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ARTICLE

Stable Isotopes as a Tool To Differentiate Eggs Laid by Caged, Barn, Free Range, and Organic Hens

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Stable carbon and nitrogen isotope values of whole yolk, delipidized yolk, albumen, and egg membrane were analyzed from 18 different brands of chicken eggs laid under caged, barn, free range, and organic farming regimes. In general, free range and organic egg components showed enrichment of ^{15}N values up to 4‰ relative to caged and barn laid eggs, suggesting a higher animal protein (trophic) contribution to the chicken's diet than pure plant-based foods and/or that the feed was organically manufactured. One sample of free range and two samples of organic eggs had δ^{15}N values within the range of caged or barn laid eggs, suggesting either that these eggs were mislabeled (the hens were raised under "battery" or "barn" conditions, and not permitted to forage outside) or that there was insufficient animal protein gained by foraging to shift the δ^{15}N values of their primary food source. δ^{13}C values of potential food sources are discussed with respect to dietary intake and contribution to the isotopic signature of the eggs to determine mixing of C_3 and C_4 diets, although they did not elucidate laying regimen. The study finds that stable nitrogen isotope analysis of egg components is potentially a useful technique to unravel dietary differences between caged or barn hens and free range hens (both conventional and organic) and could be further developed as an authentication tool in the egg industry.

KEYWORDS: Nitrogen; isotope; egg; free range; organic; diet; hen; yolk; carbon; δ^{15}N; δ^{13}C

INTRODUCTION

This study investigates the application of stable isotopes to differentiate between different egg laying regimens. Eighteen different brands of eggs sourced from local supermarkets and farmer's markets in Lower Hutt, New Zealand, were purchased in June 2008. The eggs were derived from different laying regimens: caged, barn, free range, and organically raised hens. These were analyzed using a dual carbon and nitrogen isotopic approach, along with a range of poultry food sources, to provide data to investigate product authenticity.

Eggs farmed from caged or battery hens have become less ethically acceptable in recent times, with many consumers preferring to pay a higher price for eggs raised under more humane conditions. Hen's that lay eggs classified as barn eggs are not kept caged, but are kept in large sheds with litter on the floors. They can flap their wings and partake in dust baths. Free range eggs are laid by chickens that roam freely outside and are not caged. Organic chickens are usually free range, but do not receive hormones or antibiotics and are fed an organically derived diet.

In most instances, farmers choose to exploit a specific farming regimen to produce eggs specifically from caged, barn, free range, or organic free range hens. However, when farmers choose to raise hens under multiple regimens, opportunities for fraud or mislabeling arise with larger profits and incentives to produce higher value free range eggs rather than lower value caged eggs. Farmers selling directly through farmer's markets can also fraudulently sell lower value caged or barn eggs as free range, as in New Zealand there are no quality checks by a purchasing co-operative or distributor. There is now a growing need for research to support consumer confidence in the value-added food market (*1*). This study focuses on the use of stable isotopes as a tool to detect trophic level changes in a laying hen's diet that might give an indication of their laying regimen. This can be determined by investigating the isotopic ranges of egg components and their corresponding food sources specifically associated with caged and free range chickens.

Eggs are composed of nutrients derived from the hen's diet. Commercial poultry feed consists of primarily pellets or mash, containing wheat, barley, maize, and soybeans, and also may include blood, meat, or fish meal, vitamins, and minerals. Free range chickens have the opportunity to forage in soils, picking up grubs, beetles, worms, grasses, and seeds as well as their commercial ration in mash or pellet form to ensure they have a balanced diet. Consumers believe this foraging provides the hen with extra nutrients that give their egg yolks a rich color and their eggs a fuller flavor. These extra nutrients would be expected to contribute enriched ^{15}N to their plant-based diet from the protein derived from insects that feed at a higher trophic level (*2*, *3*).

New Zealand commercial poultry feed manufacturers indicate their commercially available feed is composed of around 65% grain and 35% blood meal, meat or fish meal, vitamins, and minerals (i.e., calcium) (personal communication). The grain is predominantly wheat, with varying

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amounts of barley, bran pollard, peas, lucerne, and soybeans. In the North Island, the grain can also comprise up to 40% maize, but usually there is around 20−30% maize. The proportion of grains can change depending on the cost and availability, but in general the mix tends to favor wheat rather than maize due to cost. Maize is not grown in the South Island, and so it is not included in commercial feeds. Commercial poultry feed is used for all types of laying hens (caged, barn, and free range) and is either pellets or mash. Specialszed poultry feed is manufactured for organic hens, containing only organically grown products. Free range hens (and barn hens in some instances) also supplement their diet with insects, but mostly via the inclusion of plant material such as grass and other vegetation.

