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The identification of poultry processing in archaeological ceramic vessels using *in situ* isotope references for organic residue analysis

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Keywords

Poultry, lipid, stable isotopes, organic residue analysis, Anglo-Saxon, pottery, GC-MS, GC-c-IRMS

Abstract

Poultry products are rarely considered when reconstructing pottery use through organic residue analysis, impinging upon our understanding of the changing role of these products in the past. Here we evaluate an isotopic approach for distinguishing chicken fats from other animal products. We compare the carbon isotopes of fatty acids extracted from modern tissues and archaeological bones and demonstrate that archaeological bones from contexts associated with the pottery provide suitable reference ranges for distinguishing omnivorous animal products (e.g. pigs vs. chickens) in pots. When applied to pottery from the Anglo-Saxon site of Flixborough, England, we succeeded in identifying residues derived from chicken fats that otherwise could not be distinguished from other monogastric and ruminant animals using modern reference values only. This provides the first direct evidence for the processing of poultry or their products in pottery. The results highlight the utility of '*in-situ*' archaeological bone lipids to identify omnivorous animal-derived lipids in archaeological ceramic vessels, although due to potential variation in past animal diets it unlikely that such reference ranges can be applied globally.

Introduction

Products from omnivorous animals, such as pigs and poultry, dominate global meat production and are important for fuelling the next 'Livestock Revolution' in attempts to feed increasing global populations (Delgado et al., 1999). Undoubtedly, these were also important commodities in many past contexts, as attested by the frequent occurrence of their skeletal remains on a wide range of archaeological sites (Maltby, 2014; O'Connor, 2014; Redding, 2015; Sykes, 2012). Whilst it is generally accepted that pig bones on archaeological sites provide evidence for the consumption of pork products, the use of poultry in the past is complicated by other historically and ethnographically documented uses, from recreation to ritual (Sykes, 2012). Even as a foodstuff, poultry have been overlooked and under investigated in the past despite their undisputed importance today as a major global resource. Therefore, whilst there is clear faunal evidence attesting to the presence of domestic chicken on European archaeological sites since later prehistory (Peters et al., 2015; Serjeantson, 2009), it is unclear when, where and why poultry became routinely raised for their meat and eggs and viewed primarily as a foodstuff. One way to unequivocally demonstrate this link is by directly associating poultry products with domestic cooking vessels.

Archaeological ceramic vessels provide a wealth of information on resource use, offering a window into past production, storage, transport and processing of food and other commodities. Lipids (fats, oils and waxes) can be readily absorbed in unglazed, porous ceramic vessels (Evershed et al., 1999), and preserved for hundreds to thousands of years (Craig et al., 2013). Analytical techniques, involving gas chromatography (GC) and GC-mass spectrometry (GC-MS), provide a means to associate broad classes of compounds to their biological precursors (Evershed et al., 1999; Evershed, 2008). Stable carbon isotope analysis of palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids by GC-combustion–stable isotope ratio MS (GC-c-IRMS) provides a complementary method for animal fat identification in archaeological ceramic vessels (Copley et al., 2003; Craig et al., 2013; Evershed et al., 2002b; Mukherjee et al., 2008; Salque et al., 2013). This latter approach is routinely used to identify ruminant products (Craig et al., 2012), and dairying activity in the archaeological record. However, relatively few studies have considered using this approach to identify poultry products or distinguish these from other omnivorous animals, such as pigs (Evershed et al., 2002b).

In a first attempt to determine the processing of poultry in ceramic vessels, we investigate a pottery assemblage from the Anglo-Saxon site of Flixborough in North Lincolnshire (England). The site was chosen as its faunal assemblage shows clear evidence of mixed monogastric and omnivorous animal exploitation, i.e. geese, chickens, pigs. To distinguish these, we determined carbon isotope values of fatty acids obtained from archaeological bones of monogastric-omnivorous animals associated with the pottery to provide *in-situ* reference values. Our aim is to determine whether different monogastric commodities were processed in ceramic vessels.

Isotopic variability in monogastric-omnivorous animal fats

The stable carbon isotopic ratios of monogastric and ruminant adipose fatty acids differ due to fundamental variations in digestive physiology and metabolic processes (Copley et al., 2003; Howland et al., 2003; Jim et al., 2004; Stott et al., 1997a). Notably, ruminants incorporate specific saturated compounds (e.g. $C_{18:0}$) directly from their diet into their tissues, following biohydrogenation of the unsaturated precursors in the rumen (Harrison and Leat, 1975; Krogdahl, 1985). This process leads to measurable ^{13}C depletion compared to *de novo* synthesized components (e.g. $C_{16:0}$). However, distinguishing fat from economically important monogastric and omnivorous animals (e.g. pigs, poultry) is less straightforward using this approach. Omnivorous animals consume a broader range of food sources compared to herbivores and consequently the carbon in fatty acids can be derived from a wider range of macronutrient sources, including lipids, carbohydrates and proteins from both animals and plants (Budge et al., 2011; Howland et al., 2003; Stott et al., 1997a; Trust Hammer et al., 1998). Therefore, fatty acids from omnivores exhibit considerably larger isotopic variability (e.g. Delgado-Chavero et al., 2013; Recio et al., 2013) compared to ruminant and monogastric herbivores, depending on the animal's diet and therefore husbandry practices. As these are variable in the past and most likely different from the present, modern references for omnivores may be inappropriate and ideally site specific "*in-situ"* baselines need to be constructed.

