ‘Comparison of isotopic variability in proteinaceous tissues of a domestic herbivore: a baseline for zooarchaeological investigations’, *Rapid Communications in Mass Spectrometry* **27**:2601-2615.

von Holstein, I.C.C., Penkman, K.E.H., Peacock, E.E. and Collins, M.J. 2014. ‘Wet degradation of keratin proteins: linking amino acid, elemental and isotopic composition’, *Rapid Communications in Mass Spectrometry* **28**(19):2121-2133.

von Holstein, I.C.C., Walton-Rogers, P., Craig, O.E., Penkman, K., Newton, J. and Collins, M.J. 2016. 'Provenancing archaeological wool textiles from medieval northern Europe by light stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$)', *PLoS One* **11**(10).

Walker, J.L., Potter, C.W. and Macko, S.A. 1999. 'The diets of modern and historical bottlenose dolphin population reflected through stable isotopes', *Marine Mammal Science* **15**(2): 335-350.

Warriner, C. and Tuross, N. 2010. 'Brief communication: tissue isotopic enrichment associated with growth depression in a pig: implications for archaeology and ecology', *American Journal of Physical Anthropology* **141**(3):486-493.

Waterlow, J.C. 2006. *Protein turnover*. London: CABI Publishing.

Watson, H. 1838. 'On Southdown sheep as being well suited for the middle ranges of Highland pastures of Scotland', *The Quarterly Journal of Agriculture* **8**:468-469.

Webb, S. and Webb, B. 1963. *The Story of the King's Highway.* London: F. Cass.

Weinstock, J. 2002. 'The medieval and post-medieval remains from Heigham Street, Norwich', Centre for Archaeology Report **33**.

Welker, F., Collins, M.J., Thomas, J., Wadley, M. *et al.* 2015a'Ancient proteins resolving the evolutionary history of Darwin's South American ungulates', *Nature* **522**(7554):81-84.

Welker, F., Soressi, M., Rendu, W., Hulbin, J.J. and Collins, M. 2015b. 'Using ZooMS to identify fragmentary bone from the late middle/early upper Palaeolithic sequence of Les Cottes, France', *Journal of Archaeological Science* **54**:279-286.

West, W.M. 1830. *The history, topography and directory of Warwickshire.* Birmingham: R. Wrightson.

Weston, R. 1650. *A discourse on husbandrie used in Brabant and Flanders.* London.

White, C.D. and Schwartz, H.P. 1994. 'Temporal trends in stable isotopes for Nubian mummy tissues', *American Journal of Physical Anthropology* **93**(2):165-187.

White, C.D., Longstaffe, F.J. and Law, K.R. 1995. Isotopic analysis of hair and skin from Kharga Oasis mummies. Unpublished report to the American Museum of Natural History.

Willement, E. 1845. *A catechism on the history of things in common use, and the events which led to their discovery and improvement.* Colchester: J. B. Harvey.

Williams, J. 2008. *Investigating the diet and dietary changes using the stable isotopes of Carbon and Nitrogen in mummified tissues from Puruchuco-Huaquerones, Peru*. PhD Thesis, University of Calgary.

Williamson, T. 1998. 'Fodder crops and the 'agricultural revolution' in England, 1700-1850', *Environmental Archaeology* **1**(1):11-18.

Williamson, T. 2002. *The transformation of rural England: farming and the landscape, 1700-1870.* Exeter: University of Exeter Press.

Williamson, T. 2013. *An environmental history of wildlife in England, 1650-1950.* London: Bloomsbury.

Wint, W. and Robinson, T. 2009. *Gridded livestock of the world 2007.* FAO, Rome: Food and Agriculture Organisation of the United Nations.

Wodzicka, M. 1958. 'Studies on the thickness and chemical composition of the skin of sheep. III. Effect of shearing', *New Zealand Journal of Agricultural Research* **1**(4): 601-606.

Wodzicka-Tomaszewska, M. 1960. 'Effect of cold on the thickness and chemical composition of the skin in sheep', *Proceedings of the Australian Society of Animal Production* **3**: 195-198.

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I., Whittington, F.M. 2008. 'Fat deposition, fatty acid composition and meat quality: A review', *Meat Science* **78**(4): 343-358.

Woodward, S., Kahila, G., Smith, P., Greenblatt, C. and Zias, J. 1996. 'Analysis of parchment fragments from the Judean Desert using DNA techniques', In Parry, D.W and Ricks, S.D. (eds.) *Current research and technological developments of the Dead Sea Scrolls: Conference on the texts from the Judean Desert, Jerusalem, 30 April 1995.* Leiden: Brill, pp. 215-238.

Wrigley, E.A. 2004. 'British population during the 'long' eighteenth century, 1680-1840', In Floud, R. and Johnson, P. (ed.) *The Cambridge economic history of modern Britain, 1700-1860.* Cambridge: Cambridge University Press, pp.57-95.

YAT, York Archaeological Trust, 2015. 'Hungate species: our five-year mission', *Yorkshire Archaeology Today* **12**:1-18.

Yeomans, L. 2006. *A zooarchaeological and historical study of the animal product based industries operating in London during the post-medieval period.* PhD Thesis, University College London.

Youatt, W. 1837. *Sheep: their breeds, management and diseases; to which is added the mountain shepherd's manual.* London: Baldwin & Cradock.

Young, A. 1770. *A six months tour through the North of England.* Dublin.

Young, A. 1788. *Annals of agriculture and other useful arts.* Vol. XIV. Bury St. Edmunds: J. Rackham.

Young, A. 1793. *General view of agriculture of the county of Sussex. With observations on the means of its improvement drawn up for consideration of The Board of Agriculture and Internal Improvement.* London: J. Nichols.

Young, A. 1809. *A general view of agriculture of Berkshire. Drawn up for the consideration of the Board of Agriculture and Internal Improvement.* London: Richard Phillips.

Yun, S.I., Ro, H.M., Choi, W.J., Chang, S.X. 2006. 'Interactive effect of N fertiliser source and time of fertilisation leave specific N isotopic signatures in Chinese cabbage and soil', *Soil Biology and Biochemistry* **38**:1682-1689.

Zazzo, A., Cerling, T.E., Ehleringer, J.R., Moloney, A.P., Monahan, F.J. and Schmidt, O. 2015. 'Isotopic composition of sheep wool records seasonality of climate and diet', *Rapid Communications in Mass Spectrometry* **29**(15):1357-1369.