A recent study (*4*) on the quality characteristics of eggs laid under four various farming regimens was conducted on commercial eggs to assess physical and chemical parameters of the eggs, including parameters such as shell resistance, shell thickness, whipping capacity, and protein content. It was not possible to have clear differentiations on eggs from the four regimens, but by using a multivariate approach they could separate caged eggs from other farming regimens due to their more resistant shells.

Previous research using stable carbon and nitrogen isotopes of bird eggs has primarily centered on reconstructing diets and foraging behavior of wild birds (*5*−*8*). Rossmann (*9*) presents carbon and nitrogen isotopic ratios of chicken eggs from commercial farms in Germany, Italy, and the United Kingdom as well as some smaller farms in Bavaria. The study shows that most eggs from small farms in Bavaria have low δ^{13}C values (from −26 to −28‰), consistent with a C_3 diet, and high δ^{15}N values (from 6 to 11‰), typical of protein sourced from higher trophic levels, in contrast to larger commercial farms, which have mixed C_3 and C_4 plant diets (especially those from Italy) and lower δ^{15}N values.

Nitrogen isotope values of commercial chicken feed give an indication of whether it has been manufactured exclusively from plants, which may have been raised on soils fertilized with industrial fertilizers, or if there is animal protein included in the feed. Carbon isotopes determine if the feed is primarily derived from C_3 or C_4 plants (or marine biomass). In particular, primary commercial feed sources (laying pellets, mash, and chick crumbles) and their individual conventionally grown components (wheat, maize, and soybeans) have low δ^{15}N values, which suggest there is minimal animal protein included in the feed and that the plants are grown in soils which are fertilized with industrial fertilizers (*10*). Chickens fed exclusively on such product would produce eggs with correspondingly low δ^{15}N values.

Free range hens are often fed commercial laying pellets similar to those fed to caged and barn hens, which can also include higher protein products such as blood and bone and fish meal on the list of ingredients in minor quantities. However, free range hens are expected to have access to a larger variety of alternative food sources than caged hens, in the form of higher protein (insects) and organic matter derived from soils and vegetation fertilized with chicken manure. On the basis of this assumption, free range eggs would be expected to have more positive δ^{15}N values (due to trophic or denitrification processes) than eggs from caged or barn raised hens. Organically raised chickens must have at least 95% organic feed in their diet, which is grown under an organic regimen, and hence components in their feed should also have higher δ^{15}N values than conventionally grown products (*10*−*13*).

Table 1. Carbon and Nitrogen Isotope Values of Potential Food Sources in Chicken Diet[a]

food type (n = 6)	δ^{15}N	SD	δ^{13}C	SD	C:N ratio (atm)
chick crumbles	1.6	0.1	−19.1	0.3	18.0
laying pellets 1	3.7	0.0	−22.9	0.1	16.3
laying pellets 2	1.2	0.2	−21.9	0.2	21.8
laying pellets 3	3.6	0.6	−23.6	0.4	17.9
laying mash	1.8	0.2	−23.8	0.4	18.1
wheat 1 (organic)	6.3	0.1	−23.6	0.1	30.2
wheat 2 (conventionally grown)	3.8	0.3	−26.2	0.3	25.7
maize (conventionally grown)(*10*)	0.8	0.3	−10.4	0.2	29
maize (organic)(*10*)	4.8	0.3	−10.9	0.2	23
maize (conventionally grown)(*22*)	3.0		−11.2		
soybeans (conventionally grown)(*22*)	−0.3		−25.4		4−7 (*5, 6*)
soil (uncultivated)	4.2	0.3	−27.3	0.1	21.1
grass	2.1	0.5	−31.4	0.4	12.3
vegetation (*Tradescantia fluminensis*)	0.8	0.2	−29.9	0.1	18.7
insect (*Carabidae* sp.)(*23*)	10.5		−27.8		
insect (*Carabidae* sp.)(*23*)	10.3		−26.4		
insect (*D. hydei*, male)(*24*)	10.1	0.1	−21.8	0.2	
insect (*D. hydei*, female)(*24*)	10.2	0.1	−20.5	0.3	
insect (*D. simulans*, male)(*24*)	9.4	0.4	−22.8	0.5	
insect (*D. simulans*, female)(*24*)	8.5	0.4	−22.5	0.3	

[a] Each sample represents six replicates of each food source (apart from values taken from the literature).

In this study, the relationship between various egg components and their nutrients is investigated using the isotopic ratio of carbon (^{13}C/^{12}C) and nitrogen (^{15}N/^{14}N). The main aim is to understand if eggs laid by hens that have access to free range and organic diets have higher δ^{15}N values in order to use stable isotopes as a detection tool to differentiate laying regimens.

MATERIALS AND METHODS

Eighteen different brands of caged, barn, free range, and organic eggs were selected from local poultry farmers and several grocery stores in Lower Hutt, New Zealand, in June 2008. Two eggs from each brand were rinsed in distilled water to remove any adhered material (blood, feces, or detritus) on the outer shell and then separated into their various components (yolk, albumen, and membrane). The internal membrane was also removed from the inside of each shell for separate analysis.