Archaeological bone as an in-situ baseline for organic residue analysis

All previous attempts to interpret fatty acids from archaeological pottery rely on comparison with reference fats from modern animals raised on known diets and preferably sourced close to the archaeological sites under investigation (Copley et al., 2003; Dudd and Evershed, 1998; Dunne et al., 2012; Evershed et al., 2002b; Gregg et al., 2009; Salque et al., 2013; Spangenberg et al., 2006). In Northern Europe, for example, comparative reference fats are typically derived from animals raised on C_3 vegetation with the assumption that they represent the variety of environmental/dietary conditions experienced by past animals (Dudd and Evershed, 1998). While this is entirely reasonable for herbivore ruminants, it does not account for the greater variability in the diet of omnivores. Furthermore modern omnivore references may not be suitable analogues for animals raised in areas that have undergone substantial changes in vegetation composition during the Holocene (e.g. Near East, (Goodfriend, 1990); North Africa, (Castañeda et al., 2009)) or in cultural contexts where foddering strategies are known to have changed in the past (Hamilton and Thomas, 2012; Madgwick et al., 2012). Both geographic and temporal variability must be considered in the when deriving suitable isotopic reference ranges in order to capture environmental and cultural effects. Finally, the use of modern reference fat inherently implies that animal fat preserved in ceramic vessels originate predominantly, or exclusively, from adipose tissue. Nevertheless, other animal tissues are similarly lipid rich (e.g. bone marrow) and thus are potential sources to the pool of animal fat preserved in ceramic vessels. The boiling of bone to release nutrients, for example in soups and broths, may provide another mechanism for transferring bone lipids to archaeological ceramics.

As soft tissues are very rarely preserved in archaeological contexts, animal bone may provide an alternative or complementary source of fatty acids for comparison. Animal bones are often found directly associated with pottery (i.e. in the same contexts, stratigraphic units or site areas, and in rare

instances within the pots themselves) and are therefore chronologically coherent with cultural and environmental contexts. The lipid composition in adipose tissues and cortical bone are similar in nature (Kagawa et al., 1996; Ren et al., 2008) and studies have shown that lipids are preserved in archaeological bone (Evershed et al., 1995b; Spangenberg et al., 2014; Stott and Evershed, 1996). Recently Colonese et al. (2015) have demonstrated that endogenous palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) fatty acids can be recovered in sufficient quantity from archaeological bones from a range of environments to permit stable isotope analysis by GC-C-IRMS. In agreement with earlier studies (Stott et al., 1999, 1997a) it has been shown that stable carbon isotope composition of bone lipid covariates with bone collagen (Colonese et al., 2015), supporting the endogenous origin of fatty acids and their potential for paleodietary reconstruction.

Flixborough

The site and economy

Excavations at Flixborough, North Lincolnshire, exposed a high-status Anglo-Saxon site with an occupation sequence stretching from the 7th to 11th centuries AD (with subsequent use in the 12th- 15th centuries) (Loveluck and Gaunt, 2007; Loveluck, 1998). Over 200,000 fragments of animal bone were recovered from Flixborough, making it one of the largest assemblages of Middle to Late Anglo-Saxon date in England (Jaques et al., 2007). A hand-collected and coarse-sieved assemblage of over 41,000 mammal and bird bone fragments dating from the early 7th to late 10th century was identified to taxon. The most numerous domesticates (average %NISP) were cattle (*Bos taurus,* 29.4%), followed by sheep/goat (*Ovies aries*/*Capra hircus*, 27%), pig (*Sus scrofa domesticus*, 19.4%), chicken (*Gallus gallus domesticus*, 15%), and goose (*Anser* sp., 9.2%) respectively, although the relative proportions of these changed through time at the site (Jaques et al., 2007).

Whilst cattle and sheep/goat could have provided multiple secondary products such as milk, wool and traction, pigs would have primarily been a meat resource. Chickens would have been a useful source of meat and feathers, but also important for providing eggs and potentially fertiliser in the form of dung. However, so far the role of ceramic vessels for processing and preparing these various animal products has been only preliminarily considered (Young and Vince, 2009). In particular, the identification of domestic poultry in pottery would help clarify their role at the site and provide the first insights into the material culture associated with their processing.

Material and methods

Modern samples

In order to confirm the utility of bone lipids as reference samples, differences in $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ between adipose (subcutaneous and skin fat) and bone lipid was investigated in three distinct groups of modern chickens raised in Britain. Specimens were slaughtered for commercial purposes or died of natural causes before the experiment. Group 1 includes 10-week old, free range specimens

acquired from an organic farm in Yorkshire (CH-FR, n = 10). These specimens were fed on organic, C_3-plant derived pellets and grass; Group 2 includes 4-week old, commercial non-free range broilers (fresh class A), from a big-chain supermarket (CH-SP, n = 5); Group 3 includes >28-week old, non-commercial free-range specimens, fed *ad libitum* with occasional supplements of mealworms (CH-D, n = 8). Unfortunately the adipose tissue for the Group 3 was not available and could not be sampled. However the bone isotope data was included to explore variability between the populations. Bone lipid $\delta^{13}C$ values are also compared with previously reported values from chicken adipose tissues (Dudd and Evershed, 1998; Evershed et al., 2002b), which were 3-week old broilers fed with pellets. Chicken $\delta^{13}C$ values from Dudd and Evershed (1998) were corrected for the effect of modern atmospheric $\delta^{13}C$ values by 1.2‰ (Dudd and Evershed, 1998; Friedli et al., 1986). In chickens, *de novo* synthesis of fatty acids occurs mainly in the liver and it is transferred by lipoproteins to the site of deposition, whereas in pig it takes place mainly in the adipose tissue, similar to ruminants (Laliotis et al., 2010). In order to investigate any isotopic differences in fatty acids associated with biosynthesis, subcutaneous adipose and bone lipids were also investigated in commercial non-free range pigs from several supermarkets and a local butcher in York (Pig-SP, n = 5). The stable carbon and nitrogen isotope composition of bone collagen was also analyzed from the modern specimens and compared with bone lipid $\delta^{13}C$ values.

