Nutrient sources for offspring formation: diet–mother and mother–offspring isotope discrimination in domesticated gallinaceous birds

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ABSTRACT

Stable isotope techniques can be used to assess nutrient acquisition and allocation strategies used to produce offspring. Before stable isotope techniques can be employed, researchers need reliable isotope discrimination values. In this context, isotope discrimination compares the difference in the isotope ratio between the maternal–offspring tissue that occurs during nutrient transfer prior to egg laying. Currently, isotope discrimination values are unknown between the maternal blood constituents – that reflect different temporal scales of integration – and downy feathers of their offspring. In this study, we experimentally derive isotope discrimination relationships between maternal diet–blood constituents for egg laying, and between maternal blood constituents–down feathers of offspring in an experiment with 3 types of domesticated gallinaceous birds raised on known diets. Our experiment is the first to report isotope discrimination values for maternal blood constituents–down of offspring in avian taxa and provides a new sampling technique that is less invasive than previously available as collecting down does not require sampling viable eggs or individuals. Future researchers can use these results to assist in identifying nutrient sources used by adult birds to produce young.

1. Introduction

Stable isotope analysis (SIA) is a powerful tool in ecology that is used to gain insights into characteristics that are difficult to observe in nature such as migration, niche partitioning, and nutrient allocation [1–3]. SIA depends on knowing the isotope discrimination values for the tissues and isotopes involved. Isotope discrimination is the measured difference in the isotope value between the nutrient source and the consumer tissue, or between maternal tissue and offspring tissue [4,5]. Isotope values in the two tissue types will
differ because of the metabolic processes and isotopic routing involved in tissue synthesis [6–9]. Reliable information on isotope discrimination values and associated variation in values are critical for making accurate inferences using SIA techniques. Unfortunately, discrimination values have only been described for tissues for a few species. Thus, researchers often use values from other taxa even though discrimination values can vary by species, diet, body size, and physiology [10,11]. However, experimentally deriving discrimination values for species-specific, or closely related proxies will lead to the most robust inference using SIA techniques [12].

Income breeding animals, those who rely on local nutrient sources consumed during the time of offspring formation, route nutrients directly from their diet to the formation of their offspring. In contrast, capital breeders, who accumulate and store nutrients for future reproduction, route nutrients from endogenous (i.e. body) reserves (e.g. muscle or lipid sources) to the formation of their offspring. Most studies to date have found that birds exhibit a mixed breeding strategy [13]. The amount of income vs. capital resources used during offspring formation appears to be related to body size with larger individuals capable of storing more nutrients in endogenous reserves for reproduction to deal with environmental variability or access to food [9,14]. Using experimentally derived isotope discrimination values between maternal tissues and downy feathers from young will allow researchers to assess the degree to which dietary sources are used by females to produce offspring and can help identify the timing of when nutrients for offspring formation were acquired [15]. Chick down can be sampled noninvasively from young birds following hatch, which is preferable to destructive sampling of eggs or lethal sampling of juveniles. In addition, the collection of tissue types that reflect different periods of integration or turnover can be used to assess the timing of nutrient acquisition by breeding adults for offspring production. For instance, the turnover in blood plasma of large-bodied birds represents a period immediately prior to egg laying, whereas turnover in red blood cells (hereafter, RBC) represents a longer turnover period (e.g. 1–2 months; [6,8]). In terms of assessing which seasonal habitats were used for nutrient allocation for offspring formation, the collection of multiple tissue types with different periods of turnover is logistically easier and allows greater certainty when defining contributions of different sources used to produce young as compared to working with assumed dietary sources consumed by adult individuals during offspring formation. The collection of tissues with different periods of turnover is especially beneficial for research on migratory species and/or those with vast geographic areas for which it would be logistically difficult to collect dietary sources prior to offspring formation. Using discrimination values directly from the mother’s tissues to infer the sources of nutrient acquisition can circumvent assumptions of food preferences of breeding individuals during embryonic development, especially in migratory species that move long distances during the seasons leading up to breeding. However, researchers currently lack information on stable isotope discrimination values for mothers’ blood to chick down in avian taxa.