Delipidization was carried out on the yolk via a modified method of Bligh and Dyer (*16*) and Hobson et al. (*17*). A portion (1 g) of egg yolk was agitated for 1 h with 30 mL of a 2:1 mixture of chloroform/methanol. The delipidized yolk was separated from the lipids by filtering under vacuum on 2.5 cm GF/C filters and rinsed with 10 2-mL aliquots of a 2:1 chloroform/methanol mixture to ensure all lipids were removed. The delipidized yolk was oven-dried overnight at 30 °C.

A range of chicken feed was investigated including commercial feed (conventional and organic laying pellets, wheat, mash, maize, soybeans, and chick crumbles), soil, insects, plants, and grass. All components were freeze-dried and ground to a homogeneous powder. Between 1.5 and 30 mg of egg and food products was transferred into tin capsules. Carbon and nitrogen content and isotopic composition of egg components (n = 3) from two eggs of each brand and food samples (n = 6) were analyzed at the Stable Isotope Laboratory, GNS Science, New Zealand, using a Europa Geo 20/20 (PDZ Europa Ltd. U.K.) isotope ratio mass spectrometer, interfaced to an ANCA-SL elemental analyzer (PDZ Europa Ltd. U.K.) in continuous flow mode (EA-IRMS). The carbon dioxide gas was resolved from nitrogen gas using gas chromatographic separation on a column at 65 °C and analyzed simultaneously for isotopic abundance as well as total organic carbon and nitrogen. International and working reference standards (NIST-N1, IAEA-CH_6, leucine, wheat flour, and beet sugar) and blanks were included during each run for calibration.