Archaeological samples

Animal bones retrieved from external refuse contexts at Flixborough (5983, 5653), dated to mid-8th to early-9th century (phase 3biv), were selected for collagen and bone lipid stable isotopic analysis. These included chicken (Chk, n = 10), goose (Gos, n = 10) and pig (Pig, n = 5). Whenever possible, samples were selected to represent individual animals by sampling the same-sided portion of a specific element. Ceramic vessels (n = 60) from 25 Anglo-Saxon contexts were selected for molecular and isotopic analysis of extracted lipids (Tab. 5). These contexts were selected due to their high relative abundances of chicken remains (i.e. phase 3i) and good preservation of ceramic vessels, that allowed comparison of organic residues against pottery size, shape and form. Where possible potsherds were assigned to small, medium and large sized jars and bowls (Maxey type fabric B, E, U; Tab. 5; Young and Vince, 2009).

Collagen extraction of modern and archaeological bones

After manually removing the adipose tissue and the bone marrow, modern chicken (ulnae and femora) and pig (ribs) bones were defatted. Lipids were removed with repeated rinses (x3) of a 2:1 dichloromethane:methanol solvent solution (DCM:MeOH; 3x2 mL), ultrasonicated for 15 min and centrifuged (850 g) for 10 min. Samples were then rinsed with deionized water and dried at room temperature. Collagen extraction of archaeological and modern bone followed a modified Longin method (Brown et al., 1988) and details can be found in previous studies (e.g. (Craig et al., 2010)). Briefly, shards of modern (~100 - 186 mg) and archaeological bones (245 - 630 mg) were demineralised using 0.6 M HCl, at 4°C for several days. Samples were then rinsed with distilled water and gelatinised with 0.001 M HCl at 80°C for 48 h. Initially, the supernatant containing the collagen of modern bones were ultrafiltered (30 kDa, Amicon® Ultra-4 centrifugal filter units; Millipore, MA, USA). Given that the bones were modern, or very well-preserved, the remaining samples were filtered using Polyethylene

Ezee filters (Elkay Laboratories Ltd., 9 mL, pore size 60-90 µm). There were not isotopic differences between ultrafiltered and non-ultrafiltered samples (t-test, t = -1.44; p = 0.223; t = -1.78; p = 0.148), in agreement with previous studies (Sealy et al., 2014). Samples were then frozen and lyophilised.

Lipid extraction: bone, adipose tissue and potsherds

Lipids were extracted from archaeological (~160 - 580 mg) and modern bones free of marrow (~20 - 250 mg) following a two-step method (Colonese et al., 2015). Archaeological and modern bones were rinsed several times in distilled water. Archaeological bones were dried at room temperature while modern bones were frozen and subsequently freeze-dried. Mechanically cleaned chunks or coarsely ground bones were lipid-extracted using the same procedure as for defatting modern bones (see above). Exogenous lipids were first removed with 2:1 DCM:MeOH (3x2 mL) from each bone sample. The supernatant was removed, discarded and the bone powder was dried completely under a gentle stream of N_2. The remaining bone samples were lipid-extracted using acidified methanol (e.g. Correa-Ascencio and Evershed, 2014; Craig et al., 2013). After adding 2 mL of methanol, the samples were ultrasonicated for 15 min. Subsequently, 400 µL of H_2SO_4 was added and the samples were heated at 70°C for 4 h. The samples were then centrifuged (850 xg) for 5 min. The supernatant was extracted with hexane (3 × 2 mL) and neutralised with K_2CO_3. The extracts were then dried under a gentle stream of N_2 and an internal standard (10 µg hexatriacontane) added to each sample before further analysis by GC/MS and GC/C/IRMS. The same method (acidified methanol) was applied for the subcutaneous adipose tissue (5 and 100 mg), after tissues were rinsed repeatedly with deionized water, frozen and lyophilised.

Ceramic powder drilled (d. 2mm-5mm) from the internal sherd surface (~1 g) was lipid-extracted using the methanol (4 mL) and H_2SO_4 (800 µL) procedure as for bone lipids. A selection of samples (n = 10) were solvent extracted (DCM:MeOH; 2:1 vol/vol, 3x2 mL, 15 min) using established protocols (e.g. Dunne et al., 2012). These were silylated N,O-bis(trimethylsilyl)trifluoroacetamide at 70 °C for 1 h and analysed by high temperature GC (HT-GC) to determine the presence of any acyl lipids (mono-, di- and triglycerides).

Bone, adipose tissue and potsherd samples were screened by GC using an Agilent 7890A gas chromatograph (Agilent Technologies, Cheadle, Cheshire, UK). The injector was splitless and maintained at 300 °C and injected 1 µL of sample into the GC. The column used was a 100% Dimethylpolysiloxane DB-1 (15 m x 320 µm x 0.1 µm; J&W Scientific, Folsom, CA, USA). The carrier gas was hydrogen with a constant flow rate of 2ml/min. The temperature program was set at 100 °C for 2 minutes, rising by 20 °C/min until 325 °C. This temperature was maintained for 3 minutes. The total run time was 16.25 mins. The lipids were quantified according to the internal standard and diluted appropriately prior to GC-MS and GC-c-IRMS as described below. To avoid co-elution, samples with a higher concentration of $C_{18:1}$ were also treated with $AgNO_3$ to isolate the saturated counterpart prior to isotopic analysis. Stable isotope values of paired samples did not show evidence of isotopic fractionation between $AgNO_3$ treated and untreated samples.

