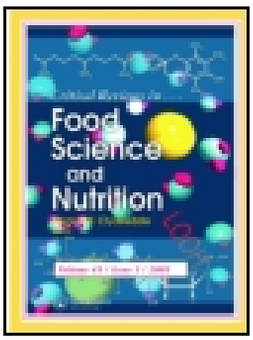

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Principles and Limitations of Stable Isotopes in Differentiating Organic and Conventional Foodstuffs: 2. Animal Products

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Abstract

In this review, we examine the variation in stable isotope signatures of the lighter elements ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{34}S$) of tissues and excreta of domesticated animals, the factors affecting the isotopic composition of animal tissues and whether stable isotopes may be used to differentiate organic and conventional modes of animal husbandry. The main factors affecting the $\delta^{13}C$ signatures of livestock are the C3 / C4 composition of the diet, the relative digestibility of the diet components, metabolic turnover, tissue and compound specificity, growth rate and animal age. $\delta^{15}N$ signatures of sheep and cattle products have been related

mainly to diet signatures, which are quite variable among farms and between years. Although few data exist, a minor influence in $\delta^{15}N$ signatures of animal products was attributed to N losses at the farm level, whereas stocking rate showed divergent findings. Correlations between mode of production and $\delta^{2}H$ and $\delta^{18}O$ have not been established, and only in one case of an animal product was $\delta^{34}S$ a satisfactory marker for mode of production. While many data exist on diet-tissue isotopic discrimination values among domesticated animals, there is a paucity of data which allow a direct and statistically-verifiable comparison of the differences in the isotopic signatures of organically- and conventionally-grown animal products. The few comparisons are confined to beef, milk and egg yolk, with no data for swine or lamb products. $\delta^{13}C$ appears to be the most promising isotopic marker to differentiate organic and conventional production systems when maize (C4) is present in the conventional animal diet. However, $\delta^{13}C$ may be unsuitable under tropical conditions where C4 grasses are abundant, and where grass-based husbandry is predominant in both conventional and organic systems. At the present time there is no universal analytical method that can be applied to differentiate organic and conventional animal products.

Keywords animal diet, animal husbandry, conventional, $\delta^{13}C$, $\delta^{15}N$, isotope discrimination, organic, stable isotopes

INTRODUCTION

Of the 37.2 million ha of agricultural land devoted to the production of organic foodstuffs in 2009, approximately two-thirds (23.2 million ha) was grassland / grazing areas mainly for beef and dairy cattle (IFOAM, 2013). Thus the production of organic animal products may represent a significant proportion of the total production of organic foodstuffs valued at USD 62.9 billion in 2011 (IFOAM, 2013), although hard statistical data are lacking. Organically-produced animal products attract a value-added price in the market place, and therefore it is important to have protocols in place to monitor and detect illegal mislabelling of the products.

In a previous article, Inácio et al. (2013) reviewed the principles and limitations of using stable isotopes to differentiate plant products from organic and conventional production regimes. Interest in the application of stable isotopes to differentiate mode of production stems from their demonstrated usefulness in identifying adulteration of foodstuffs (Rossmann, 2001) and appellation of origin (Kelly et al., 2005). For example, the isotopic composition of hair of mammals may help to access information on the animalôs diet ($\delta^{13}C$, $\delta^{15}N$), nutritional status ($\delta^{15}N$), and location and movements ($\delta^{2}H$, $\delta^{13}C$, $\delta^{18}O$, $\delta^{34}S$, $\delta^{87}Sr$) (West et al., 2004).

The most useful isotopic signature for identifying mode of production of plant products is ŭ^{15}N (Inácio et al., 2013), because the organic fertilizers (e.g. manures, composts) used in organic systems are significantly enriched in this isotope compared with synthetic N fertilizers used in conventional systems. However, the situation regarding organic animal products is likely

to be quite different, as animal husbandry in organic systems (e.g. free range) differs markedly from the husbandry practiced in confined animal feeding operations (feedlots, cages), which by definition are considered to be non-organic.

The use of ŭ^{15}N to establish mode of production of animal products may be less useful than for plant products because of the relatively narrow range in animal diets. For example, Schwertl et al. (2005) reported mean δ^{15}N values of +2.3 ± 2.1 for pastures and silage, +1.2 ± 0.5 for legumes and +3.3 ± 1.4ă for commercial concentrates. On the other hand, the δ^{13}C composition of animal products may be a more useful index of mode of production, since the diet will consist of grains or fodder originating from C3- or C4-species that differ markedly in δ^{13}C signatures (Inácio et al., 2013), which could enable the animal husbandry system to be identified. The objective of the present review is to synthesize and analyze the published literature to determine whether the mode of production of foodstuffs derived from domesticated animals can be differentiated on the basis of stable isotope composition alone or in combination with statistical and / or other analytical techniques.

STANDARDS FOR ORGANIC ANIMAL PRODUCTS

Organic livestock production is mainly based on the physiological and behavioral needs of animals and access to organically-grown feedstuffs. Therefore, appropriate stocking rates and natural grazing or foraging traits are perceived to promote normal behavioral patterns, health and welfare, while reducing stress, disease and parasitism. The use of veterinary drugs and

antibiotics is avoided. According to IFOAM (2005) animals may be fed with a limited percentage of non-organic feed under specific conditions for a limited time, and in no case may the percentage of non-organic feed exceed 10% dry matter per ruminant and 15% dry matter per non-ruminant (annual basis). In addition, ruminants should not be fed a diet that consists entirely of silage and concentrates. Many substances are prohibited in the diet: farm animal by-products (e.g. abattoir waste) for ruminants, all types of excrement, urea and other synthetic nitrogen compounds, synthetic growth promoters or stimulants. Derogations are reported for both diet and animal health. e.g. legal vaccination, extreme climatic conditions.

STABLE ISOTOPIC SIGNATURES OF ANIMAL TISSUES AND EXCRETA

The intake of diverse herbaceous materials (forages, grains) and water are the primary determinants of the isotopic signatures of animal tissues and excreta, whereas physical, chemical and biological fractionation processes during metabolism are the secondary and final determinants of isotopic composition. Due to these two principal and interacting factors, animal tissues and excreta exhibit wide variations in stable isotopic signatures of the lighter elements ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{34}S$).

Therefore, the isotopic discrimination i.e. the difference in isotopic composition expressed in per mil between the animal diet and tissue ($\Delta_{diet\text{-}tissue}$) vary in a wide range for $\delta^{13}C$ (–8.8 to +6.1ă) and for $\delta^{15}N$ values (–3.2 to +9.2ă), with an average of 0.75ă (SE = 0.11) and 2.75ă (SE = 0.10), respectively (Caut et al., 2009). Caut et al. (2009) state that the parameters which

explain the variability of discrimination ñcan be grouped at two scales: (i) the *individual scale*, which includes the consumer class and species, tissues and organs, physiological stress, and the form of N excretion; (ii) the *diet scale*, which includes the diet protein quality, the type of food and the diet isotopic ratioò. In addition, the same authors point to a significant negative relationship between both $\Delta^{13}C$ and $\Delta^{15}N$ discrimination and their corresponding diet isotopic ratios, and also propose a method to calculate discrimination based on diet isotope ratios (Diet-Dependent Discrimination Factor, DDDF).

$\delta^{13}C$

The ŭ^{13}C signatures of animal tissues and excreta will reflect the livestock diet which is related to the mode of production i.e. it will reflect the distinct ŭ^{13}C signatures of C3 or C4 plant material in the diet (Table 1). The ŭ^{13}C signatures of carbonate in mammalian dental bioapatite have been used to reconstruct diet on archaeological and geological time-scales (Passey et al., 2005). However, the tissue isotopic composition may be enriched or depleted in relation to diet. On average, the animal body is enriched in ^{13}C in relation to diet (respired CO_2 is depleted in ^{13}C), but the δ^{13}C values may differ by up to 2ă among individuals of a species fed with the same diet (DeNiro and Epstein, 1978).

Diet-tissue ^{13}C discrimination is caused by physical and chemical processes involved in the synthesis and construction of tissues (Hatch et al., 2002). Passey et al. (2005) showed the relationship of δ^{13}C enrichment of tooth enamel bioapatite in relation to diet (ŭ^{13}C = 11.5 to 14.6ă) with inter-species differences due to digestive physiology i.e. ^{13}C-depleted methane

production in the digestive tract. Furthermore, different tissues and compounds may not reflect the bulk $\delta^{13}C$ diet values, but may reflect the isotopic signatures of different dietary components, due to the differential nutrient routing and digestibility of the diet (Gannes et al., 1998).

Mammalian herbivore tissues (hair and muscles) and blood tend to be enriched in ^{13}C relative to the diet. An exception is the depletion in ^{13}C during the synthesis of lipids. e.g. muscle or kidney fat (Kim et al., 2012; Sponheimer et al., 2003a; De Smet et al., 2004). This depletion in ŭ^{13}C of lipids (e.g. glucose ŭ^{13}C = ï9.5ă into lipid ŭ^{13}C = ï15.7ă) results from isotopic fractionation during the oxidation of pyruvate to acetyl CoA which concentrated the depletion of ^{13}C in the carbonyl-C atom (DeNiro and Epstein, 1977). These authors also demonstrated the temperature dependence and source effect on the C isotopic composition of lipids in *in vitro* assays. The animal species also has an influence on ^{13}C discrimination. e.g. diet-muscle discrimination was reported as +1.9ă in lamb (Harrison et al., 2011), +3.0ă in beef (Bahar et al., 2009) and ï2.1ă in swine (Nardoto et al., 2006), probably due to the different intramuscular lipid contents.