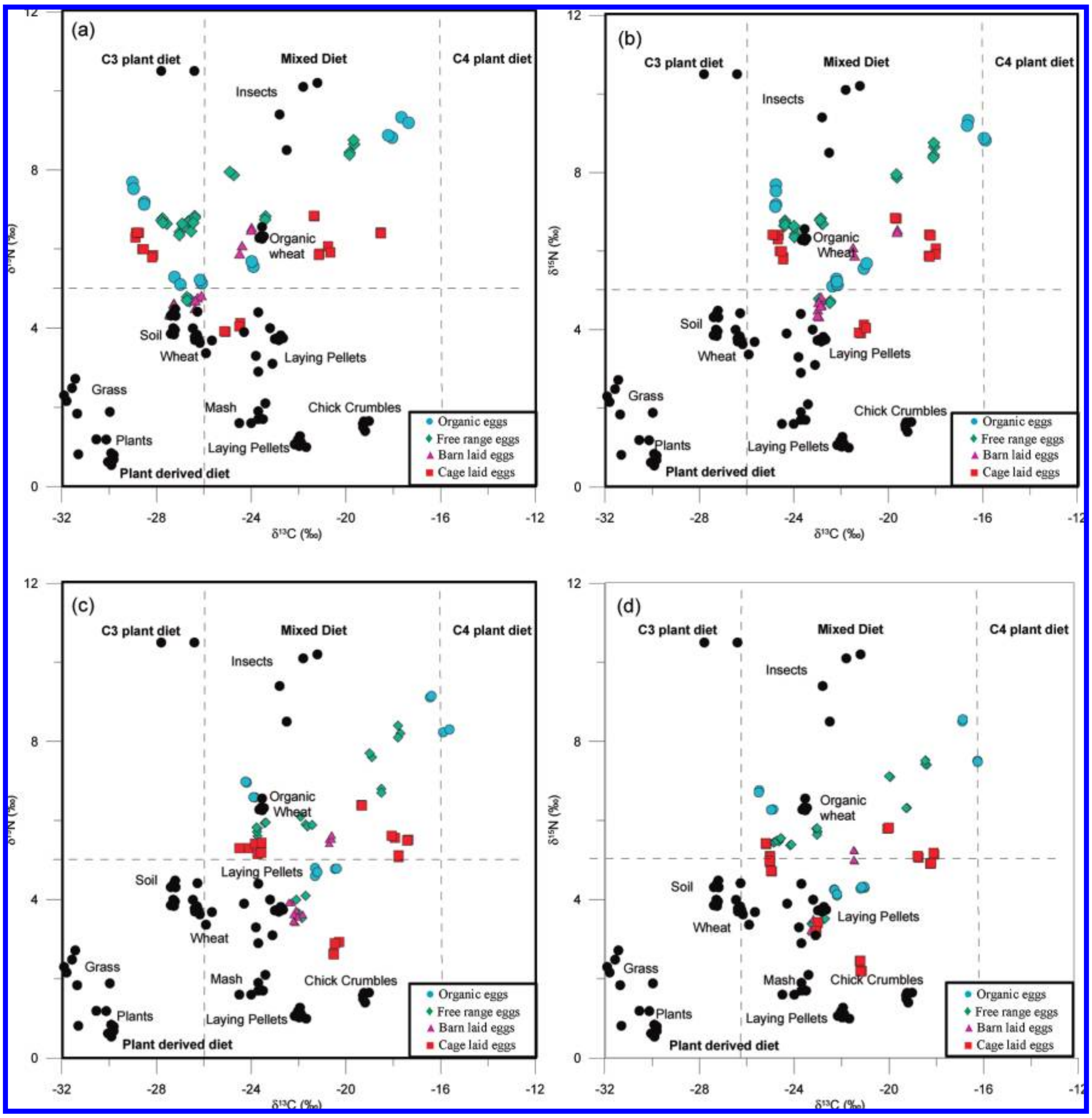

Figure 1. Scatter plots of δ^{13}C and δ^{15}N isotope values of diet and (**a**) whole egg yolks, (**b**) delipidized egg yolks, (**c**) egg membrane, and (**d**) albumen from various laying regimens to show plant and animal protein contributions.

Isotopic ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) are expressed as isotopic deviations δ defined as

$$\delta\ (‰) = \frac{R_s - R_{ref}}{R_{ref}} \times 1000$$

where R_s is the isotopic ratio measured for the sample and R_{ref} that of the international standards. The δ^{13}C value is relative to the international Vienna Pee Dee Belemnite (VPDB) standard, and the δ^{15}N value is relative to atmospheric air. Results are expressed in δ (‰) versus the specific reference. Analytical precision is within ±0.2‰ for carbon and within ±0.3‰ for nitrogen (1 σn).

RESULTS

Diet. Food sources investigated in the study had a range of δ^{15}N values from −0.3 to 10.5‰ and δ^{13}C values from −10.4 to −31.4‰ (including literature values) (**Table 1**). These foods plot into regions (**Figure 1**) whereby more positive δ^{15}N values represent a mixed protein diet and lower δ^{15}N values represent only plant protein (*18*). More negative δ^{13}C values are representative of C_3 plants, and less negative δ^{13}C values are indicative of a C_4 plant (maize) and/or marine (fishmeal) contribution (*19*).

Commercial chicken feed is primarily derived from wheat and maize (which are usually cultivated with the use of agricultural fertilizers) and soybeans (which are leguminous, fixing N from air). Mean δ^{15}N values of commercial laying pellets and laying mash from this study were between 1.2 and 3.7‰. Conventionally grown wheat and maize had δ^{15}N values between 0.8 and 3.8‰. Two samples of organically grown wheat and maize were also included in the study. These had higher δ^{15}N values (6.3 and 4.8‰, respectively) than their conventionally grown counterparts (**Table 1**), typical of crops cultivated on soils fertilized with organic manure (*10*).

Mean δ^{13}C values of laying pellets, mash, and chick crumbles were between −19.1 and −23.8‰, suggesting they have a variable C_3/C_4 plant (maize) or marine (fishmeal) contribution. Wheat and soybeans had δ^{13}C values between −23.6 and −27.3‰, typical of C_3 plant contribution (*2*); however, maize had more positive δ^{13}C values between −10.4 and −11.2‰, reflecting its C_4 origin.

Table 2. δ^{15}N and δ^{13}C Values of Egg Components from Various Commercial Laying Regimens[a]