Carbon and nitrogen stable isotope analysis of bulk collagen

Collagen samples (1 mg) were analysed in duplicate or triplicate by EA-IRMS in a GSL analyser coupled to a 20-22 mass spectrometer (Sercon, Crewe, UK) at the University of York. The analytical error for both $\delta^{13}C$ and $\delta^{15}N$ values, calculated from repeated measurements of each sample and measurements of the bovine control from multiple extracts, was <0.2‰ (1σ). $\delta^{13}C$, $\delta^{15}N$ = [(Rsample/Rstandard−1)] × 1,000, where R = $^{13}C/^{12}C$ and $^{15}N/^{14}N$. The standard for $\delta^{13}C$ is Vienna PeeDee Belemnite (V-PDB), the standard for $\delta^{15}N$ is air N_2. In-house collagen standards (bovine control) were exchanged between laboratories (University of Bradford) to ensure accuracy.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS was carried out on all samples using a 7890A Series chromatograph attached to a 5975C Inert XL mass-selective detector with a quadrupole mass analyser (Agilent Technologies, Cheadle, UK). The carrier gas used was helium, and the inlet/column head-pressure was constant. A splitless injector was used and maintained at 300°C. The GC column was inserted directly into the ion source of the mass spectrometer. The ionisation energy of the mass spectrometer was 70 eV and spectra were obtained by scanning between m/z 50 and 800. Three different analytical columns were used.

General screening was performed using a DB-5ms (5%-phenyl)-methylpolysiloxane column (30 m × 0.250 mm × 0.25 µm; J&W Scientific, Folsom, CA, USA). The temperature for this column was set at 50°C for 2 min, then raised by 10°C min^{-1} to 325°C, where it was held for 15 min. This column was also used with the MS in Single Ion Monitoring (SIM) mode to selectively detect ions characteristics of alkylphenyl alkanoic acids (APAAs; *m/z* 105, *m/z* 262, *m/z* 290, *m/z* 318) in order to increase the sensitivity for the detection of lipids derived from aquatic products (Evershed et al., 2008; Hansel et al. 2004). For this purpose, the temperature was set at 120°C for 2 min, raised by 6°C min^{-1} to 260°C, and then raised by 20°C min^{-1} to 325°C where it was held for 10 min.

A second, more polar column (DB-23, 50%-cyanopropyl-methylpolysiloxane, 60 m × 0.25 mm × 0.25 µm; J&W Scientific) was used to provide better resolution of isoprenoid fatty acids. The temperature was set at 50°C for 2 min, raised by 10°C min^{-1} to 100°C, then raised at 4°C min^{-1} to 250°C, where it was held for 20 min. This column was also used with the MS in Single Ion Monitoring (SIM) mode to selectively detect ions characteristics of APAA (*m/z* 105, *m/z* 262, *m/z* 290, *m/z* 318).

Solvent extracted samples were analyzed with a HT-DB1, 100% Dimethylpolysiloxane (15 m x 0.320 mm x 0.1 µm) (J&W Scientific, Folsom, CA, USA). The injector was maintained at 350°C. The temperature of the oven was set at 50°C for 2 min, and then raised by 10°C min^{-1} to 350°C, where it was held for 15 min.

Gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS)

Carbon stable isotopes were determined on two fatty acid methyl esters; methyl palmitate ($C_{16:0}$) and methyl stearate ($C_{18:0}$) in each extract using an Isoprime 100 (Isoprime, Cheadle, UK) linked to a Hewlett Packard 7890B series gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a Isoprime

GC5 interface (Isoprime, Cheadle, UK). The gases eluting from the chromatographic column were split into two streams. One of these was directed into an Agilent 5975C inert mass spectrometer detector (MSD), for sample identification and quantification, while the other was directed through the GC5 furnace held at 850°C to oxidise all carbon species into CO_2. All samples were diluted with hexane and subsequently 1 µL of each sample was injected into a DB-5MS fused-silica column. The temperature was set for 0.5 minute at 50°C, and raised by 10°C min^{-1} until 300°C was reached, at which it stayed for 10 minutes. The carrier gas was ultra-high purity grade helium with a flow rate of 3 mL min^{-1}. Eluted products were combusted to CO_2 and ionized in the mass spectrometer by electron impact. Ion intensities of m/z 44, 45, and 46 were monitored in order to automatically compute the $^{13}C/^{12}C$ ratio of each peak in the extracts. Computations were made with IonVantage Software (Isoprime, Cheadle, UK) and were based on comparisons with a standard reference gas (CO_2) of known isotopic composition that was repeatedly measured. The results from the analysis are reported in parts per mil (‰) relative to an international standard (V-PDB). Replicate measurements of each sample and a mixture of fames fatty acid methyl esters (FAMEs) with $\delta^{13}C$ values traceable to international standards were used to determine instrument precision (<0.3‰) and accuracy (<0.5‰). Values were also corrected subsequent to analysis to account for the methylation of the carboxyl group that occurs during acid extraction. Corrections were based on comparisons with a standard mixture of $C_{16:0}$ and $C_{18:0}$ fatty acids of known isotopic composition processed in each batch as a sample.

Statistical analysis

Fatty acid $\delta^{13}C$ values from bone and adipose tissue in modern chicken and pig samples were compared using a paired t-test (after checking for equality of variance with F test) in PAST 3.x (Hammer et al., 2001).