In this study, we experimentally derived isotope discrimination values between (1) diet–blood constituents (i.e. plasma and RBC) of mothers and (2) maternal blood constituents–down feathers of offspring using 3 types of domesticated gallinaceous birds (2 chickens and 1 quail). Researchers can use these values to assess dietary sources in wild gallinaceous birds to elucidate income foraging strategies for egg production. All
female captive birds were raised on a consistent, known diet from hatching until they laid eggs. We sampled plasma and RBC from the adult females when they reached breeding age and down feathers from their offspring upon hatch, to estimate isotope discrimination values for diet–maternal blood constituents, and maternal blood constituents–down feather of offspring.

2. Methods

2.1. Experimental design

We raised Australorp and Barred Rock (Gallus gallus domesticus spp.) chickens from chick stage and incubated Japanese quail (Coturnix japonica) eggs at 37.5°C until hatch to comprise our experimental flock. We housed the captive flock in 3 holding stalls (1/bird type) and provided ample floor, roosting, and nesting space (floor space: 0.25 m²/chicken, 0.01 m²/quail; roost space: 20.3 cm/chicken [ground-dwelling quail did not require roosting space]; nesting space: 1 nest box/4 females for each spp., nest box = 35.6 cm wide × 35.6 cm long × 30.5 cm tall). We fed chickens with Purina® Medicated Start and Grow Feed®, and we fed quail with Purina® Non-Medicated Start and Grow Feed®, which was formulated with prebiotics, probiotics, and yeast to support digestive health and immune function. Both chicken and quail feed contained 3% crude fat and 18% crude protein. We tested the isotope values of the feed throughout the duration of the experiment by collecting 3 random samples per bag of feed for both chicken (12 bags) and quail (3 bags). The isotopic composition of the chicken feed was $-19.7 \pm 1.5$ ‰ (mean ± SD) for carbon stable isotope (hereafter, $\delta^{13}$C) and $1.4 \pm 0.5$ ‰ for nitrogen stable isotope (hereafter, $\delta^{15}$N), while the isotopic composition of the quail feed was $-17.2 \pm 1.2$ ‰ for $\delta^{13}$C and $1.2 \pm 0.3$ ‰ for $\delta^{15}$N. The C/N ratio of the feed by atomic mass was 18.3/1. We allowed birds to feed ad libitum throughout the duration of the experiment, which represents an income breeding strategy.

Because body size can influence the period of integration or turnover [8], we raised all birds from the juvenile stage to the breeding age to ensure that body tissues from all bird types were derived entirely from the consistent, known diet source. Birds were fed the consistent diet source for five months for the chicken breeds and two months for the quail type. When females reached breeding age, we placed a male of the breed with the females (1 male/8 female chickens: 1 male/3 female quail) for mating and egg fertilization. After fertilization, we collected all eggs that were laid and placed them in a forced air incubator with an automatic egg turner. We set the incubator temperature at 37.5°C and kept the humidity between 55 and 65 % until 3 days before hatching when we raised the humidity to 70–80 %. Three days before hatch, we removed the eggs from the automatic egg turner and placed them directly on the bottom of the incubator until hatch. Once the chicks hatched, we allowed them to dry (~5 h) before collecting their down feathers.

2.2. Sample collection

We raised 27 female chicks to breeding age (9 black Australorp, 9 Barred Rock, and 9 Japanese Quail), and collected and incubated their eggs, which produced 40 chicks (12 Barred
Rock, 13 Australorp, and 15 Japanese quail). We obtained tissue for the isotope analysis from all females and their chicks. Once adult females reached breeding age, we collected blood samples. We collected a 2–3 ml sample of whole blood from each female by brachial venipuncture. We placed blood samples in plastic vacutainer containing spray coated sodium heparin to avoid clotting. We stored the whole blood samples on ice in a cooler until being centrifuged to separate plasma and RBC within 3 h of collection. For chick tissue samples, we collected ∼5 mg of down feathers from the breast region of individual birds.