Discrimination of ^{13}C in cattle hair and other mammalian herbivores ranges from +2.6 to +3.5ă (De Smet et al., 2004; Schnyder et al., 2006; Zazzo et al., 2007; Osorio et al., 2011), whereas animal feces are slightly depleted (e.g. ï0.8ă , Sponheimer et al., 2003a; ï0.9ă , Norman et al., 2009) and urine is slightly enriched in ^{13}C (+0.8ă , Norman et al., 2009). In addition, feces and rumen samples, which represent the indigestible parts of the plant diet, provided a better prediction of short-term (18 days) diet changes (C3 to C4) than wool of sheep,

that has a longer turnover rate, being an integrator of long-term dietary intake (Zazzo et al., 2008; Norman et al., 2009).

$\delta^{15}N$

The ^{15}N isotopic composition of animal tissue and excreta depends on the ŭ^{15}N signature of the animal diet as well as fractionation during assimilation and metabolism (McCutchan et al., 2003). Fractionation during deamination and transamination and the synthesis of protein with ^{15}N-enriched amino acids lead to ^{15}N enrichment in tissues of terrestrial animals (diet-tissue ^{15}N discrimination) (DeNiro and Epstein, 1981; Gannes et al., 1998). Hence, animal-derived products and animal feces tend to show higher values of ŭ^{15}N than the diet, and urine tends to be depleted in ^{15}N (Table 2).

The magnitude of the ^{15}N discrimination is linked to the main biochemical form of nitrogenous excretion, tissue-specific composition and the protein content of the diet. Ureolitic (urea) animals (bovines) had mean ^{15}N enrichment (+3.1ă) compared with uricoletic (uric acid) animals such as poultry (+2.7ă), but the difference was not statistically significant (Vanderklift and Ponsard, 2003). Ammonium (NH_4^+) is produced in protein metabolism, and hence the reactions to transform ammonium into urea or uric acid involve ^{15}N isotope fractionation with urea and uric acid waste products being depleted in ^{15}N compared to ammonium. For instance, Knobbe et al. (2006) reported ŭ^{15}N mean values of ï 3.2 ± 0.25ă and +3.7 ± 0.27ă for dairy urine and the milk, respectively, and Wittmer et al. (2011) reported ŭ^{15}N mean values of +3.6 ± 0.12ă and +5.9 ± 0.12ă for sheep feces and wool, respectively (Table 2). The tissue ŭ^{15}N

value might be amplified in starving animals which tend to preferentially void ^{15}N-depleted excreta and resynthetise protein with ^{15}N-enriched amino acids (Gannes et al., 1998). In addition, for mammals but not for birds, the ^{15}N discrimination shows a significant negative correlation with the diet N isotopic ratio (Caut et al., 2009).

$\delta^{2}H$ and $\delta^{18}O$

The global hydrological cycle has a direct effect on the incorporation of ^{18}O and ^{2}H into the body water and tissues of terrestrial herbivores mainly through the drinking water, the free water in the feed and water in the air (Kohn, 1996). Bound O and H in the feed also contribute, while O_2 in the air also affects ^{18}O composition. The δ^{2}H and δ^{18}O composition of meteoric water follows a predictable geographical pattern that is related to latitude, altitude, distance from the coast and the amount of precipitation at the location (Dansgaard, 1964). The ŭ^{2}H and ŭ^{18}O composition of meteoric water is affected by physical phenomena such as condensation and evaporation. Temperature is the main variable that inversely influences fractionation of ^{2}H and ^{18}O (Dansgaard, 1964; Gat et al., 1996). Ocean water is defined as 0ă for each isotope, and depleted δ values are measured in the meteoric water and ocean vapor, while relatively enriched δ values are expected for body waters such as lakes (Gat, 1996). The annual mean ŭ^{18}O in meteoric water ranges from +2 to –2ă in equatorial regions to as low as –22ă in the north polar region (Gat, 1996).

There is no isotopic fractionation during water uptake by terrestrial plants, and therefore water in the stem shows the same isotopic composition as the water source i.e. shallow *vs*. deep soil water (Dawson et al., 2002). However, due to transpiration plant leaf water is enriched in ^{2}H and ^{18}O relative to surface water (Dawson et al., 2002). Low water content in plants causes a decrease in herbivore ŭ^{18}O because the intake of lower ŭ^{18}O surface water must increase to maintain water balance. C3 and C4 plants can exhibit differences in ŭ^{18}O signatures (Kohn, 1996). C4 plants may be enriched in ŭ^{18}O compared with C3 plants, with small differences (< 1ă) in cool and humid environments, but with larger differences (10ă) in hot and arid areas (Kohn, 1996).

The relative contribution of drinking water and diet to the isotopic composition of animal tissues or body water appears to be quite variable, and depends on the type of animal and the tissue or product sampled. For example, Hobson et al. (1999) demonstrated that while both drinking water and diet contributed to the non-exchangeable ^{2}H of both metabolically active (muscle, liver, blood, fat) and inactive (feather, nail) tissues of quail, diet had the major effect. The drinking water was shown to be the main source of muscle oxygen in lambs, while the feed was the main source of muscle H (Harrison et al., 2011). On the other hand, Chesson et al. (2010) found highly significant correlations ($R^2 > 0.99$) for both δ^2H and δ^{18} between paired samples of milk and drinking water across eight locations in the USA, within a range of δ^2H_{milk} of ī 110 to 0ă , δ^2H_{water} of ī 120 to 0ă , $\delta^{18}O_{milk}$ of ī 12 to ī 1ă and $\delta^{18}O_{water}$ of ī 15 to ī 2ă .

δ³⁴S

The ŭ^{34}S signatures of animal tissues and products reflect the organic and inorganic S composition of the diet. Fractionation of ^{34}S has been reported as negligible in animals (González-Martín et al., 2001; McCutchan et al., 2003; Harrison et al., 2011). However, Tanz and Schmidt (2010) reported an enrichment of +2 to +5ă for hoof and hair (goat) and +1 to +2ă for muscle tissue which is almost free of sulfate, but a depletion of ^{34}S in cartilage of –5 to –6ă relative to diet (^{34}S-enriched sulfate is excreted in the urine). Schmidt et al. (2005) found higher values of ŭ^{34}S in organic (+7.9 ± 0.6ă) than conventional Irish beef (+7.2 ± 0.4ă) and they speculated about the influence of the use of seaweed as a fertilizer or feed supplement in organic animal husbandry. Osorio et al. (2011) also found significantly (P<0.001) higher values of ŭ^{34}S (+6.0 ± 0.4ă) in muscle of concentrate-fed beef cattle than those growing under a pasture-containing diet (+4.6 to +4.8ă) reflecting the high ŭ^{34}S values of the concentrate mix (+6.5ă). In fact, some commercial pelleted maize concentrate may contain seaweed which had a high ŭ^{34}S signature of +8.3 ± 0.8ă (Harrison et al., 2011). Nevertheless, ŭ^{34}S values vary markedly among forages, with higher values for some legumes (+7.9ă) and sunflower (+6.3ă) than cereal grains (e.g. maize, +4.4ă ; barley, +5.1ă) (González-Martín et al., 2001), and some temperate grasses (+4.9 ± 3.0ă) and grass silage (+3.9 ± 0.5ă) (Osorio et al., 2011). In contrast, the ŭ^{34}S value of milk did not differ significantly between organic and conventional farms (Molkentin and Giesemann, 2007, 2010).

In general, seasonal variations in the ŭ^{34}S signatures of animal tissues or products are expected to follow the seasonality of the ŭ^{34}S signatures of feedstuffs, location (sea spray)

(Zazzo et al., 2011), use of dietary supplements, atmospheric deposition related to industrial production and other sources of sulfate or elemental S° (Schmidt et al., 2005). In addition, the half-life of S (~50 days) was similar to C in ovine *longissimus dorsi* (LD) (Harrison et al., 2011), but much longer (219 days) than C (151 days) in bovine LD (Bahar et al., 2009). Another example of the influence of diet is the Iberian-breed swine diet based on acorns which have a higher ŭ^{34}S signature (+9.1ă) than commercial feedstuffs based on grains (+5.0ă) (González-Martín et al., 2001). Therefore, as discussed for plant products (Inácio et al., 2013), it is unlikely that the ŭ^{34}S signatures of animal products can be used to differentiate organic and conventional production systems, except in specific instances.

FACTORS AFFECTING THE ISOTOPIC COMPOSITION OF ANIMAL TISSUES

δ^{13}C

Composition of the diet

The proportion of C3 and C4 plant materials in the diet affects the $\delta^{13}C$ signatures of animal tissues and products. A strong positive linear relationship has been reported between $\delta^{13}C$ values of animal tissues / products and maize (grain and silage) content (Bahar et al., 2005, 2009; Knobbe et al., 2006; Camin et al., 2008; Moreno-Rojas et al., 2008; Rhodes et al., 2010) or the proportion of C4 forages in the diet (Schmidt et al., 2005; Norman et al., 2009). Less negative $\delta^{13}C$ values in animal products have been found when C4 plants and derived products (e.g. corn

or sugar cane bagasse) are present in the animal diet (Table 3) compared to animals fed exclusively with C3 plants and derived products. Nevertheless, concentrates with a high proportion of C3 grains and derived products (e.g. rolled barley, soybean meal, beet pulp), which are non-photosynthetic organs of the plant, also show less negative $\delta^{13}C$ values (ï 27.2 ± 1.3ă) than C3 pasture (ï 30.9 ± 0.8ă) (Osorio et al., 2011). In this case, the introduction of concentrate into the diet of beef cattle made the $\delta^{13}C$ values of the muscle samples less negative than from pasture fed animals (Table 3). In addition, Schnyder et al. (2006) reported that seasonal variations in soil water availability which can influence the plantôs rate of photosynthesis were significantly related to variations in $\delta^{13}C$ signatures in pastures ($R^2 = 0.86$, $P<0.001$) and in cattle hair samples ($R^2 = 0.84$, $P<0.001$). It should also be noted that $\delta^{13}C$ values of leaf lipids (n-alkanes) are depleted relative to the bulk tissue $\delta^{13}C$ value, and the discrimination may be different among C3 (ï 5.9ă), C4 (ï 9.9ă) and CAM (ï 11.0ă) plants (Collister et al., 1994).