egg			yolks				membrane		albumen	
			non-delipidized		delipidized					
sample	regimen	*n*	^{15}N	^{13}C	^{15}N	^{13}C	^{15}N	^{13}C	^{15}N	^{13}C
10	cage	6	6.1 ± 0.3	−28.5 ± 0.4	5.8 ± 0.2	−24.6 ± 0.1	5.3 ± 0.1	−24.0 ± 0.4	5.0 ± 0.1	−25.0 ± 0
11	cage	6	5.9 ± 0.1	−20.9 ± 0.3	5.8 ± 0	−18.1 ± 0.2	5.3 ± 0.2	−17.6 ± 0.2	5.0 ± 0.1	−18.2 ± 0.1
13	cage	6	6.6 ± 0.2	−19.9 ± 1.6	6.5 ± 0.3	−19.0 ± 0.8	6.0 ± 0.5	−18.7 ± 0.8	5.4 ± 0.4	−19.4 ± 0.7
14	cage	6	4.0 ± 0.1	−24.8 ± 0.4	3.8 ± 0.1	−21.1 ± 0.1	2.8 ± 0.2	−20.4 ± 0.1	2.3 ± 0.2	−21.2 ± 0
16	cage	6	6.2 ± 0.2	−28.7 ± 0.1	6.0 ± 0.2	−24.7 ± 0.2	5.3 ± 0.1	−23.7 ± 0.1	5.1 ± 0.4	−25.1 ± 0.1
8	barn	6	4.7 ± 0.1	−26.3 ± 0.1	4.5 ± 0	−22.9 ± 0.1	3.8 ± 0.2	−22.3 ± 0.1	3.3 ± 0.2	−23.2 ± 0.1
9	barn	6	6.2 ± 0.3	−24.2 ± 0.3	6.5 ± 0.2	−21.5 ± 0	5.5 ± 0.1	−20.7 ± 0	5.1 ± 0.1	−21.5 ± 0
15	barn	6	4.4 ± 0.2	−27.3 ± 0.1	4.4 ± 0	−22.9 ± 0.1	3.5 ± 0.1	−22.0 ± 0.2	3.3 ± 0.1	−23.0 ± 0
5	free range	6	4.7 ± 0	−26.7 ± 0.1	4.6 ± 0	−22.7 ± 0.2	4.1 ± 0.1	−21.9 ± 0.3	3.5 ± 0.1	−23.0 ± 0.3
6	free range	6	8.6 ± 0.2	−19.7 ± 0.1	8.4 ± 0.1	−18.1 ± 0	8.2 ± 0.2	−17.8 ± 0.1	7.5 ± 0.1	−18.5 ± 0
7	free range	6	7.9 ± 0.1	−24.1 ± 0.8	7.9 ± 0	−19.6 ± 0	7.2 ± 0.5	−18.7 ± 0.3	6.7 ± 0.5	−19.6 ± 0.4
12	free range	6	6.7 ± 0.2	−26.4 ± 0.1	6.8 ± 0	−22.9 ± 0	6.0 ± 0.1	−21.8 ± 0.1	5.7 ± 0.1	−23.0 ± 0.1
17	free range	6	6.7 ± 0.1	−27.7 ± 0.1	6.6 ± 0	−24.4 ± 0	5.7 ± 0.1	−23.7 ± 0	5.5 ± 0	−24.8 ± 0.1
18	free range	6	6.5 ± 0.1	−27.0 ± 0.1	6.6 ± 0	−24.0 ± 0	5.9 ± 0	−23.4 ± 0	5.5 ± 0.1	−24.4 ± 0.2
1	organic	6	7.4 ± 0.3	−28.8 ± 0.3	7.2 ± 0.2	−24.8 ± 0	6.8 ± 0.2	−24.0 ± 0.2	6.5 ± 0.3	−25.2 ± 0.3
2	organic	6	5.2 ± 0.1	−26.6 ± 0.6	5.2 ± 0.1	−22.2 ± 0.1	4.7 ± 0.1	−21.3 ± 0	4.2 ± 0.1	−22.2 ± 0.1
3	organic	6	9.1 ± 0.2	−17.8 ± 0.4	9.0 ± 0.2	−16.3 ± 0.4	8.7 ± 0.5	−16.1 ± 0.4	8.0 ± 0.6	−16.6 ± 0.4
4	organic	6	5.6 ± 0.1	−23.9 ± 0	5.7 ± 0.1	−21.0 ± 0.1	4.8 ± 0	−20.4 ± 0	4.3 ± 0	−21.1 ± 0.1

[a] Each sample represents three replicates on two eggs (*n* = 6) from a discrete egg producer under a particular regimen.

Grass and vegetation had low δ^{15}N values between 0.8 and 2.1‰ and the lowest δ^{13}C values found in the study, which lie between −29.9 and −31.4‰, whereas a corresponding soil sample (uncultivated) shows typical δ^{15}N and δ^{13}C values around 4.2 and −27.3‰, respectively. A range of insects reported from literature studies (**Table 1**) are included to show the range of possible isotopic values of extra protein that could be ingested by free range chickens. Their δ^{15}N and δ^{13}C values range between 8.5 and 10.5‰ and between −20.5 and −27.8‰, respectively.

C:N ratios of laying pellets have an intermediary value relative to their main components. Wheat and maize have high C:N ratios, whereas soybeans (which are high in protein and hence nitrogen) have low C:N ratios. The C:N ratios of commercial laying pellets appear to be dependent on the proportion of each component used.

Eggs. Carbon and nitrogen isotope values of egg components (yolk, delipidized yolk, membrane, and albumen) for each laying regimen are presented in **Table 2** and **Figure 1**.

Yolks. Egg yolk from caged and barn eggs have δ^{15}N values between 4.0 and 6.6‰ and a range of δ^{13}C values between −19.9 and −28.7‰. Egg yolk from free range and organic eggs have δ^{15}N values between 4.7 and 9.1‰ and δ^{13}C values between −19.7 and −28.8‰. Yolks have high C:N ratios (between 13 and 17%) relative to the other egg components (**Table 3**) due to their higher lipid (carbon-rich) content.