Theoretical mixing curves were computed in order to estimate the effect of mixing of different animal fats on the fatty acid $\delta^{13}C$ values of ceramic sherds (e.g. Dudd 1990; Evershed 2008; Mukherjee et al., 2008). Four mixing curves were computed between chicken and pig, goose, ruminant adipose and milk fat. This approach takes into account the average $\delta^{13}C$ values and the relative abundance of $C_{16:0}$ and $C_{18:0}$ fatty acids from each animal fat. Fatty acid $\delta^{13}C$ values for goose, pig, and chicken were taken from the archaeological bones, while fatty acid $\delta^{13}C$ values for ruminant adipose and milk were sourced from the literature (Dudd and Evershed 1999). The relative proportions of $C_{16:0}$ and $C_{18:0}$ fatty acids (average % of free fatty acid distribution) were taken from Dudd (1990).

Results and discussion

Molecular and stable isotope composition of modern bone and adipose tissue

Derivatized fatty acids from bone and adipose tissues were similar for both chicken and pig samples (Fig. 1A-B), except for a lower concentration of unsaturated and polyunsaturated fatty acids recovered in bone, which can be in part attributed to the solvent wash. Similar results were obtained for pig samples. In chicken and pig the main lipids extracted were $C_{18:1}$, $C_{16:0}$, $C_{18:0}$, $C_{18:2}$, $C_{20:4}$. In chicken these were followed by other saturated ($C_{12:0}$ to $C_{24:0}$), monounsaturated ($C_{14:1}$, $C_{17:1}$), polyunsaturated fatty acids

($C_{20:2}$, $C_{20:3}$, $C_{20:5}$) and cholesterol derivatives (particularly in bone; Evershed et al., 1995b). The fatty acid distribution is consistent with poultry fat reported in the literature (Givens et al., 2011; Koizumi et al., 1991) and some variations in relative abundance between groups most likely reflect diversification in dietary components (Crespo and Esteve-Garcia, 2002). In pig samples, other extracted lipids also include saturated ($C_{12:0}$ to $C_{24:0}$), monounsaturated ($C_{17:1}$ to $C_{24:1}$), polyunsaturated fatty acids ($C_{20:2}$, $C_{20:3}$, $C_{20:5}$, $C_{22:4}$, $C_{22:5}$, $C_{22:6}$) and cholesterol derivatives (bone).

The $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ in bone and adipose tissues are reported in Table 1. Free range organic C_3 specimens (Group 1) have average $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ in subcutaneous fat (-29.2±1.0‰ and -28.9±1.2‰ respectively) statistically indistinguishable from bone (-29.0±0.9‰ and -28.5±0.7‰) (paired t-test, t =-1.018, p = 0.335 and t = -1.659, p = 0.131 respectively), with mean pairwise differences in $\delta^{13}C$ between adipose and bone lipids of 0.2‰ and 0.4‰ for $C_{16:0}$ and $C_{18:0}$ respectively. In commercial non-free range chickens (Group 2), the average $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ are significantly lower in adipose tissue (-29.2±0.6‰ and -29.4±0.4‰) compared to bone (-28.5±0.3 and -28.3±0.3‰) by 0.8‰ (paired t-test, t =-6, p = 0.003) and 1.1‰ (t =-7.78, p = 0.001) respectively. No significant differences were observed in the distribution of $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ between adipose tissue (-29.3±0.6‰ and -28.4±0.6‰) and bone (-29.5±0.7‰ and -28.6±0.3‰) from commercial pork samples (paired t-test, t =1.15, p = 0.327 and t = -1.17, p = 0.304 respectively). The small isotopic offset between adipose and bone lipid in chicken noticed in Group 2 might reflect differential turnover rate and isotopic fractionation during *de novo* synthesis and assimilation from diet of fatty acid in liver, bone and adipose tissue (Nir et al., 1988). However, we note that the magnitude of the adipose/bone pairs is not great.

Comparisons between bone fatty acid $\delta^{13}C$ values reveal no overall differences between specimens from Groups 1 and 2 (organic free range and battery) for both $C_{16:0}$ (t = -1.18, p = 0.256) and $C_{18:0}$ (t = -0.61, p = 0.549; Fig. 2). By contrast, non-commercial free-range specimens (Group 3) have $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ in bone (-25.4±0.6‰ and -25.8±0.9‰) significantly higher than specimens from both groups 1 and 2 ($p < 0.005$ for both groups). Average $\delta^{13}C$ values of $C_{16:0}$ (-30.2‰) and $C_{18:0}$ (-29.4‰) in adipose chicken fat reported by Dudd and Evershed (Dudd and Evershed, 1998) also differ statistically from bone lipid from groups 1, 2 and 3 ($p < 0.005$ for all groups). The dietary regimes the animals were reared under have a clear effect on the isotopic composition of their fatty acid values.

In agreement with previous studies (Colonese et al., 2015; Stott et al., 1997b) both $C_{16:0}$ and $C_{18:0}$ are depleted in ^{13}C relative to bulk collagen from the same samples (Tab. 2). However, the range of the offset (ca. 3‰ to 7‰) is greater than previously observed. The isotopic offset fundamentally reflects differences between the biosynthesis of collagen and fatty acids, and particularly the kinetic isotopic fractionation associated to the oxidative decarboxylation of pyruvate to acetyl Co-A during lipid biosynthesis (DeNiro and Epstein, 1977). Strong positive correlations between bone lipid and collagen $\delta^{13}C$ and $\delta^{15}N$ values confirm that the lipids are endogenous (Fig. 3).

The results indicate that the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ in bone reflect the isotopic composition of adipose fat in chicken and pig samples. Therefore, we conclude that fatty acids from bone are a suitable reference for comparison with adipose derived fatty acids extracted from archaeological pottery.