The $\delta^{13}C$ values of milk were more negative under grass feeding (C3) than under maize-based feeding (mix C3 / C4 plants) (Knobbe et al., 2006; Table 3). Camin et al. (2008) found that each 10% increment of maize content corresponded to a shift increase of 0.7 to 1.0ă in the $\delta^{13}C$ value of milk casein, and suggested a threshold $\delta^{13}C$ value of ï 23.5ă , above which it is not possible to exclude the presence of maize in the diet. Hence, the $\delta^{13}C$ signature of dairy products could determine the amount of maize in the diet (Camin et al., 2008). A similar relationship was found in beef cattle which reflected the $\delta^{13}C$ values of the maize silage diet (Bahar et al., 2005). Besides beef and dairy cattle, the strong positive linear relationship between the proportion of maize in the diet and the $\delta^{13}C$ value of animal products has permitted the period (days) of maize feeding in poultry and the corn-fed status of commercial samples of chickens to be estimated

(Rhodes et al., 2010). Also the $\delta^{13}C$ signatures for lamb (Piasentier et al., 2003; Moreno-Rojas et al., 2008) and swine (González-Martín et al., 1999, 2001) allowed a variety of diets with different $\delta^{13}C$ values to be differentiated, although small differences were found for swine (Tables 4 and 5).

In tropical and sub-tropical climates, C4 grasses (e.g. *Brachiaria*, *Panicum*, *Pennisetum*) are abundant in native and improved pastures. Therefore it would not be possible under such climates to distinguish free-range animal products from those originating from maize-based confined feeding, since the $\delta^{13}C$ signatures of C4 maize and the C4 grasses are the same. For example, similar $\delta^{13}C$ values were reported for Brazilian cattle raised on C4 pasture grasses and maize-based feedstuffs (Heaton et al., 2008). A similar situation exists in the USA where it would not be possible to differentiate a maize based intensive animal product from one raised on improved pastures of hybrid Bermudagrass (*Cynodon dactylon* L. var. Coastal), a C4 species (Chen et al., 1971) widespread across the southern states. Thus beef samples from the USA (ï 12.3 ± 0.1ă) and Brazil (ï 10.0 ± 0.6ă) showed similar but less negative $\delta^{13}C$ values than samples from northern Europe (ï 21.6 ± 1.0ă) mainly because of contrasting proportion of C3 and C4 plants in the cattle diets (Schmidt et al., 2005; Table 3). A worldwide survey of fast-food (burger) isotope composition showed that $\delta^{13}C$ values varied in a range of ï 25.4ă (UK, only C3 pasture) to ï 11.1ă (Brazil, where C4 pasture is abundant), with intermediate values representing cattle-rearing based on a mixture of C3- and C4-feedstuffs (Martinelli et al. 2011).

Digestibility of the diet

The digestibility of dietary components (% DM) is a factor that influences the $\delta^{13}C$ signatures of animal products because feedstuffs with low digestibility will contribute less to tissue formation, and consequently $\delta^{13}C$ signatures of tissues or animal products will not mirror the bulk $\delta^{13}C$ value of the diet (Hatch et al., 2002; Camin et al., 2008; Norman et al., 2009). It is significant in the case of monogastric animals (e.g. swine) which do not digest cellulose (Hatch et al., 2002). As a consequence, cellulose will not contribute to the $\delta^{13}C$ signatures of swine tissues, but its signal will be expressed in the feces. Codron et al. (2011) studied the $\delta^{13}C$ signatures of feces and blood of goats (i.e. a ruminant) and demonstrated the influence of digestibility and protein content of the C3 and C4 feed sources. Feces and blood over-expressed the $\delta^{13}C$ signature of the C3 feed source because of the lower fiber digestibility than the C4 grass, and blood under-expressed the $\delta^{13}C$ signature of the C4 feed source due to the low protein content of the grass (Codron et al., 2011).

The prediction of $\delta^{13}C$ values of sheep rumen solids and feces samples due to short-term dietary changes was improved by taking into account the differences in digestibility among C3 and C4 forages (Norman et al., 2009). In the same way, energy allowance (EA) in the diet of sheep affected $\delta^{13}C$ signatures of muscle and wool samples with significantly (P = 0.0003) higher $\delta^{13}C$ values when higher EA (MJ kg^{-1} DM, ~DM digestibility) was available in the animal diet (Harrison et al., 2011).

Tissue and compound specificity

Different animal tissues show differential discrimination related to diet (either enriched or depleted). Tieszen et al. (1983) found decreasing values of $\delta^{13}C$ in the order hair > brain >muscle >liver> fat (gerbil), when hair was enriched by 1.0ă and fat was depleted by 3.0ă from the diet (C4), which were related to tissue lipid content. Based on these findings, it was proposed that dietary reconstruction studies should consider more than one tissue or product, with different half-lives. However, to trace stable isotopes a single tissue with minimum deviation (discrimination) of δ values from the diet might be enough (e.g. hair).

Isotopic discrimination during oxidation of pyruvate to acetyl CoA in the process of lipid synthesis resulted in more negative $\delta^{13}C$ values in milk fat (Camin et al., 2008; Molkentin and Giesemann, 2007) and adipose tissue in beef cattle (Bahar et al., 2005) and lamb (Moreno-Rojas et al., 2008) compared with milk casein and muscle samples. Thus, milk lipid $\delta^{13}C$ values poorly reflected the different $\delta^{13}C$ values of dairy cattle diets compared with other components such as proteins and lactose, even compared to whey ethanol resulting from lactose fermentation (Masud et al., 1999). Moreover, relationships between $\delta^{13}C$ values of protein fractions and maize content have been reported to be higher than relationships between $\delta^{13}C$ values of fat fractions and maize content (Bahar et al., 2005; Camin et al., 2008).

Despite the lipid discrimination effect, adipose tissue $\delta^{13}C$ was influenced by the diet more rapidly than chicken muscle protein $\delta^{13}C$ (Rhodes et al., 2010). This effect suggests a tendency of preferential nutrient routing (Hatch et al., 2002) of maize-C into intramuscular lipids or a relatively higher rate of fat deposition than protein production in maize-fed animals, i.e. beef

cattle (Bahar et al., 2005). Another example of nutrient routing is the more marked effect in $\delta^{13}C$ values of protein samples than in fat samples of poultry caused by maize gluten (protein) (Rhodes et al., 2010). Furthermore, animal protein tends to reflect dietary protein and not bulk $\delta^{13}C$ values when animals are fed high-protein diets, whereas with low-protein diets the protein $\delta^{13}C$ values tend to be close to the bulk diet values because of the effect of non-protein compounds of the feed on the amino acid metabolism (ñisotopic routingò). However, as gut symbiosis produces most of the ruminantôs protein from bulk diet through urea recycling, protein C skeletons may reflect the bulk $\delta^{13}C$ value of the diet (Gannes et al., 1998). In addition, carbonate in dental bioapatite derived from respiratory CO_2 (dissolved inorganic C in blood), integrates the isotopic composition of the whole C used for energy metabolism, and represents the best average isotopic composition of the animal diet (Gannes et al., 1998). In addition, Zazzo et al. (2005) showed a sampling strategy with which it is possible to identify large variations in $\delta^{13}C$ in tooth enamel (cattle) corresponding to the dietary history (diet-switch C3 / C4).

Nardoto et al. (2006) found different ^{13}C isotopic discrimination in swine tissues, where liver, muscle and fat tissues were significantly depleted relative to diet, while nail, hair and cartilage were not significantly enriched in ^{13}C relative to diet. However, adipose tissue (*vs.* liver and muscle) showed the best differentiation of $\delta^{13}C$ values in swine under different diets (González-Martín et al., 1999). In contrast to experimental trials, analysis of commercial poultry samples suggests that chicken lipid was a less reliable indicator of dietary status than protein (Rhodes et al., 2010). In chicken eggs, delipidized yolk showed less negative $\delta^{13}C$ values than corresponding whole yolk due to differences in lipid content, but albumen, membrane and yolk

did not show significant differences (Rogers, 2009). Despite the differences in $\delta^{13}C$ values in different tissues and compounds (e.g. protein *vs.* fat) highly significant positive correlations ($r >$ 0.9) have been reported among them (Piasentier et al., 2003; Camin et al., 2008; Moreno-Rojas et al., 2008; Molkentin and Giesemann, 2010).

Amino acids show different $\delta^{13}C$ values both in the diet and tissue protein. Also diet-tissue discrimination may be different for C3 (+1.4ă) and C4 (+3.0ă) diets, and among different amino acids e.g. glutamate and aspartate in collagen (protein) were enriched in ^{13}C relative to the diet by 6ă and 3ă , respectively (Hare et al., 1991). Thus, protein of animal tissue will have a bulk $\delta^{13}C$ value close to the most abundant amino acid e.g. collagen is 33% glycine which has a $\delta^{13}C$ value 8.0ă more positive than the bulk protein of the diet (C3). Therefore, fractionation during metabolism and the isotope composition of the most abundant amino acid influence the $\delta^{13}C$ value of collagen (Hare et al., 1991).