We dried blood samples in a freeze drier, and then crushed into small pieces. We removed surface oils from offspring downy feathers using a 2:1 chloroform to methanol rinse, solution then decanted, and samples allowed to dry. Feather samples were then chopped into small pieces using high-precision scissors. All samples were encapsulated in tin cups (3 × 5 mm in dimension) to a weight of 0.6 mg (± 0.1 mg) prior to SIA. Isotope samples were analyzed for stable carbon (δ¹³C) and stable nitrogen (δ¹⁵N) isotopes using a Thermo Scientific Delta V continuous-flow isotope-ratio mass spectrometer with a dual inlet and Conflo IV interface connected to a Costech 4010 elemental analyzer at the University of New Mexico Center for Stable Isotopes. Stable isotope values were reported in parts per thousand (‰), relative to various in-lab organic protein standards including tuna muscle, casein, soy protein, whey protein, and elemental protein. Estimated analytical error was 0.1 and 0.3 ‰ for δ¹³C and δ¹⁵N, respectively, based on n = 200 replicate within-run measurements of various standards.

2.3. SIA and statistical analysis

We conducted analyses directed at assessing the differences in δ¹³C and δ¹⁵N between diet–blood constituent, and maternal blood–down feathers of their offspring for three types of Galliformes. Isotope ratios for both analyses were continuous, and both covariates, bird type and tissue type, were categorical. We implemented the analyses using an ANOVA model within the rstanarm package in R [16]. Each model was run using 4 chains with 1000 warm-up iterations and 1000 subsequent iterations per chain. We assessed model convergence using the Gelman–Rubin (R̂) statistic [17]. The model was validated using posterior predictive checks obtained using the R package shinystan [18] to compare predicted values based on the model to observed values. We built one a priori model that included an interaction with species and tissue type for both δ¹³C and δ¹⁵N as a means of quantifying isotope discrimination values.

We derived isotope discrimination values (hereafter: Δ) in diet–blood constituents and blood constituents–chick down feathers to create differences across bird type that approximates isotope discrimination values. To do so, we summarized (mean ± 90 % credible intervals) estimates based on all stored values in the posterior distribution of coefficients for each bird type x tissue type combination. We evaluated the magnitude of each estimated discrimination value by assessing whether the 90 % credible interval for the estimated discrimination value overlapped zero. We then subtracted these estimates from the summarized estimates for the diet and the chick down for a given bird type. Finally, we derived average discrimination values across the three bird types by
combining all rows stored in the posterior distribution of coefficients for a given tissue type and using the same method as described above.

3. Results

3.1. Model validation

Inspection of model diagnostics indicated that models converged for all estimated parameters ($\hat{R} < 1.01$). Posterior predictive checks indicated that the two models could produce predicted values with characteristics similar to those for the observed data.

3.2. Diet–blood constituent discrimination

Isotope discrimination for both $\delta^{13}$C and $\delta^{15}$N between diet–blood constituents differed depending on tissue type (i.e. RBC or plasma) but showed a similar pattern across bird types (Table 1). Relative to diet, the $\delta^{13}$C values of plasma and RBC were negative (Figure 1), which resulted in negative discrimination values between diet–blood constituent (Table 1). Overall difference of discrimination values for $\delta^{13}$C between diet–plasma and diet–RBC were similar (Table 1). For $\delta^{15}$N, we found that both plasma and RBC had higher isotope values than their diet (Figure 1), which resulted in positive discrimination values between diet–blood for both constituents (Table 1). However, discrimination for $\delta^{15}$N in diet–blood constituent was greater in plasma than in RBC (Figure 1). Variation in discrimination between diet–blood constituents was less in $\delta^{15}$N than $\delta^{13}$C (Table 1).

3.3. Blood–down feather discrimination

Isotope discrimination for both $\delta^{13}$C and $\delta^{15}$N between maternal blood constituents–down feathers of chicks differed depending on blood constituent type but showed a similar pattern across bird types (Table 1). Relative to chick feathers, the $\delta^{13}$C values of plasma and RBC were negative for the adult females across the 3 bird types (Figure 2).