Molecular and stable isotope composition of archaeological bone

The lipid extracts from archaeological bones (chicken, goose, pig) are in general dominated by saturated fatty acids ($C_{12:0}$ to $C_{28:0}$), notably $C_{16:0}$ and $C_{18:0}$, and by lower concentrations of monounsaturated fatty acids (particularly $C_{18:1}$), along with some products of cholesterol oxidation (cholesta-3,5-diene; Evershed et al., 1995b). Traces of linoleic acid ($C_{18:2}$) were present in chicken, pig and goose bones, while phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) was recorded in all goose bones. These compounds are compelling evidence for the endogenous origin of lipids in the bone as they can only be incorporated through diet and are rare in the depositional environment. Phytanic acid in the goose samples is likely to be attributable to the direct ingestion of aquatic organisms (zooplanktons) (Lucquin et al., 2016; van den Brink et al., 2004; Wanders et al., 2011).

Archaeological bones yielded sufficient amounts of fatty acids for stable carbon isotopic analysis (Tab. 3). No significant correlations were observed between total fatty acid yield (µg g^{-1}) and the $\delta^{13}C$ values for both $C_{16:0}$ (R^2 = 0.04, p = 0.30) and $C_{18:0}$ (R^2 = 0.10, p = 0.11) respectively. Similarly, no significant correlation was observed between $C_{16:0}$ yields and their $\delta^{13}C$ values (R^2 = 0.13, p = 0.06), while significant but a very week correlation was found between $C_{18:0}$ yields and their $\delta^{13}C$ values (R^2 = 0.20, p = 0.02). The results confirm that degradation of fatty acids have no effect on their stable carbon isotope composition, as already demonstrated for fatty acids preserved in archaeological ceramics (e.g. Evershed et al., 1999). Furthermore moderate to high correlations between fatty acid and collagen $\delta^{13}C$ values (Fig. 4) support the preservation of lipids in our archaeological bone assemblages, as observed in previous studies (Colonese et al., 2014). The $\delta^{13}C$ values of archaeological bone lipids are compared with modern authentic carcass fat and milk for the UK (Copley et al., 2003; Dudd and Evershed, 1998; Evershed et al., 2002a) from ruminant and monogastric-omnivorous animals raised on C_3 plants (Fig. 5). As the diet between ancient and modern herbivores is unlikely to be very different in this context, we consider these values to be a suitable proxy.

The $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ in archaeological goose bone differ to those from UK modern references. There is larger variability in archaeological samples, which probably reflects different management strategies and environments, or potentially the presence of both wild and domestic geese in the archaeological sample (Budge et al., 2011; Trust Hammer et al., 1998). Finally, substantial isotopic differences are also observed between omnivorous animals, notably chickens and pigs. Although pigs and chickens were being raised at elite settlements, historical documents indicate that pigs were often supplied by lower status people to elites as food rents, with chickens also being provided in this manner (Hagen, 1995). Accordingly, the observed isotopic differences may in part reflect different management strategies by groups not resident at Flixborough.

Fatty acids from archaeological pig bones have $C_{16:0}$ and $C_{18:0}$ consistently depleted in ^{13}C by ~3‰ compared to those from modern pigs originating from the UK, but are within the observed range of $\delta^{13}C$ values for Central Europe (Spangenberg et al., 2006). Archaeological pig bones also have narrower $\delta^{13}C$ values in both $C_{16:0}$ (0.4‰) and $C_{18:0}$ (0.6‰) than the modern reference fat. By contrast, the $\delta^{13}C$ values of $C_{16:0}$ in chicken bones from Flixborough are higher by ~2‰ compared to the modern chicken sample.

Differences between archaeological and modern samples may be related to differences in foddering practices and access to food sources with variable lipid content and isotopic signatures. Most likely, archaeological pigs and chickens were raised on a mixture of plants and animal products, however measurable differences between pigs and chickens also point toward species-specific husbandry practices. This is further supported by collagen $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 6), which reveal that chickens had access to food sources at a higher trophic position than pigs or their diet contained a higher proportion of animal to plant products. Pathological data provide further information about how pigs were raised at Flixborough. Frequencies of Linear Enamel Hypoplasia on pig teeth indicate the Flixborough pigs were kept in a semi-natural husbandry regime, including use of a woodland environment, which provided suitable foraging opportunities for the pigs (Dobney et al., 2002). Interestingly, O'Connell and Hull's (2011) synthesis of animal isotope data from the Anglo-Saxon period (predominantly Early and Middle-Saxon) indicated that the degree of omnivory among the pigs was not high, showing some overlap with sheep and cattle. This was consistent with the pigs being raised in a free-range system, including pannage. The chickens and geese in their samples also have elevated $\delta^{15}N$ values compared to other species. Data from Flixborough thus supports the view that chickens were generally raised in local/household settings, while pigs had greater access to woodland products and higher plant content in their diet.

The contribution of animal products to chicken diet would be further confirmed by the correlations between $\delta^{13}C$ of fatty acids and collagen (Fig. 4). The high correlation between $\delta^{13}C$ values of $C_{16:0}$ and collagen reveals that the $C_{16:0}$ fatty acid in pig, chicken and goose bones was predominantly synthesized *de novo* from carbohydrates and protein precursors. By contrast the lower correlation observed between $\delta^{13}C$ values of $C_{18:0}$ and collagen, mainly due to an overall ^{13}C-depletion in the $C_{18:0}$ fatty acid of chickens is more difficult to explain. It could imply some degree of direct incorporation of $C_{18:0}$ fatty acid in chicken bone from diet (Baião and Lara, 2005; Villaverde et al., 2006).