Metabolic turnover

Different animal tissues or products have different metabolic turnover rates or half-lives. Because of slow turnover, skeletal muscles contain isotopic information on dietary inputs integrated over a long period of time (months to years) (Bahar et al., 2009; Harrison et al., 2011). The C half-life was estimated as 134ï 157 days in different muscles in beef cattle, and isotopic equilibrium ($\delta^{13}C$) was not reached in muscle after 168 days of feeding in beef cattle (Bahar et al., 2009). Hence, less negative $\delta^{13}C$ values of meat in spring and early summer is a delayed

result of a high proportion of concentrates in the diet during winter (Bahar et al., 2008). Harrison et al. (2010, 2011) showed that C turnover in lamb was strongly affected by the energy allowance, being faster with a higher EA diet (75.6 ± 1.6 days) than with a lower EA diet (95.5 ± 7.7 days). Also the EA of the diet had a small although significant impact on intra-muscular $\delta^{13}C$ variation and there was an inter-muscular C turnover variation (Harrison et al., 2010). These same authors speculated that due to the possible re-esterification of free fatty acids, ovine intramuscular lipid may never be completely turned over and will therefore always retain the signatures of past diets (Harrison et al., 2011). However, dairy milk $\delta^{13}C$ values changed rapidly (3ï 10 days) according to a diet switch (Knobbe et al., 2006), but the complete turnover cannot be reached after two weeks of a diet switch (Camin et al., 2008). Samples of cattle hair (keratin) showed a much faster turnover with a half-life of 16 days (Schwertl et al., 2003), and horse hair showed short-term (1-7 days) switches (1.0 to 5.6ă) from the diet $\delta^{13}C$ values, called ôhigh resolutionô (daily) information about diet (West et al., 2004). Turnover of cattle hoof (keratin) was also estimated to be faster than for bovine muscle (Harrison et al., 2007). In poultry, which are smaller animals with very rapid growth, the C in breast muscle may be replaced in approximately 17 days (Carrijo et al., 2006).

The $\delta^{13}C$ tissue value integrates the exogenous (diet) and endogenous (metabolism) sources of the isotope. West et al. (2004) described three sources (pools) as (i) an initial fast pool (half-life < 1 day) reflecting C (amino acids to build keratin of hair) sourced directly from the diet, (ii) a second fast pool (~4 days) reflecting a source from biosynthesis or the breakdown products of metabolic proteins, (iii) a slow pool (~136 days) representing a source from the breakdown of

structural proteins, with a half-life of months. Horse tail hair $\delta^{13}C$ values showed a contribution of three different pools with different turnover rates (t½) of ~0.5, ~4, and 140 days for ~41%, ~15% and ~44% of the $\delta^{13}C$ signal, while breath CO_2 (which is linked to blood bicarbonate) of horses had faster t½ of ~0.2, ~3, and 50 days for ~67%, ~17% and ~16% of the $\delta^{13}C$ (Ayliffe et al., 2004). In order to identify whether more than one isotope turnover pool is present, Cerling et al. (2007) applied the reaction progress variable [ln(1-F)] which normalizes isotope data into a linear function rather than an exponential function, allowing comparison of experiments with different initial and final isotope δ values.

Growth rate and animal age

To our knowledge there are no specific studies relating growth rate and livestock age to $\delta^{13}C$ signatures of animal tissues and products. Rhodes et al. (2010) related differences in the intercept of the regression line of corn content of feed *vs.* $\delta^{13}C$ of breast muscle of two breeds of chickens to differences in the growth rates of the two breeds. Bahar et al. (2005) argued that $\delta^{13}C$ of lipids could overestimate total C4 intake due to the natural tendency for more tissue lipid deposition with advancing age in animals. Differences in $\delta^{13}C$ signatures of hair samples were negligible when growing animals (heifers and steers) and adult cattle were fed similar diets (Schwertl et al., 2005).

$\delta^{15}N$

Diet composition and digestibility

The ^{15}N composition of feedstuffs is determined by the ^{15}N signature of available soil N, by symbiotic N_2 fixation in the case of legumes and by N fertilization, particularly in regard to whether the N fertilizer source is synthetic or organic (Inácio et al., 2013). N_2 fixing plants have lower and distinct ŭ^{15}N values. e.g. legume forages, +0.5 to +1.1ă ; soybean meal, +1.5ă ; whereas non-fixing plants such as maize silage have ŭ^{15}N values of +3.2 to +5.1ă (Camin et al., 2008). The ŭ^{15}N values in cattle tissues and products are affected by the ŭ^{15}N values of constituents in the diet including pastures (ï 1.5 to +7ă), maize (+3.2ă), grass silage (+6.3ă) and concentrates (+3.3 ± 1.4ă) (Bahar et al., 2005, 2009; Schwertl et al., 2005). The ŭ^{15}N signatures of animal products will reflect the seasonality of diet composition and its pattern of ŭ^{15}N values (Schwertl et al., 2003; Bahar et al., 2008; Molkentin and Gieseman, 2010; Osorio et al., 2011).

The ŭ^{15}N values of different poultry tissues were strongly related to the inclusion of animal by-products in the diet (Carrijo et al., 2006; Móri et al., 2007; Denadai et al., 2008; Table 6), which tend to have higher values of ŭ^{15}N due to trophic level fractionation (McCutchan et al., 2003). However, samples of lamb from different European countries did not show significant differences related to diet, but differences in ŭ^{15}N values were related to geographical origin (Piasentier et al., 2003). Despite the influence of diet on isotopic composition of animal tissue,

Camin et al. (2008) and Knobbe et al. (2006) reported the ŭ^{15}N signature of milk was unsuitable to differentiate feeding regimen (e.g. maize content).

Different tissues show different ŭ^{15}N enrichment according to the diet. Osorio et al. (2011) found that different diets (pasture *vs.* concentrate) did not significantly alter the diet-muscle discrimination (Δ^{15}N of +2.8 and +2.9ă , respectively) in beef cattle, while diet-hair discrimination was higher for both diets (+4.6 and +3.3ă , respectively), which was attributed to the different protein contents of the diets (pasture = 21.5%; concentrate = 13.4%). Also, Sponheimer et al. (2003b) reported that diet-hair discrimination was 1.5 to 2.8ă greater for the high-protein diet in an experiment with mammalian herbivores, as a consequence of an increase in the proportion of the ^{15}N-depleted urinary N as the main N efflux. However, these findings are in contrast to the statement of Vanderklift and Ponsard (2003) that a low C: N ratio in the diet tends to translate to lower ŭ^{15}N enrichment in the animal (varied species), although N content in the animal diet showed no relationship with diet-tissue discrimination. Also, Wittmer et al. (2011) found no effect of N content (range 0.9 to 3.7%) on diet-wool discrimination. For Robbins et al. (2005, 2010), whose findings showed that diet-tissue discrimination did not vary significantly with either diet N content or C: N ratio (varied species), the protein quality (percentage of absorbed protein that is retained) accounted for most of the variation of discrimination between diet-groups, while protein intake relative to animal requirements explained the within-group variation.

Cheng et al. (2011) found the lowest ^{15}N enrichment of milk protein when the N use efficiency (NUE) by dairy cows was low (different diets), in contrast with the findings of

Sponheimer et al. (2003b). In addition, growing animals (cattle) showed larger ^{15}N enrichments relative to diet than adult animals (Schwertl et al., 2005), probably due to the different net N (protein) retention between these animal groups (Robbins et al., 2005). Clearly, the role of diet protein content in diet-tissue discrimination needs to consider feeding patterns of domesticated animals which are quite distinct from wild life or controlled experiments. Especially for ruminants, the contribution of the incorporation of depleted ammonia by rumen bacteria must be considered under different feeding regimes (Cheng et al., 2011).

Sponheimer et al. (2003c) found that N is more readily available in C3 than in C4 grasses, even when they have similar N concentrations. Apparent N digestibility was about 9% higher for all animal species (mammals) with the C3 grass in comparison with the C4 grass mainly due to anatomical differences (highly-vascularized bundle sheath cells of C4 plants). Therefore, in a feed with mixed C3 and C4 sources, the C3 source may contribute more to the $\delta^{15}N$ values of animal tissues and products.

Tissue and compound specificity

Tissue metabolic fractionation will imprint differences in $\delta^{15}N$ values observed in animal products or sample tissues. Cattle, lamb and swine (mammals) are expected to show higher ^{15}N enrichment in brain (+4.8ă) and lower enrichment in kidneys (+1.3ă), while poultry (birds) are expected to show lower ^{15}N enrichment in muscles (+0.9ă) than in feathers (+3.5ă). These variations may be attributed to turnover rate, type of biochemical reactions and biochemical

composition (Vanderklift and Ponsard, 2003). Nevertheless, delipidized yolk, albumen and membrane in chicken eggs showed no significant differences in ŭ^{15}N values (Rogers, 2009). Also, little variation in ŭ^{15}N values was found among swine tissues (hair, nail, liver, muscle, fat and cartilage), with the exception of liver (+2.2ă) that was significantly (P<0.05) less enriched in ^{15}N than the nail (+3.0ă) relative to diet, probably due to their different protein composition (Nardoto et al., 2006). Cattle hair was the best indicator of changes in diet than muscle samples, allowing discrimination between animals fed pasture and animals fed grass silage + pasture, diets with ŭ^{15}N values of +6.4 ± 1.8ă and +5.0 ± 1.0ă , respectively (Osorio et al., 2011). In this case, as the diets had different crude protein contents, the authors pointed out that the ŭ^{15}N values of cattle hair keratin was affected by both the ŭ^{15}N values of the diet and the proportion of protein in the diet. Knobbe et al. (2006) analyzed animal urine in order to observe significant differences related to the feeding regimen (maize- *vs*. grass-based diet; ŭ^{15}N = ï 1.5 ± 0.3ă and ï 2.9 ± 0.3ă , respectively), which were not distinct in animal products (ŭ^{15}N = +4.1 ± 0.2ă and +3.7 ± 0.2ă , respectively).