Table 1. Average (± 1SD) discrimination values (denoted $\Delta^{13}$C and $\Delta^{15}$N) for diet–blood and blood constituent–down feather of chickens (Barred Rock and Black Australorp) and Japanese Quail.

| Source to Tissue | Species (n) | $\Delta^{13}$C | $\Delta^{15}$N |
|------------------|-------------|----------------|----------------|
| Diet to Plasma   | Barred Rock (9) | $-0.6$ (0.4) | $4.0$ (0.2)$^a$ |
|                  | RBC         | $-0.6$ (0.4) | $2.7$ (0.2)$^a$ |
| Plasma to Down feather | Barred Rock (9) | $1.0$ (0.2)$^a$ | $0.3$ (0.1)$^a$ |
| Down feather     | Black Australorp (9) | $0.6$ (0.1)$^a$ | $0.4$ (0.1)$^a$ |
| Down feather     | Japanese Quail (9) | $0.3$ (0.2)$^a$ | $0.1$ (0.1) |
| Down feather     | Overall (27)   | $0.7$ (0.3)$^a$ | $0.3$ (0.2) |
| Red blood cells to Down feather | Barred Rock (9) | $1.0$ (0.2)$^a$ | $1.6$ (0.1)$^a$ |
| Down feather     | Black Australorp (9) | $0.6$ (0.2)$^a$ | $1.7$ (0.1)$^a$ |
| Down feather     | Japanese Quail (9) | $0.7$ (0.2)$^a$ | $1.5$ (0.1)$^a$ |
| Down feather     | Overall (27)   | $0.8$ (0.2)$^a$ | $1.6$ (0.1)$^a$ |

Birds were fed ad libitum a consistent source of grain diet.

$^a$Indicate coefficients that had 90 % credible intervals that did not include 0.
resulting in positive discrimination values between maternal blood–down feathers of offspring (Table 1). For δ₁⁵N, offspring down feathers had consistently higher isotope values than maternal RBC regardless of bird type (Table 1, Figure 2), resulting in positive discrimination values. Discrimination value for δ₁⁵N overlapped zero between plasma–down feathers. Variation in discrimination between blood constituent–feather was less in δ₁⁵N than δ₁³C (Table 1).

4. Discussion

Our study experimentally derived discrimination values for diet–blood constituents and blood constituents–down feather for three types of domesticated gallinaceous birds. We found evidence that the pattern in isotope discrimination was similar for all the three species in terms of whether discrimination was positive or negative for each of the various diet–blood and blood–down feather comparisons. By establishing diet–blood and blood–down discrimination values, the current study allows researchers to

Figure 1. Box plots summarizing observed carbon (δ₁³C; A) and nitrogen (δ₁⁵N; B) values (points), empirical means for each tissue x bird combination, and modelled estimates (error bars: mean ± 90% credible interval) for diet and adult female plasma and red blood cell (RBC) tissues for different domesticated gallinaceous bird types. Black dot shows the observed mean value for each cluster.
assess nutrient allocation strategies of females during offspring formation by sampling blood constituents with different periods of integration and down feathers of their young. The current study also provides a sampling technique that is less invasive than previously available methods, which avoids sampling viable whole eggs (but see [19] for alternative techniques to sample egg tissue while avoiding whole egg collection) or lethal sampling of juveniles. Previous studies established discrimination values between diet to egg constituents (i.e. albumen, yolk protein, and membrane; [20]) and egg constituents to chick feather [21,22]. However, the current study avoids the use of multiple discrimination values for a single egg by providing only one discrimination value per blood constituent to assess nutrient strategies used by females to produce down feathers of their young. The physiological processes that are encapsulated in the difference between the blood constituent of the mother to the down feather of their young are likely to be quite complex. Future studies could assess how variable these physiological processes are across different species and diet types.