Molecular and stable isotope composition of organic residues in archaeological ceramics

Appreciable amounts of absorbed lipids could be extracted from the vast majority of analyzed potsherds (90%, n = 54), demonstrating relatively good preservation, with concentrations up to ~2 mg g^{-1} (average 1.02 mg g^{-1}) compared to other studies (Evershed, 2008; Evershed et al., 2008). They revealed a range of saturated and unsaturated mid-chain length n-alkanoic acids (fatty acids) with even numbers of carbon atoms, particularly dominated by $C_{16:0}$ and $C_{18:0}$ (Tab. 5; Fig. 7). Most of these samples contain trace amounts of isoprenoid fatty acids (phytanic acid), short chain diacids, scarce amounts of cholesterol and mid-chain ketones. These compounds demonstrate that the vessels were used for heating animal fat, possibly along with plant resources (Baeten et al., 2013; Evershed et al., 1995a; Raven et al., 1997). Traces of C_{16} and C_{18} ω-(o-alkylphenyl) alkanoic acids (APAAs), likely formed from mono- di and tri-unsaturated fatty acid precursors (Hansel et al., 2004) were found in most of the sherds, along with short-chain alkanes. Significantly however, longer chain length APAAs typically formed from processing aquatic organisms (Evershed et al. 2008) were absent, even when the MS was used to selectively monitor ions from these compounds thereby maximising the chance of detection. Other lipids at high

concentration in aquatic tissues, such as 4,8,12-TMTD (Hansel et al. 2004), were also absent. Therefore there is no evidence that fish were processed in pottery from Flixborough.

Triacylglycerols (TAGs) were recovered from only two sherds out of ten that were tested using solvent extraction. The acyl carbon distribution of TAGs in these samples (C_{42}-C_{50} and C_{46}-C_{54}) is consistent with dairy and ruminant adipose fat respectively, and this inference is supported by their $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$. Apart from these observations, there were no other compounds that could be used to further resolve the source of the residue. Most likely, extensive degradation has substantially altered the original lipid distributions.

Identification of the degraded animal fats recovered from the pottery was assessed through the determination of the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ (Tab. 5). The results were compared with fatty acid $\delta^{13}C$ values from archaeological bones (pig, chicken, goose) and modern ruminant adipose and milk reference for the UK (Dudd and Evershed 1999). Over half of the potsherds (62.9%) have fatty acid $\delta^{13}C$ values broadly consistent with modern ruminant adipose fat (Fig. 8). However, as these values fall between the ellipses of modern ruminant adipose and dairy as well as archaeological monogastric-omnivore bone fats, mixing of these commodities would produce similar results. Such equifinality cannot be easily resolved. The remaining potsherds have fatty acid $\delta^{13}C$ values that fall within the reference ranges for archaeological bone fat from pig and goose (16.6%), chicken (9.25%) and modern ruminant milk (7.4%). Notably, the three vessels that produced almost identical values to archaeological chicken bones are from the 8-9th century phase (Fig. 8). These data are not as easily explained by mixing of different fats. From theoretical mixing curves, it is crudely estimated that these three ceramic sherds must contain at least 90% chicken fat if mixed with pig/goose or ruminant carcass fat (Fig 8). Modelling mixtures of other fats (curves not shown) does not produce values that plot within the chicken ellipse. The results therefore would represent the first direct identification of poultry lipids in archaeological ceramic vessels and implies that these three vessels, at least, were used largely for this purpose. However, further consideration of uncertainties associated with the reference ranges of different fatty acids in the source fats and their concentration is needed to discern the degree to which other commodities may have been mixed in these vessels. The deconvolution of multiple sources requires the development and application of more sophisticated mixing models.

The vessel typology was only evident in approximately half of vessels that produced interpretable lipid residues but some preliminary observations could still be made (Tab. 5). For example, residues from the omnivorous animals were only clearly distinguishable in jars, which generally had a wider range of uses compared to bowls. Similar results were observed in the early medieval site of West Cotton, in Britain, where jars contained the largest range of lipid compounds and may have had a more versatile function compared to other vessels (Charters et al., 1993). By contrast, pottery size and fabric did not influence lipid distribution and isotopic composition.

Study of the faunal bone assemblage from Flixborough has shown that animal exploitation was mainly focused on cattle and sheep, followed by pigs and poultry (chicken, geese) in the mid-8th to early 9th century. Organic residue analysis demonstrates that all these animals were indeed processed in ceramic

containers, as well as dairy products, as may have been expected. The pottery evidence shows that poultry were processed in the same way and probably mixed with multiple products from other livestock and perhaps perceived similarly, at least in terms of their culinary value. However, it is important to point out that using this isotopic approach we cannot differentiate lipids derived from chicken eggs or meat. Ruminant products (meat and/or dairy) appears to be the most frequently processed animal resource in ceramic containers, followed by dairy products and then omnivorous animals, including chickens, which to some extent show some similarities with the relative abundance of faunal remains (Jaques et al., 2007). However, further determination of the proportional contribution of poultry and other products to the pottery at Flixborough currently lacks accurate quantification using the approach we have described.

Archaeological implications

This study emphasises further the value of conducting isotopic analyses of residues and integrating zooarchaeological remains and ceramic artefacts. Compared to larger mammals such as cattle, sheep and pig, bird bones are often overlooked during excavation because of their small size and therefore potentially under-represented in many zooarchaeological assemblages. Similarly, avian products are rarely considered in studies of pottery use or when reconstructing human diet using stable isotopes. As the wider zooarchaeological evidence from England reveals a considerable increase in chicken remains from the early to the Late Anglo-Saxon period (Holmes, 2014), poultry products may have been a common commodity to consider when reconstructing both diet and pottery use from this period. These changes were not uniform across all of society however. Greater numbers of bones of chickens, and domestic birds more generally, are found at high status and ecclesiastical sites during the Middle and Late Anglo-Saxon periods (Poole and Lacey, 2014). Comparison of different sites thus has real potential to demonstrate differences in dietary intake and consumption practices between different parts of society. In earlier periods, residue analysis using the approach we describe is essential to understand the culinary role of poultry and distinguish this from other uses of chickens, such as in ritual practices or for fighting.