Amino acids show different isotope composition (ŭ^{15}N) related to bulk protein in the diet, and the ŭ values may be different in a C3 or C4 diet for the same amino acid. Non-essential amino acids are enriched relative to the animal diet (diet-tissue discrimination). An exception is threonine, an essential amino acid for mammals, that is depleted in the diet (–1.3 and –0.1ă in C3 and C4 species, respectively) and in bone collagen (–6.0ă) (Hare et al., 1991). In the case of collagen, glutamate showed the greatest discrimination (enrichment) relative to diet. Transamination (e.g. glutamate synthesis) and deamination (e.g. urea excretion) reactions play a

key role in the fractionation of N of animal protein. Despite this complexity, ŭ^{15}N values of amino acids (glutamate, aspartate, threonine, serine) in muscles were similar to those of swine bone collagen (Hare et al., 1991).

Metabolic turnover, growth rate and animal age

Similar to ^{13}C turnover in cattle muscles, the turnover curve of ^{15}N did not reach steady state even after 168 days of feeding (Bahar et al., 2009). However, samples of cattle hair showed an N turnover with a half-life of 19 days, and even in this case, the isotopic signal of the feed will be strongly diluted by the body metabolic pool in the first week (Schwertl et al., 2003).

Few studies appear to have focused on the influence of growth rate and age on the ŭ^{15}N values of animal products or tissue samples. Bahar et al. (2009) adjusted the growth rate of cattle fed different diets to determine the turnover of the components of the diet. Schwertl et al. (2005) observed higher values of ŭ^{15}N (0.3 to 1.1ă ; mean 0.7ă) in growing dairy heifers compared to adult cows when fed a similar diet. Metabolic turnover of ^{15}N is expected to be faster when the growing rate of the animal is higher, as found for free-range poultry with additional energy and protein available (t½ = 34 days) compared to only corn-fed poultry (t½ = 53 days) (Coletta et al., 2012). Growth rate is also related to animal age, and therefore adult animals can show distinct metabolic turnover of ^{15}N and ŭ^{15}N values in their tissues than growing animals due to different N assimilation rates (Robbins et al., 2005; Schwertl et al., 2005). Although it may not be an issue for conventional animal husbandry with short production periods (e.g. less than 45 days for poultry or less than two years for beef cattle), it could be a

factor contributing to variation for alternative animal husbandry systems that adopt longer production periods or lower yield goals (e.g. liters of milk per day).

N balance

N balance has been reported to explain variations in $\delta^{15}N$ values in hair samples (Schwertl et al., 2005) and animal products including beef (Schmidt et al., 2005) and milk (Molkentin and Giesemann, 2007). Schwertl et al. (2005) reported significant relationships for adult cows between $\delta^{15}N$ values of hair samples and stocking rate (kg of live-weight ha^{-1}) ($R^2 = 0.55$), and between $\delta^{15}N$ and farm N input ($R^2 = 0.78$), but the latter relationship was not as pronounced ($R^2 = 0.54$) when growing animals (bulls, steers, heifers) were included in the regressions. Higher N inputs lead to increasing N losses which influenced the $\delta^{15}N$ signatures of farm crops and pastures probably due to fractionation of ^{15}N via NH_3 volatilization, nitrification and denitrification (Schwertl et al., 2005).

Nevertheless, Wittmer et al. (2011) working with a 3-year experiment with six stocking rates (0.375 to 2.25 sheep ha^{-1} $year^{-1}$) in a semi-arid grassland did not find an effect of stocking rate on $\delta^{15}N$ values of feces and wool, or even in the vegetation and soil, although vegetation N content increased with stocking rate. These authors speculated that the semi-arid climatic conditions could have reduced N losses, and consequently ^{15}N discrimination. In addition, ^{15}N discrimination values (e.g. pasture-wool) were constant even with different stocking rates.

δ²H and δ¹⁸O

Geographic origin

Several authors have shown that the ŭ^{2}H and ŭ^{18}O signatures of beef are potential tools to predict geographic origin among countries (e.g. Heaton et al., 2008) and even regions within a country (e.g. Nakashita et al., 2008), as a consequence of the direct relationship between isotope signatures of drinking water and animal diet to the geographical patterns of meteoric water. Nakashita et al. (2008) found a positive correlation (R^2 = 0.88) between Japanese beef with drinking water isotopic composition, which shows latitude dependence within the country. Beef lipid samples with more depleted δ^{2}H and δ^{18}O values were obtained from Scotland, New Zealand and England (higher latitude), whereas more enriched δ^{2}H and δ^{18}O values were found from Southern Africa, Australia, Brazil and Uruguay (lower latitudes and / or warmer climate) (Heaton et al., 2008). Also, beef samples (muscle) from Germany and Argentina were differentiated using δ^{2}H and δ^{18}O values which were related to groundwater isotope signatures (Boner and Förstel, 2004).

However, Boner and Förstel (2004) cautioned that seasonal variation overlaps geographical patterns of isotope signatures, and it needs to be clearly identified to allow the correct designation of beef geographical origin. Moreover, the ratio of rainfall / evaporation or ósurface water turnoverô of a location is an additional factor that influences the isotope composition of milk rather than the production site latitude, as found for Australian samples, which showed

more enriched $\delta^{18}O$ values than most European dairy products (Crittenden et al., 2007). In addition, manufacturing processes may alter the isotope signature of animal products. For example, Silva et al. (2014) found that the $\delta^{18}O$ and $\delta^{2}H$ values of cheese samples were depleted (mean of ī 6ă and ī 40ă , respectively) relative to natural buffalo milk from the North of Brazil.

Diet

Osorio et al. (2011) found that the ŭ^{2}H values of different diets (concentrates vs. pasture and silage) influenced the ŭ^{2}H values of the muscles of beef cattle, but there was no clear separation. Biondi et al. (2013) found that $\delta^{18}O$ of blood plasma of lambs was sensitive to change of diet (proportion of pasture / concentrate), while ŭ^{2}H values overlapped between different diets. Although most of the H of animal tissue comes from the diet (Hobson et al., 1999; Harrison et al., 2011), ŭ^{2}H has not been a reliable parameter to discriminate between diets (i.e. pasture vs. concentrate) (Camin et al., 2008; Biondi et al., 2013). C3 pasture plants show lower values of ŭ^{18}O (22.5 ±1.0ă) and ŭ^{2}H (ī 97 ±6ă) than concentrates (28.7ă and ī 58ă , respectively) (e.g. Biondi et al., 2013). However, different proportions of C4 maize silage and C3 pasture in the diet had only a slight effect the ŭ^{18}O value of milk water and the ŭ^{18}O and ŭ^{2}H values of casein (Camin et al., 2008), although the C4 maize silage had significantly higher values of ŭ^{18}O and ŭ^{2}H (+25.4ă and ī 69ă , respectively) than C3 pastures (+22.6ă and ī 106ă , respectively).

The oxygen and hydrogen isotopic ratio of animals kept on pastures or fed with fresh pasture forage reflected more the free water in leaves (enriched in ŭ^{18}O) than the drinking water isotope

composition (Biondi et al., 2013; Renou et al., 2004). Additionally, for the same geographical origin, a high isotopic enrichment of δ^2H and $\delta^{18}O$ in the tissue water may indicate cattle reared on biomass where the water isotopic composition is influenced by precipitation (Boner and Förstel, 2004). The lower water content of concentrates, silage and dry fodder than fresh pasture causes the animal to increase drinking water intake, which will influence the isotopic composition of the animal more than the diet (Biondi et al., 2013; Boner and Förstel, 2004).

Mode of production

The geographical patterns of variation in $\delta^{18}O$ and δ^2H signatures imprinted in feed and drinking water are the primary factors affecting the $\delta^{18}O$ and δ^2H composition of unprocessed organic and conventional animal products. However, restrictions in the feeding regimen in organic systems (i.e. limitations in the use of silage, concentrates, non-organic feed) could conceivably lead to differences in isotopic composition between modes of production. Further isotopic discrimination may occur during food processing. There is presently a lack of data which permit a direct and statistically verifiable comparison of the $\delta^{18}O$ and δ^2H signatures of conventionally- and organically-produced animal products. However, it could be hypothesized that only in exceptional circumstances where the sources of the drinking water and diet differed markedly between the two modes of production would $\delta^{18}O$ and δ^2H be of value in identifying the animal husbandry system.

DIFFERENTIATION OF ORGANIC AND CONVENTIONAL ANIMAL PRODUCTS

$\delta^{13}C$

Organic and conventional cattle products may differ significantly in $\delta^{13}C$ values (Table 7) mainly because organic production standards limit the use of maize in animal feeding. Some authors have suggested a maximum threshold $\delta^{13}C$ value of ï 20ă in beef (Boner and Förstel, 2004), ï 23.5ă for defatted dry matter (DDM) and ï 26.5ă for milk fat (Molkentin, 2013). The proportion of maize (% of DM) in the diet explained 96% of the variation of the $\delta^{13}C$ value of cattle hair, showing less negative values for conventional production, which allows maize-based feedstuffs (Schwertl et al., 2005). Hence, more negative values of $\delta^{13}C$ are expected for organic cattle and dairy products than conventional production.

Molkentin and Giesemann (2010) found a significant difference between organic (ï 27.0 to ï 23.8ă) and conventional (ï 23.3 to ï 21.2ă) milk without overlap in $\delta^{13}C$ signatures in an annual time series (Table 7). They also observed a seasonal tendency related to the reduction of the maize content in the diet of conventional animal production and the availability of pasture for both husbandry systems in summer. Discrimination appeared to be higher in organic milk production due to lower metabolic turnover associated with the lower energy intake and milk yield (Molkentin, 2009; Molkentin and Giesemann, 2010).