Isotope discrimination values for $\delta^{13}$C between mother-to-offspring down did not differ between the three bird types for RBC, but the quail differed from the chicken.

Figure 2. Box plots summarizing observed carbon ($\delta^{13}$C; A) and nitrogen ($\delta^{15}$N; B) values (points), empirical means for each tissue x bird combination, and modelled estimates (error bars: mean ± 90% credible interval) for adult female plasma and red blood cells (RBC) and down feathers of offspring for different domesticated gallinaceous bird types. Black dot shows the observed mean value for each cluster.
types for plasma. However, isotope discrimination followed a similar pattern across the considered bird types. Specifically, blood-to-offspring down for $\Delta^{13}C$ in RBC and plasma were consistently positive across the bird types. This pattern reflects biochemical and metabolic routing involved in tissue formation, which indicates that relatively uniform patterns exist for isotope discrimination between maternal tissues to chick down formation [20]. The change in $\Delta^{13}C$ between maternal blood constituents–down feather formation of chicks ranged from 0.3–1.0‰, which is similar to values reported in other studies ($\Delta^{13}C$ from −1.3–1.5‰; [20,22]). However, previous studies assessed isotope discrimination between female diet-to-eggs, or eggs-to-chick down, so previous findings are not directly comparable to our current study. For comparison, variation in blood–down $\Delta^{15}N$ were more similar across bird types, with plasma having no measurable difference in discrimination than RBC across all bird types. Values for blood–down $\Delta^{15}N$ in our study ranged from 0.1–1.7‰, which is lower, on average, than values from previous studies, which reported $\Delta^{15}N$ for diet–egg to range from 2.8–3.6‰ and egg–chick down to a range from 1.7–3.1‰. Results for $\Delta^{15}N$ are, however, more comparable to the isotope discrimination of turtles for mother epidermis–blood of offspring (epidermis: −0.2–1.4‰; whole blood: 2.1–2.3‰; [23]). Our results suggest that researchers wanting to apply SIA to wild gallinaceous species for which discrimination values are lacking could potentially use plasma and RBC to assess foraging patterns along with timing of nutrient acquisition for offspring formation as the patterns of isotope discrimination were largely similar for a given tissue type across a suite of different gallinaceous bird types that vary in body size.

It is important to note that individuals in our study had unlimited access to food, which might make the inferences less applicable to wild birds residing in seasonal environments. Variation in discrimination values is primarily driven by diet, however other factors can also influence the variation in discrimination values including air temperature [6], diet quality and quantity [24], and nutritional stress (e.g. fasting; [25]). One potential way to lessen the impacts of these known sources of variation associated with using only one isotope is to use both $\delta^{13}C$ and $\delta^{15}N$ simultaneously when estimating dietary contributions to offspring formation. It is also important to note that blood plasma contains lipids, which can influence discrimination in $\delta^{13}C$, given lipids are more depleted in $\delta^{13}C$ than proteins. Removing lipids from plasma can standardize lipid content across blood constituents, which will further reduce variation in isotope discrimination values [9,26]. Future studies that include a greater diversity of gallinaceous species with different breeding strategies (e.g. income vs. capital breeders) fed a variety of diet types would be useful. Future studies can also assess compound specific analyses of essential and non-essential amino acids to determine how isotopes routing between diet–blood and blood–feather and corresponding changes to isotope discrimination [8,27]. For now, the discrimination values developed for bulk tissues described herein can help avian researchers to address foraging patterns, along with timing of nutrients acquisition, for offspring formation [13].

**Acknowledgments**

All handling and sampling of animals were conducted under an Agricultural Animal Care and Use Committee permit (2016-AA10). We thank The Nature Conservancy of Montana for use of the experimental facility.
Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was funded by the U.S. Fish and Wildlife Service Inventory and Monitoring Program (FXRS1261066RIM0). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Geolocation information

Coordinates of experimental laboratory: 44.697573 °N, 111.881760 °E

Data availability statement

Data will be deposited in the Dryad Digital Repository once accepted for publication.

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