Conclusion

In this study we show that:

- $\delta^{13}C$ values of fatty acids ($\delta^{13}C_{FA}$) and collagen ($\delta^{13}C_{coll}$) from modern omnivorous animal bone are correlated and reflect diet
- $\delta^{13}C_{FA}$ values in bone reflects the isotopic composition of adipose fat in modern omnivorous animals and serves as a good proxy. However, it is reasonable to expect that different animal tissues (e.g. adipose, bone and bone marrow) were processed in the past and should be considered.
- $\delta^{13}C_{FA}$ ($C_{16:0}$ fatty acid) and $\delta^{13}C_{coll}$ values in archaeological bone are highly correlated. The former are therefore endogenous and both reflect diet

- there is substantial variation in $\delta^{13}C_{FA}$ values between archaeological bone and modern adipose tissues for monogastric-omnivorous animals reflecting different husbandry practices between past and present. Therefore it is highly unlikely that any global $\delta^{13}C_{FA}$ ranges can be derived for distinguishing monogastric-omnivorous animal fats
- archaeological bone $\delta^{13}C_{FA}$ values from omnivorous animals therefore complement or provide an alternative to modern reference fat for interpreting $\delta^{13}C_{FA}$ values from archaeological ceramic to determine their use
- fatty acids extracted from several vessels from the Anglo-Saxon site of Flixborough have similar $\delta^{13}C$ values as those from chicken bones in the same deposits providing the first compelling evidence of their processing
- substantial mixing of different ruminant and monogastric-omnivore products needs to be resolved in order to determine the importance and nature of poultry processing in the past

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Figure caption

Fig. 1. Partial gas chromatogram of adipose and bone lipid extracts from modern chicken tissues (CH-SP1) and archaeological chicken bone (CHK1). $C_{n:x}$ indicates fatty acid with n carbon atoms and x double bonds; P, phthalates. IS indicates internal standard.

Fig. 2. Plot of $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids from modern chicken bones and adipose tissue (Dudd and Evershed, 1998).

Fig. 3. Plot of $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids from modern chicken bones against their respective collagen $\delta^{13}C$ and $\delta^{15}N$ values.

Fig. 4. Plot of $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids from pig, chicken and geese bones against their respective collagen $\delta^{13}C$ and $\delta^{15}N$ values.

Fig. 5. $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids from archaeological bones. Data are compared with ellipses (68% confidence) calculated for fatty acid $\delta^{13}C$ values of modern samples from the UK (Dudd and Evershed, 1998).

Fig. 6. Collagen $\delta^{13}C$ and $\delta^{15}N$ values of archaeological bones.

Fig. 7. Partial gas chromatogram of main lipid extracts from Anglo-Saxon potsherds at Flixborough. Fatty acid $\delta^{13}C$ values are indicative of dairy (A), ruminant fat (B), possible chicken fat (C), and goose/pig fat (D). Partial HT chromatogram of a solvent extract (E). $C_{n:x}$ indicates fatty acid with n carbon atoms and x double bonds. Phy. - phytanic acid, Alk - *n*-alkanes, K - mid-chain ketones, Ch - Cholesterol derivatives. MAGs are monoacylglycerols of carbon chain length x, DAGs are diacylglycerols and C_x TAGs are triacylglycerols of carbon chain length x. IS indicates internal standard.

Fig. 8. $\delta^{13}C$ values for the $C_{16:0}$ and $C_{18:0}$ fatty acids extracted from Anglo-Saxon potsherds at Flixborough. Each data point represents an individual vessel. Data are compared with ellipses (68% confidence) calculated for the $\delta^{13}C$ values of archaeological bone lipids (dotted) and modern adipose and milk (Dudd and Evershed, 1998). The theoretical mixing curves illustrate the $\delta^{13}C$ values which would result from the mixing of chicken fat with ruminant adipose and milk, goose and pig fat (unfilled circles represent 10% increments between mean values).

Table caption

Table 1. $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids of bone and adipose tissues from modern chicken (CH) and pig samples.

Table 2. The $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen extracted from modern chicken and pig samples and the offset with the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids in bone. Samples extracted with Ultrafiltration method*

Table 3. $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids of archaeological bones.

Table 4. The $\delta^{13}C$ and $\delta^{15}N$ values of archaeological bone collagen.

Table 5. The description of the pottery sherds submitted for analysis and details of the absorbed lipid residues detected. Composition of lipid extracts. Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; DUFA, diunsaturated fatty acid; K, mid-chain ketones; ALK, *n*-alkanes; ALC, *n*-alcohols; Di, diols; D, diacids; CH, cholesterol, Phy, Phytanic acid; APAA, C16 and C18-ω-(o-alkylphenyl) alkanoic acids; MAG, monoacylglycerols; DAG, diacylglycerols; TAG, triacylglycerol. Phases, contexts and relative age (AD) from Young and Vince (Young and Vince, 2009). Samples trimethylsilylated*. Attributions have been made according to the $\delta^{13}C_{FA}$ proximity to the reference ranges shown in Figure 5 and therefore indicate only the most prominent source of animal fat contributing to the contents.

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