Organic and conventional milk fractions (protein and fat) showed a pattern in the difference ($\delta^{13}C_{protein}$ ï $\delta^{13}C_{fat}$) which could be used to detect a mixture of conventional milk with organic

milk (Molkentin and Giesemann, 2007, 2010). Molkentin (2013) points out that $\varphi^{13}C$ ($\delta^{13}C_{DDM}$ï $\delta^{13}C_{fat}$) values of less than 1.0ă indicates different origins for protein and fat i.e. possibly a fraudulent mixture. In addition, plotting $\delta^{13}C_{DDM}$ against $\delta^{13}C_{fat}$ shows different quadrants (organic, conventional and non-compliant sample) separated by the threshold values. However, the threshold value may deviate depending on the population sampled, which can be influenced by the production conditions found in different countries or locations (Molkentin, 2013).

Differences of $\delta^{13}C$ signatures in cattle and dairy products may be smaller or not significant when conventional animal husbandry is also based in C3 pastures and the use of maize is low. The relative content of maize in the diet can vary from 8% of DM (Schwertl et al., 2005) to around 60% of DM (Molkentin and Giesemann, 2007). Conventional Irish beef (mainly C3 pasture) showed less negative and more variable $\delta^{13}C$ values (ï 24.5 ± 0.7ă) than organic beef (ï 26.0 ± 0.2ă) (Schmidt et al., 2005; Table 7). One conventional grassland based husbandry showed a $\delta^{13}C$ value of ï 25.5ă , which fell within the range of organic grassland based husbandry (ï 26.3 to ï 23.5ă), although most confinement systems had less negative values (>ï 22.0ă) (Schwertl et al., 2005).

The limitations of ^{13}C isotopic composition to differentiate organic and conventional animal systems might be greater for poultry. Apparently the common combinations of C3 and C4 diets fed to chickens result in completely overlapping $\delta^{13}C$ signatures between organic and conventional eggs (Rogers, 2009). Coletta et al. (2012) also pointed out that C4 grasses, predominant in tropical conditions, are a confounding factor for distinguishing free-range and

barn-raised corn-fed poultry using $\delta^{13}C$ values of breast muscle. Standards for organic poultry products stipulate the use of organically-produced feedstuffs and a free-range environment. Thus, there is no restriction on the use of maize in the diet that could be clearly detected as discussed before. A similar limitation could be expected for differentiation of mode of production of swine, although the Iberian-breed showed small differences in $\delta^{13}C$ signatures among a variety of diets (González-Martín et al., 1999, 2001; Table 5). ^{13}C isotopic composition would be unsuitable to differentiate organic and conventional systems in tropical environments (e.g. Brazil) because of the high proportions of tropical C4 pasture grasses in grazing animal diets, which could imprint $\delta^{13}C$ values similar to confined maize-fed beef (e.g. Schmidt et al., 2005; Table 3).

δ¹⁵N

Higher $\delta^{15}N$ signatures in hair samples were found for conventional confined dairy and bull fattening farms (+5.0 to +7.4ă), whereas $\delta^{15}N$ signatures in grassland based systems (organic and conventional) varied considerably and overlapped (+3.3 to +6.8ă) (Schwertl et al., 2005). One conventional farm had $\delta^{15}N$ values probably lowered by feeding high amounts of imported legume seeds (Schwertl et al., 2005). These two groups could be distinguished also according to N inputs: organic and conventional (<120 kg ha^{-1} y^{-1}) and confinement (conventional) farms (>220 kg ha^{-1} y^{-1}), despite the large variations in the isotopic signatures of feedstuffs. A similar tendency of $\delta^{15}N$ signatures was reported for predominantly pasture based Irish beef (Bahar et

al., 2008), whereas conventional beef had higher $\delta^{15}N$ values (+7.8 ± 0.4ă) than organic beef (+6.6 ± 0.4ă) (Schmidt et al., 2005; Table 7).

$\delta^{15}N$ signatures of organic and conventional milk overlapped substantially (18 months time series) and did not permit the unequivocal distinction between modes of production, although conventional milk predominantly exhibited higher ^{15}N values and organic milk never exceeded a maximum $\delta^{15}N$ value of +5.5ă (Table 7; Molketin and Giesemann, 2010; Molkentin, 2013). Therefore, in the case of dairy products the influence of organically-grown feedstuffs that are expected to have higher $\delta^{15}N$ signatures due the type of fertilization was not sufficient to distinguish the mode of production. Overall, organic farms may show strong variability of $\delta^{15}N$ signatures in feedstuffs and a higher proportion of leguminous material in the diet than conventional farms and confinement systems (Schwertl et al., 2005; Molketin and Giesemann, 2010).

Similarly, organic eggs showed highly variable $\delta^{15}N$ signatures that overlapped with conventional eggs (Rogers, 2009; Table 7). In this study, the highest values in organic eggs suggested a higher animal protein contribution due to the ingestion of insects ($\delta^{15}N$, +8.5 to +10.5ă), reflecting the availability of indigenous food sources in a free-range environment. The grass and soil isotopic signatures, i.e. +8.9ă and +8.8ă , respectively, also seemed to affect the $\delta^{15}N$ value of breast muscle of free-range chickens (+4.0ă), as distinct from barn-raised chickens (+3.0ă) (Coletta et al., 2012). However, inclusion of animal by-products in the chicken diet also imprinted high $\delta^{15}N$ values in chicken muscle (Table 6) that could be a factor

confounding the use of $\delta^{15}N$ signatures to distinguish organic poultry products. On the other hand, Coletta et al. (2012) stated that it would be most unlikely that chickens with low $\delta^{15}N$ value (breast muscle) would come from a free-range regime (e.g. organic).

Special attention should be given to the effect of additives on the $\delta^{15}N$ signatures of foodstuffs. Nitrite and nitrate are added to cured meat as sodium or potassium salts, to prevent microbial growth and its positive effect on the flavor and color of meat, especially ham (Honikel, 2008). Shearer et al. (1974) reported that the $\delta^{15}N$ signatures of two KNO_3 laboratory reagents from the same supplier were quite different (1.3 ± 1.2ă and −7.5 ± 0.7ă). These additives are not allowed in the processing of organic food (IFOAM, 2008), which can contain natural sources of nitrite/nitrate. e.g. celery powder, celery juice and sea salt (Neibuhr et al., 2010). Therefore, the use of nitrate/nitrite additives can lower the $\delta^{15}N$ values of meat products, and hence the adulteration of organic processed meat could be detected. However, the presence of other permitted natural additives of nitrite/nitrate could confound the presumption of adulteration.

COMPLEMENTARY MARKERS

Capuano et al. (2012) recently reviewed potential chemical and biochemical markers for differentiating mode of production of both plant and animal products, and therefore a detailed treatment in the present review is unnecessary. A few examples of such markers for a variety of animal products are given in Table 8.

Organic milk has been reported to have a high content of α-linolenic acid (C18:3ω3) and eicosapentaenoic acid (C20:5ɤ 3) (Aulrich and Molkentin, 2009; Schröder et al., 2011). A threshold value of 0.5% for C18:3ω3 in milk fat and dairy products using German retail products has been proposed, but noncompliant results were influenced by geographical origin (i.e. different feeding regimen) and by atypical lipid composition (Molkentin, 2013). In addition, linolenic acid concentration was strongly and negatively correlated with $\delta^{13}C$ and $\delta^{15}N$ values (r = ï 0.93 and ï 0.61, respectively) (Molkentin and Giesemann, 2007, 2010), and a threshold value of C18:3ω3 may be used together with a threshold value of $\delta^{13}C$ to differentiate organic and conventional milk and dairy products (Molkentin, 2013). However, the fatty acid content in animal products could be manipulated with inclusion in concentrates of linseed which is very rich in linolenic acid (Scollan et al., 2001).

Another promising parameter to differentiate organic and conventional dairy products is phytanic and pristanic acid analysis because of significantly higher values in grass-based feedstuffs (source of chlorophyll) (Vetter and Schröder, 2010). In addition, hippuric acid content was significantly different between organic and conventional goats milk (Carpio et al., 2010), while organic eggs were distinguishable by carotenoid profiling (van Ruth et al., 2011). Iodine concentration has been reported as significantly lower in organic than conventional milk, and also significant for geographical origin (Bath et al., 2011). Overall, terpenes and phenolic compounds were cited as tools for traceability of geographical origin and diet of small ruminants, respectively (Prache et al., 2005).

CONCLUSIONS

Differentiation of organic and conventional animal products using stable isotopes appears to be more complex than for plant products. Knowledge of the isotopic composition of the diet and a detailed understanding of diet-tissue isotopic discrimination processes are required. The diet exerts an overriding influence on tissue isotopic composition, but there are intrinsic factors related to the tissue itself, the digestibility and compound specificity of the diet, metabolic turnover, growth rate and animal age. While there are many studies which report the isotopic composition of animal excreta and tissues of domesticated animals (beef and dairy cattle, sheep, swine, poultry) in relation to diet, there are very few studies which allow a direct and statistically-verifiable comparison of animal products produced under conventional and organic husbandry systems.

For ruminants (beef and dairy cattle, sheep), $\delta^{13}C$ appears to be the most promising isotopic marker when maize (C4) is present in the conventional diet. Nevertheless, $\delta^{13}C$ signatures may be unsuitable under tropical conditions where C4 grasses are widely available, and where grass-based husbandry is predominant in both conventional and organic production. $\delta^{15}N$ may be an additional parameter in separating mode of production of beef and dairy products as a consequence of the N balance at the farm-level, although as the sole criterion it has failed to differentiate between grass-based husbandry systems. In fact, the cause of differentiation of $\delta^{15}N$ values between organic and conventional cattle and dairy production (e.g. thresholds for milk) is still unclear and ought to be the subject of more research under controlled conditions and

different environments (e.g. tropical, subtropical, and temperate climates). $\delta^{13}C$ and $\delta^{15}N$ appear to be unsuitable markers for organic and conventional poultry and swine products because of the common combination of C3 and C4 dietary components and the high dispersion in the values of organic samples, respectively. $\delta^{2}H$ and $\delta^{18}O$ signatures of animal products are correlated with geographical origin, and only in one specific situation was a $\delta^{34}S$ signature a positive marker of mode of production.