Delipidized Yolks. Delipidized yolks from caged and barn eggs have δ^{15}N values between 3.8 and 6.5‰ and δ^{13}C values between −18.1 and −24.7‰. Delipidized yolks from free range and organic eggs have δ^{15}N values between 4.6 and 9.0‰ and δ^{13}C values between −16.3 and −24.8‰. Delipized egg yolk had a mean C:N ratio of 3.9% across all egg types. Relative to its corresponding whole yolk, delipidized yolk has a more positive δ^{13}C value, as lipids are substantially more depleted in ^{13}C relative to diet (*14*). Hobson (*14*) suggests that minimal isotopic fractionation exists between diet and the protein content of the yolk, so it is necessary to delipidize yolks to enable useful comparisons with dietary inputs.

Table 3. Elemental Composition of Various Egg Components

egg component	% C	% N	C:N ratio (atm)
yolk	60−64	4.4−5.5	13−17
delipidized yolk	35−43	10−13	3.9
membrane	42−46	13−14	3.6
albumen	41−48	12−13	4.1

Membrane. Shell membranes from caged and barn eggs have δ^{15}N values between 2.8 and 6.0‰ and δ^{13}C values between −17.6 and −24.0‰. Membranes from free range and organic eggs have δ^{15}N values between 4.1 and 8.7‰ and δ^{13}C values between −16.1 and −24.0‰. Egg membrane had a mean C:N ratio of 3.6% across all egg types.

Albumen. Albumen from caged and barn eggs has δ^{15}N values between 2.3 and 5.4‰ and δ^{13}C values between −18.2 and −25.1‰. Albumen from free range and organic eggs has δ^{15}N values between 3.5 and 8.0‰ and δ^{13}C values between −16.6 and −25.2‰. Egg albumen had a mean C:N ratio of 4.1% across all egg types.

DISCUSSION

Separation of Laying Regimen Based on Stable Isotopes. Box and whisker plots (**Figure 2**) clearly show the separation of the various farming regimens based on diet using δ^{15}N and δ^{13}C values of egg components (yolk, delipidized yolk, membranen and albumen). In generaln caged and barn egg components can be separated from free range egg components on the basis of δ^{15}N values. Organic eggs cannot be separated from the other regimens due to their large overlap of δ^{15}N values with caged, barn, and free range eggs. Caged eggs have the narrowest box plot for δ^{15}N values of all egg components, suggesting that the nitrogen contribution to their diet is mostly uniform across eggs sampled in this study and that they are eating a mass-produced laying pellet diet.

Comparisons between laying regimens (**Figures 1** and **2**; **Table 1**) show that sample 5 (free range eggs) and samples 2 and 4 (organic eggs) are classed as outliers. They do not plot

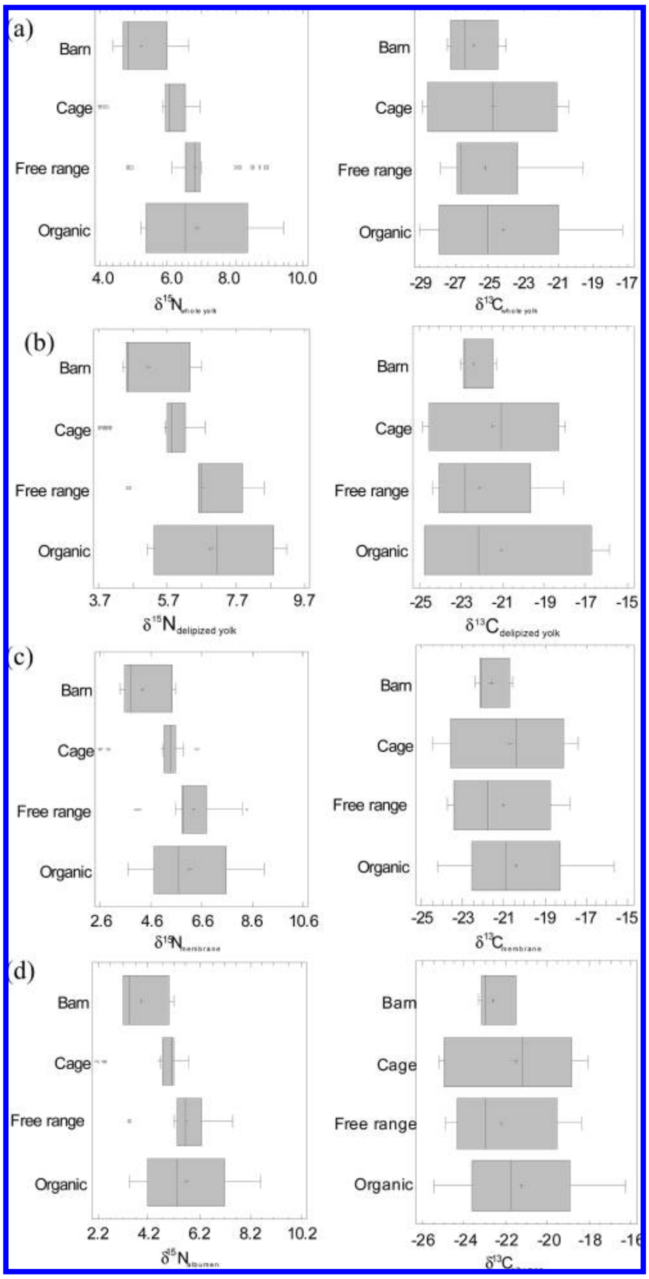

Figure 2. Box and whisker plot showing median, lower, and upper quartile and range of carbon and nitrogen isotope values of (**a**) whole egg yolks, (**b**) delipidized egg yolks, (**c**) egg membrane, and (**d**) albumen for each laying regimen.

with higher δ^{15}N trends seen in the other free range and organic eggs,and lie within the range found for caged and barn eggs in this study. This could be due to mislabeling or the fact that these hens do not have access to the same type of diet as other free range and organic hens in this study. Discussion with a free range farmer suggested his interpretation of his farming methods was no different from those farmers producing barn laid eggs, and the chickens were kept fenced in an enclosure at all times (personal communication). They may also be primarily eating vegetation with low δ^{15}N values such as grass and plants sampled in this study (**Table 1**).

Organic eggs have the largest δ^{15}N value range, including the highest δ^{15}N values in this study. This can be attributed to the large variety of different foods available to organic chickens. A mixture of plant material supplemented with

Table 4. Isotopic Differences (Δ^{15}N) between Delipidized and Non-delipidized Yolk

sample	regimen	Δ^{15}N delip−non-delip	Δ^{13}C delip−non-delip
10	cage	0.4	4.0
11	cage	−0.1	2.8
13	cage	0.2	1.1
14	cage	−0.2	3.7
16	cage	−0.2	4.0
8	barn	−0.2	4.1
9	barn	0.3	2.5
15	barn	0	4.4
5	free range	−0.1	4.0
6	free range	−0.2	1.7
7	free range	0.6	2.5
12	free range	0.1	3.6
17	free range	−0.1	3.3
18	free range	0.2	2.9
1	organic	−0.2	4.0
2	organic	0	4.4
3	organic	−0.1	1.5
4	organic	0.3	2.9

organically grown feed would provide this large range of δ^{15}N values and would be largely based on each organic farmer's interpretation of a suitable organic diet.

It is not possible to separate the egg farming regimens using δ^{13}C values of various egg components (**Table 2**; **Figure 2**). Caged, free range, and organic eggs have the largest δ^{13}C range across each egg component, suggesting that a combination of C_3 and C_4 diets is commonly fed to these chickens. Barn-raised hen eggs had the narrowest δ^{13}C range and did not exhibit the less negative δ^{13}C values found in caged, free range, and organic eggs. This may reflect either the particular sample set or the fact that the eggs were from producers which used feed primarily C_3-containing products such as wheat, with minimal C_4 maize products and/or fish products.

On average, yolk, albumen, and shell membranes of caged and barn eggs were enriched in ^{15}N by 2.5−3‰ relative to commercial poultry feed. However, free range and organic eggs were enriched in ^{15}N up to 5−6‰ relative to commercial poultry feed, signifying these chickens are obtaining nutrients from a higher trophic level or from organically grown produce contributing enriched nitrogen from organic fertilizers through the soil and plant into their diet. Hobson (*14*) reconstructed avian diets using stable isotopes and suggested a nitrogen isotopic fractionation factor between diet and albumen of 3.1‰, similar to other trophic enrichment factors (*3*, *18*). The δ^{15}N values of conventionally produced feed (nonorganic) in the diet of hens in this study range from −0.8 to 3.8‰. As the commercial laying pellets and mash have δ^{15}N ranges from 1.2 to 3.8‰, those eggs with δ^{15}N values above 6.9‰ (3.8‰ diet plus 3.1‰ trophic contribution) are definitely receiving additional protein from higher trophic levels, whereas those eggs in the range of 4.3−6.9‰ may be receiving additional protein from higher trophic levels. Eggs with δ^{15}N values of <4.3‰ are unlikely to be from hens receiving additional protein from higher trophic levels, unless mixed with a ^{15}N-poor diet. Caution should be taken in the interpretation of single eggs that have δ^{15}N values between 4.3 and 6.9‰. Further study is required to understand metabolic differences that may arise on the stable isotope values of egg components from caged (potentially low metabolism) and uncaged (potentially higher metabolism) hens. The use of other isotopes (such as ^{34}S to determine marine food sources) and examination of protein profile