At the present time there is no universal analytical method that can be applied to differentiate organic and conventional animal-derived foodstuffs. Based on the potentials and limitations of each marker tool, Capuano et al. (2012) concluded that authentication of organic food products might not be attainable by a single analytical marker, and it is likely that an array of analytical strategies would be necessary for authentication of different food products. Furthermore, for the establishment of a universal analytical tool, it will be necessary to consider the differences in organic standards among different countries and also the international market established for these products. The combination of stable isotope analysis and other markers (e.g. multi-element and biochemical compounds) might be a robust approach for the authentication of plant and animal products.

Besides one-off stable isotope analyses of organic products from retail or on-farm samples, time-series sampling in organic farms could be considered as part of the certification process. Non-destructive samples of feces, urine, hair, breath or blood might be used in the same way as those taken for detection of short-term changes in diet. In any event, stable isotope analysis is

not a substitute for *in locus* verification of organic farming, but may improve the robustness of organic labeling in some cases, especially with respect to the inclusion of maize in animal diets.

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Table 1 $\delta^{13}C$ composition of animal tissues and excreta and discrimination ($\Delta^{13}C$) in relation to diet

Animal	Sample	Diet[a]	Isotopic signature ŭ^{13}C (ă)[b]		$\Delta^{13}C$	Reference
			Feed	Tissue or excreta (ă)[b,c]		
Cattle	Tail hair	Hay/silage to pasture	--	ï 27.0 to ï 25.4	+2.6	Schnyder et al., 2006
Cattle	Tail hair	Concentrate	ï 27.5	ï 24.1 to ï 27.1	+3.1	Osorio et al., 2011
Cattle	Hair	Hay/silage to pasture	--	ï 27.2 to ï 24.3	--	Schwertl et al., 2003
Cattle	Tail hair	Conv. Organic	--	ï 26.3 to ï 23.5 ï 25.5 to ï 14.1	+2.7	Schwertl et al., 2005
Cattle	Tail hair Hoof		ï 28.7	ï 25.1 to ï 26.0 ï 25.3 to ï 26.1	+3.0 +2.8	Zazzo et al., 2007
Dairy	Urine	Maize + mix. Grass + hay	ï 19.9 (0.7) ï 27.4 (0.1)	ï 20.6 (0.4) ï 24.0 (0.5)	--	Knobbe et al., 2006
Goat	Feces Blood	C3 alfalfa C4 grass C3 alfalfa C4 grass	ï 27.4 (0.6) ï 13.2 (0.7)	ï 27.7 to ï 27.2 ï 14.1 to ï 13.7 ï 24.5 to ï 23.2 ï 17.0 to ï 14.9	0.0 ï 0.7 +3.7 ï 2.2	Codron et al., 2011
Swine	Nail Hair Cartilage Muscle Liver Fat	Corn + Soybean	ï 11.2 ï 25.4	--	ï 0.3 +0.2 +0.9 ï 1.6 ï 2.4 ï 2.3	Nardoto et al., 2006
Various	Hair Feces	Alfafa CBG	ï 27.0 (0.4) ï 13.3 (0.3)	ï 23.9 ï 14.3	+3.2 ï 1.4	Sponheimer et al., 2003a

[a] Conv., conventional farms with confinement and grassland based; Organic, pasture based animal husbandry; Maize + mix.; diet of 44% maize silage, 25% concentrate, 20% sugar beet, and 11% others; CBG, coastal Bermuda grass hay

[b]--, data not reported; data in parentheses are standard deviations

[c]Mean values

Table 2 $\delta^{15}N$ composition of animal tissues and excreta and discrimination ($\Delta^{15}N$) in relation to diet

Animal	Sample	Diet[a]	Isotopic signature ŭ^{15}N (ă)[b]		$\Delta^{15}N$ (ă)[b, c]	Reference
			Feed	Tissue or excreta		
Cattle	Tail hair	Concentrate	+3.6	+4.7 to +9.5	+3.3	Osorio et al., 2011
Cattle	Hair	Hay/silage to pasture	--	+1.7 to +9.0	--	Schwertl et al., 2003
Cattle	Tail hair	Conv. Organic	--	+3.3 to +6.8 +5.0 to +7.4	--	Schwertl et al., 2005
Dairy	Milk Feces Urine	Silage	+1.8 to +8.4	+5.2 to +8.7 +4.2 to +8.8 ï 0.9 to +4.1	+2.9 +1.5 ï 3.3	Cheng et al., 2011
Dairy	Urine	Maize + mix. Grass + hay	+3.3 (0.2) +1.4 (0.2)	ï 1.5 (0.3) ï 2.9 (0.3)	ï 4.0	Knobbe et al., 2006
Sheep	Feces Wool	Grass	ï 0.3 to +1.3	+3.6 (0.1) +5.9 (0.1)	+3.0 +5.3	Wittmer et al. 2011
Swine	Nail Hair Cartilage Muscle Liver Fat	Corn + Soybean	+3.0 ï 0.3		+3.0 +2.7 +2.5 +2.8 +2.2 +2.6	Nardoto et al., 2006
Swine	Feces Urine	Not specified	+1.7 (1.0)	+2.4 (0.9) ï 0.1 (1.1)		Mariappan et al., 2009
Various	Hair	Alfafa CBG	+0.1 (0.4) +7.8 (0.9)		+2.8 to +6.4	Sponheimer et al., 2003b

[a] Conv., conventional farms with confinement and grassland based; Organic, pasture based animal husbandry; Hay/silage, red clover/corn silage; Silage, nine individual silages were made from forage grasses (3 types), red clover, red clover mixed with corn or oats in different

proportions; Maize + mix.; diet of 44% maize silage, 25% concentrate, 20% sugar beet, and 11% others; CBG, coastal Bermuda grass hay;

[b]--, data not reported; data in parentheses are standard deviations

[c]Mean values

Table 3 ŭ^{15}N and ŭ^{13}C composition of beef and dairy cattle products in relation to diet

Product[a]	Origin	Diet	Isotopic signature (ă)[b]		Statistical tool[c]	*P*[d]	Reference
			ŭ^{15}N	ŭ^{13}C			
Beef (LTL)	Ireland	Grass silage	+6.3	ï 28.5	ANOVA, *t*-test	***	Bahar et al., 2005
		Grass + maize	+5.0	ï 22.4			
		Maize silage	+3.2	ï 15.8			
Beef (LD)	Ireland	Barley	+6.1	ï 26.0	ANOVA, nonlinear regression	***	Bahar et al., 2009
		Maize + ^{15}N-urea					
		14-d	+6.7	ï 25.1			
		28-d	+6.9	ï 24.2			
		56-d	+7.5	ï 23.1			
		112-d	+8.7	ï 21.0			
		168-d	+10.0	ï 19.0			
Beef (LD)	Ireland	Pasture	+9.2(0.4)	ï 27.7 (0.2)	ANOVA, CDA	***	Osorio et al., 2011
		P + silage	+8.9 (0.4)	ï 27.6 (0.1)			
		P, S + concentrate	+7.9 (0.2)	ï 26.4 (0.2)			
		Concentrate	+6.3 (0.3)	ï 25.0 (0.1)			
Beef (SL)	USA,	C4 plants		ï 12.3 (0.1)	MANOVA	***	Schmidt et al., 2005
	Brazil,	C4 plants		ï 10.0 (0.6)			
	Europe,	C3 plants		ï 21.6 (1.0)			
	Ireland	C3 plants		ï 24.5 (0.7)			
Beef (LD)	Various[e]	C4 plants	+6.0 to +7.3	ï 11.0 to –16.8	CDA	*	Heaton et al., 2008
		C3 plants	+4.7 to +7.1	ï 20.2 to –26.3			
Dairy (milk)	Germany	Maize (44%)	+4.1 (0.2)	ï 22.0 (0.4)		--	Knobbe et al., 2006
		Grass and hay	+3.7 (0.2)	ï 26.3 (0.4)			
Dairy (milk casein)	Italy	23% (corn)	+5.8 (0.1)	ï 20.3 (0.0)	ANOVA, *t*-test, Tukey	**	Camin et al., 2008
		42%	+5.4 (0.1)	ï 19.2 (0.2)			
		51%	+5.7 (0.1)	ï 18.3 (0.2)			
		55%	+5.5 (0.1)	ï 17.5 (0.2)			

[a]LTL, *longissimus thoracis et lumborium*; LD, *longissimus dorsi*; SL, strip loin and round steak

[b]Data in parentheses are standard deviations of the mean

[c]ANOVA, analysis of variance; MANOVA, multivariate ANOVA; CDA, canonical discriminant analysis

[d]**, $P<0.01$; ***, $P<0.001$; --, no test applied

[e]17 countries from all continents; other isotopic analysis were carried out (ŭ^{2}H and ŭ^{18}O)