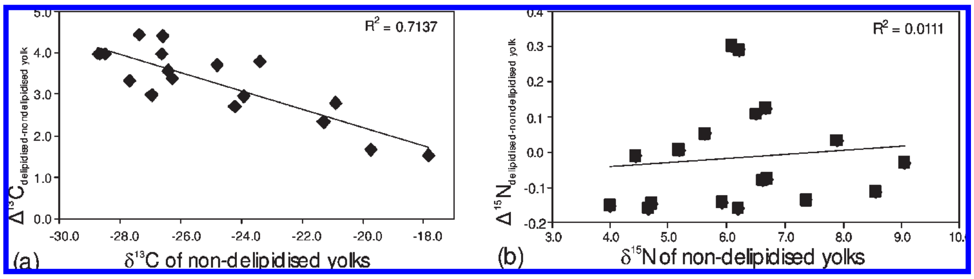

Figure 3. Relationship between (**a**) δ^{13}C and Δ^{13}C delipidized−non-delipidized yolk and (**b**) δ^{15}N values of yolks and Δ^{15}N delipidized−non-delipidized yolk, respectively.

characteristics using NMR or mass spectrometry could better elucidate nutrient transfer from diet to egg.

Egg yolks (both whole and delipidized) have the highest δ^{15}N values from the egg components analyzed in this study and were on average 1−1.2‰ more enriched than the corresponding membrane and albumen. Hobson (*14*) also found the yolk to be more enriched than other egg components.

Albumen has isotopic values very similar to those of its corresponding membrane for both carbon and nitrogen isotopes, suggesting that these components are not significantly fractionated during the process of nutrient ingestion to egg formation. Albumen and membrane from caged eggs have the lowest δ^{15}N values at 2.2 and 2.6‰, respectively. This suggests the maximum fractionation factor (Δ^{15}N) between diet and egg for chickens without exterior food sources and a single source commercial feed is around 2.5−3.0‰ (assuming their diet is 100% soybeans with a δ^{15}N value of −0.3‰).

Albumen and shell membranes did not show a significant enrichment in ^{13}C relative to diet, so it was not possible to relate δ^{13}C ratios of these egg components directly to diet. However, the yolk showed a 2−3‰ depletion due to the contribution of δ^{13}C-rich lipids in the yolks. Once delipidized, the yolk showed a similar trend to the membrane and albumen. The δ^{13}C values of various types of eggs changed according to the type of diet and reflected the use of C_3 and C_4 products in feed such as wheat and maize.

Lipid Removal. Lipid removal from the yolks did not significantly change the δ^{15}N values of delipidized yolks from that of the non-delipidized yolks (R^2 = 0.01, P value = 0.54) (**Table 4**; **Figure 3**). The isotopic difference (Δ^{15}N delipidized−non-delipidized yolk) ranges between −0.2 and 0.3‰, which is within experimental precision for these analyses. Other egg studies (*20*, *21*) have also found that lipid removal did not change the δ^{15}N values for the various egg components, supporting this finding. However, Ricca et al. (*21*) recommended using a diethyl ether for lipid extraction in preference to a chloroform/methanol mix as it is lipid specific and will not bind to and remove substantial amounts of protein, which may cause small positive changes in δ^{15}N values. This study suggests that a 2:1 chloroform/methanol mix is a suitable solvent for lipid extraction of egg yolks and does not remove or fractionate the δ^{15}N values of the remaining protein in these samples.

Delipidized yolks are found to be more positive by up to 4.4‰ in ^{13}C than their corresponding whole (non-delipidized) yolks. The isotopic difference (Δ^{13}C delipidized−non-delipidized yolk) ranges between 1.1 and 4.4‰ (**Table 4**; **Figure 3**). Whole yolks that have more negative δ^{13}C values tend to have larger Δ^{13}C delipidized−non-delipidized values (R^2 = 0.71, P value = 0.004), suggesting a higher lipid content than those with more positive δ^{13}C values. However, there appears to be no correlation to laying regimen with this trend (R^2 = 0.01, P value = 0.5).

Traditionally, delipidization is performed to remove lipids from lipid-rich tissues to enable isotopic comparison with nonfatty tissues in trophic web studies (*18*). Ricca et al. (*21*) suggest lipid interference can bias estimation of foraging locations and diets of higher trophic organisms when samples with variable lipid contents are compared. Results from this study suggest that lipid removal may not reduce any confounding variation in δ^{13}C values, especially within similar samples which display variable lipid contents. Furthermore, a simple, "across the board" correction factor for delipidized egg yolks would bias ^{13}C trophic level food studies, as corrections must be made according to the original δ^{13}C isotopic composition of egg yolk.

In conclusion, this study finds that the nitrogen isotope composition of chicken eggs show promise as an indicator to differentiate eggs laid in a caged and barn-raised regimen and eggs laid by free range hens. Stable nitrogen isotopes of egg components (delipidized yolk, albumen, and membrane) are found to reflect the trophic level of the chickens and can be related to a commercial pellet diet, organic grain diet, or ingestion of higher protein. The carbon isotope composition of chicken eggs, although not a useful indicator of laying regimen, gives insight to the type of product fed to chickens. This technique could also differentiate the laying location of eggs, as maize (C_4 plants) is not used in commercial feed in the South Island of New Zealand.

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