Table 4 ŭ^{15}N and ŭ^{13}C composition of sheep products in relation to diet

Product[a]	Origin	Diet[b]	Isotopic signature (ă)		Statistical tool[c]	*P*[d]	Reference
			ŭ^{15}N	ŭ^{13}C			
Lamb (LD)	Italy	Vetch	+4.2	ï 28.3	GLM, ANOVA	***/ ŭ^{15}N, ns	Moreno-Rojas et al., 2008
		Barley	+5.4	ï 25.6			
		Maize	+5.5	ï 21.1			
Lamb (LT)[e]	Europe	Pasture	+1.9 to +8.0	ï 32.5 to ï 31.3	ANOVA, K-means, CDA	*/ ŭ^{15}N, ns	Piasentier et al., 2003
		Cereals	+3.7 to +7.8	ï 28.7 to ï 24.6			
		Milk	+5.3 to +6.1	ï 25.4 to ï 25.0			
Lamb (LD)[f, g]	Ireland	Control		ï 21.0	ANCOVA, GLM, *t*-test	**	Harrison et al., 2011
		C4-diet					
		30 d		ï 18.0			
		100 d		ï 14.0			
		160 d		ï 13.0			
		234 d		ï 12.0			

[a]LTL, *longissimus thoracis et lumborium*; LD, *longissimus dorsi*

[b]Control diet, barley, maize, cane, molasses, oats (ï 22.6 ă); C4-diet, pelleted concentrate and maize (ï 12.5 ă); Free-range, diet of grass, stubble, acorns; grains, commercial concentrate of barley, wheat and soy flour

[c]GLM, general linear model; ANOVA, analysis of variance; ANCOVA, analysis of covariance; CDA, canonical discriminate analysis; K-means, non-hierarchical clustering

[d]*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns, not significant; --, no test applied

[e]ŭ^{15}N signature of muscle protein and ŭ^{13}C signature of peri-renal fat

[f]Approximate values from regression analysis

[g]Additional isotopic analysis were carried out (ŭ^{2}H, ŭ^{18}O, ŭ^{34}S)

Table 5 ŭ^{15}N and ŭ^{13}C composition of swine products in relation to diet

Product	Origin[a]	Diet	Isotopic signature (ă)[e]		Reference
			ŭ^{15}N	ŭ^{13}C	
Swine	Spain-Iberian	Free-range		ï 23.2	González-Martín et al., 2001
(liver)		Grains		ï 21.4	
(hams)		Free-range		ï 20.5	González-Martín et al., 1999
		Grains		ï 21.0	
(rear fat)		Free-range		ï 23.9	
		Grains		ï 22.1	
(loin)		Free-range[b]	+4.6		Delgado Huertas et al., 2007
		Commercial	+5.3		
		Free-range[c]	+3.8		
		Commercial[d]	+5.9		

[a]Iberian, Certified Brand of Origin of pork meat

[b]Scarce acorns

[c]Abundant acorns

[d]Product included sunflower high in oleic (fatty) acid

[e]Statistical analyses of the data were not provided

Table 6 ŭ^{15}N and ŭ^{13}C composition of poultry products in relation to diet

Product	Origin	Diet[a]	Isotopic signature (ă)[b]		Statistical tool[c]	*P*[d]	Reference
			ŭ^{15}N	ŭ^{13}C			
Chicken (breast)	UK	Corn-fed		ï 20.7 (0.3) ï 20.8 (0.4)	Regression, analysis of covariance	*	Rhodes et al., 2010
		Corn-free		ï 24.7 (0.2) ï 24.9 (0.4)			
Chicken (breast)	Brazil	Corn-soybean-fed	+0.6 to 0.1	ï 16.7 to ï 18.8	ANOVA, Tukeyôs	**	Coletta et al., 2012
		Corn-fed	+3.0	ï 12.4			
		Free-range	+4.0	ï 12.2			
Chicken (breast)	Brazil	Base diet	+0.9 to +1.1	ï 17.5 to ï 17.0	GLM Multivariate	*	Carrijo et al., 2006
		+ 1% MBM	+1.0 to +1.4	ï 17.8 to ï 16.9			
		+ 2% MBM	+1.1 to +1.3	ï 16.4 to ï 16.6			
		+ 4% MBM	+1.6 to +1.5	ï 16.4 to ï 16.5			
		+ 8% MBM	+2.1 to +2.2	ï 15.9 to ï 16.3			
Eggs (yolk)	Brazil	Soybean meal	+4.4 (0.1)	ï 18.4 (0.1)	MANOVA, GLM	*	Denadai et al., 2008
		MBM	+5.1 (0.1)	ï 17.2 (0.2)			
		Poultry offal meal	+4.9 (0.2)	ï 17.4 (0.2)			
		Feather meal	+4.8 (0.1)	ï 17.2 (0.2)			
		POM + FM	+5.0 (0.2)	ï 17.2 (0.1)			
		MBM+ POM + FM	+5.2 (0.2)	ï 17.5 (0.1)			
Quail (breast)	Brazil	Corn and soybean	+3.6 (0.22)	ï 19.4 (0.2)	MANOVA, GLM	*	Móri et al., 2007
		Poultry offal meal	+3.9 (0.01)	ï 18.4 (0.2)			
		MBM	+4.0 (0.0)	ï 18.7 (0.2)			
		Feather meal	+3.9 (0.2)	ï 18.0 (0.1)			
		MBM+ POM + FM	+3.9 (0.1)	ï 18.1 (0.2)			

[a]Corn-fed, at least 50 % (w/w) corn for the greater part of the fattening period; corn-free, diet without corn, but with wheat, rye, barley and/or oats; Base diet, maize + soybean + supplements (results for 1 to 21 days and 22 to 42 days); MBM, bovine meat and bone meal (ŭ^{13}C = ï 12.82 to ï 12.97 ă and ŭ^{15}N = +7.43 to +8.06 ă); POM, poultry offal meal (ŭ^{13}C = ï 16.28 ă and ŭ^{15}N = +4.30 ă), FM, poultry feather meal (ŭ^{13}C = ï 16.98 ă and ŭ^{15}N = +4.44 ă)

[b]Data in parentheses are standard deviations of the mean

[c]GLM, general linear model; MANOVA, multivariate analysis of variance

[d]*, $P<0.05$; --, no test applied

Table 7 Isotopic composition of organically- and conventionally-grown animal products

Product	Origin	Isotope	Isotopic signature (ă)[a]		Statistical tool[b]	*P*[c]	Reference
			Organic	Conventional			
Cattle[d] (meat)	Germany	^{13}C	ï 25.0 to ï 27.0[e]	ï 20.0 to –29.0	PCA	--	Boner and Förstel, 2004
Cattle (strip loin)	Ireland	^{15}N	+6.6 (0.4)	+7.8 (0.4)	MANOVA, Pillaiôs test	**	Schmidt et al., 2005
		^{13}C	ï 24.5 (0.7)	ï 26.0 (0.2)		**	
		^{34}S	+7.9 (0.6)	+7.2 (0.4)		**	
Cattle (meat)	Ireland	^{15}N	+6.4	+7.0	*t*-test	***	Bahar et al., 2008
		^{13}C	ï 26.0	ï 25.2		***	
		^{34}S	+7.8	+7.8		ns	
Dairy (milk)	Germany	^{13}C	ï 28.0	ï 26.6		--	Molkentin and Giesemann, 2007
		^{13}C	ï 28.6 (0.9)	ï 24.4 (0.9)		--	Molkentin, 2009
		^{15}N	+3.9 to +5.4	+4.7 to +6.4	Time series	--	Molkentin and Giesemann, 2010
		^{13}C	ï 27.0 to ï 23.8	ï 23.3 to ï 21.2			
Eggs (yolk)[g]	New Zealand	^{15}N	+5.2 to +9.1	+4.0 to +8.6	Box-whisker	--	Rogers, 2009
		^{13}C	ï 28.8 to ï 17.8	ï 28.7 to ï 19.7			

[a]Data in parentheses are standard deviations of the mean

[b]PCA, principal components analysis; ; MANOVA, multivariate ANOVA

[c]**, $P<0.01$; ***, $P<0.001$; ns, not significant; --, no test applied

[d]Additional isotopic analyses were carried out (ŭ^{15}N, ŭ^{34}S, ŭ^{2}H, ŭ^{18}O) to assign geographical origin

[e]85 % of samples

[g]18 brands of eggs from supermarkets and farmersômarkets derived from caged, barn, free-range and organically-raised hens

Table 8 Complementary markers for mode of production of animal products

Product	Marker	Technique[a]	Statistical tool[b]	*P*[c]	Reference
Chicken (eggs)	Carotenoid profiling	HPLC	ANOVA, LSD, PCA, kNN	**	van Ruth et al., 2011
Dairy (milk)	Ů-linolenic acid (C18:3ɤ 3)	GC	SD, time series	--	Molkentin, 2009, Molkentin and Giesemann, 2007
Dairy (milk)	Ů-linolenic acid (C18:3ɤ 3), eicosapentaenoic acid (C20:5ɤ 3)	NIRS	SD	--	Aulrich and Molkentin, 2009
Dairy (milk)	Iodine	ICP-MS	ANOVA, *t*-test	**	Bath et al., 2011
Dairy (milk)	Metabolomics	LC /GC-MS	ANOVA, PCA	*	Boudonck et al., 2009
Dairy (products)[d]	Phytanic acid, pristanic acid	GC/EI-MS	ANOVA	**	Vetter and Schröder, 2010
Goat (milk)	Hippuric acid	Capillary eletrophoresis	ANOVA, PCA, Tukeyôs test	***	Carpio et al., 2010

[a]HPLC, high performance liquid chromatography; GC, gas chromatography; NIRS, near infrared reflectance spectroscopy; LC, liquid chromatography, GC-MS, gas chromatograph coupled to mass spectrometer; ICP-MS, inductively coupled plasma mass spectrometer; GC/EI-MS, gas chromatography coupled to electron ionisation mass spectrometry

[b]SD, standard deviation; ANOVA, analysis of variance, CDA, canonical discriminate analysis; LSD, Fisher least significant difference, PCA, principal component analysis; kNN, *k*-nearest neighbour classification

[c]* $P<0.05$, ** $P<0.01$; ***, $P<0.001$; --, no test applied.

[d]Milk, cheese, cream, butter