FORAGING ECOLOGY OF RING-NECKED PHEASANTS RECORDED IN THE STABLE ISOTOPE SIGNATURE OF FEATHERS

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ABSTRACT

Thirty six male ring-necked pheasants *(Phasianus colchinus)* were harvested in South Dakota and Wisconsin from a variety of habitats supporting differing plant communities. Feeding habits of the birds were evaluated by visually identifying gross crop contents and stable isotope ratios of carbon ($^{13}C/^{12}C$) from different regions of tail feathers. Carbon isotope ratios are useful tracers in food webs since the isotopic signals of ingested food become incorporated and expressed in the tissues of consumers.

We found that pheasant crop contents are significantly correlated with the $\delta^{13}C$ values of the calamus (Fall growth) of the feather. Their was no significant correlation between the $\delta^{13}C$ of the rachis (Summer growth) and crop contents. The findings demonstrate that crop content analysis is accurate only over short periods of time, whereas, stable isotope analysis of feathers can show diet changes over several months. Our study also found that pheasants shift their diet toward a higher C_4 plant consumption in the Fall as compared to Summer. The significance of pheasant diet shifting appears to depend on land-use and C_4 plant source availability. When $\delta^{13}C$ values are compared to C4 crop land-use, a significant logarithmic relationship exists. The pheasant diet appears to be saturated in C_4 at approximately 40% C_4 crop land-use. The techniques used may have applications for non-destructive diet studies in pheasants and help provide information for pheasant habitat management.

INTRODUCTION

Ring-necked pheasants *(Phasianus colchicus)* are known to feed on a wide variety of sources. They prefer plants, especially agricultural grain such as corn and soybeans, as well as a variety of seeds of weeds and grasses, buds and soft parts of herbaceous plants (Trautman 1982). Pheasant populations tend to thrive in mixed habitats that include grain fields interspersed with areas of heavier cover supporting permanent vegetation such as native grasses and cattails. In the present study, we were interested in studying seasonal patterns in pheasant foraging habits. Traditional dietary studies involve examining crop contents from birds (Trautman 1982). The crop is a membranous sac utilized to store excess granules of food until digestion can ensue (Welty 1962). However, crop content analyses provide only a snapshot of recent food ingestion. Furthermore, food ingestion is not necessarily synonymous with food assimilation.

An increasing number of studies are using stable isotope analysis of various tissues as a tool for diet reconstruction (Tieszen 1994). The technique has been used substantially in migratory birds to trace origins and relationships (Chamberlain et al. 1997; Hobson et al. 1998), in mammals to trace seasonal diet changes (Ben-David et al. 1997), and in seabirds as a measure of environment and habitat conditions (Furness and Camphuysen 1997). Chamberlain et al. (1997) used stable isotope data from feather samples of black-throated blue warblers *(Dendroica caerulescens)* to trace bird migration patterns. Romanek et al. (2000), analyzed stable isotopes of feathers from wood storks *(Mycteria americana)* to identify foraging areas, since differences in isotopic signature of the feathers could be traced to differences in food sources being consumed when the feathers were being produced.

Stable isotopes are best utilized as tracers in food webs when there are measurable differences in isotopic ratios between different food sources. Many food web studies make use of the fact that C_3 and C_4 plants have distinct carbon isotope signatures (Hobson 1999). Such differences result from differential fractionation of stable carbon isotopes during photosynthesis by C_3 and C_4 plants (O'Leary 1988).

In the present study we attempted to reconstruct pheasant foraging habits using stable isotope analysis of tail feathers together with more traditional analysis of crop contents. We chose to analyze feathers since previous studies indicated that the isotopic composition of different regions of a feather reflect the foods and water consumed by the bird at the time that portion of the feather was being produced (Chamberlain et al. 1997). Although stable isotope studies have been conducted on a variety of bird species, we are not aware of any such studies using pheasants.

In the present study, we first set out to confirm whether the recent diet of pheasants, indicated by crop content analysis, would be reflected in isotope ratios of recently synthesized portions (the calamus) of the tail feathers. We expected that birds feeding primarily on C_3 food sources would have more negative $\delta^{13}C$ values than birds feeding on predominantly C_4 food sources (Fig. 1). Secondly, by analyzing various parts of the feather we hoped to be able to reconstruct the pheasant diet over the course of the year. The study was conducted on pheasants collected from a wide variety of habitats in Codington, Jones, Moody, and Spink counties, South Dakota and St. Croix county, Wisconsin.

METHODS

Male pheasants were collected via upland hunting techniques from October 21 - November 11, 2001. Birds were collected from a wide range of habitats. After each bird was collected, the general plant cover-type within a one half-mile (804.65m) radius of the collection site was recorded. Following col-

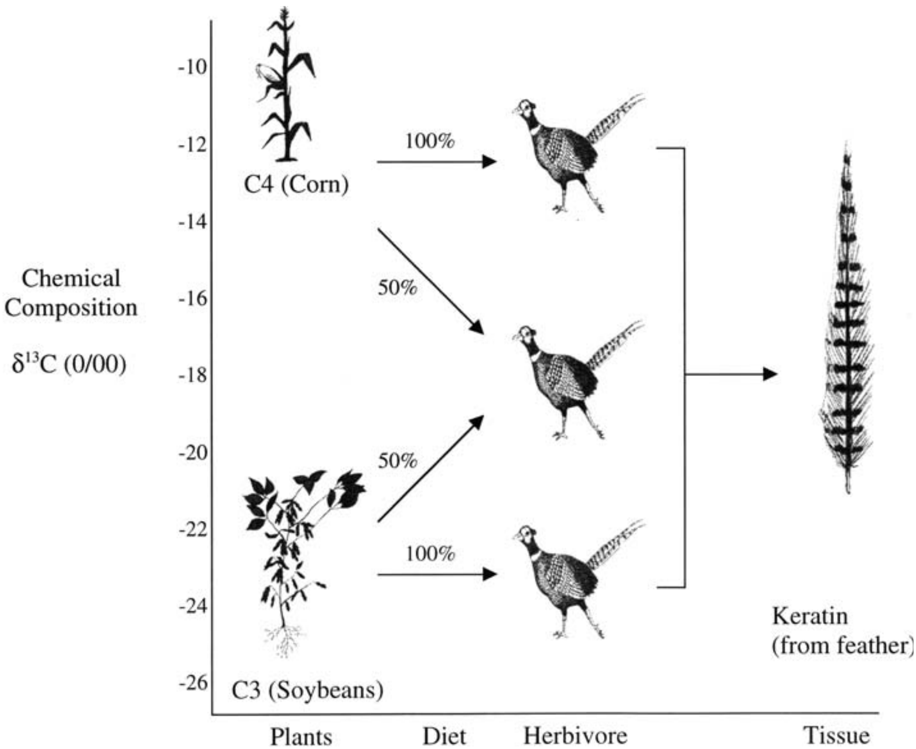

Figure 1. The general relationship between pheasant diet and $\delta^{13}C$ values as taken from the feather. The $\delta^{13}C$ values of the feather can be expected to change according to the ratio of C_3 and C_4 plants in the pheasant diet. Fractionation factors are not figured into the general $\delta^{13}C$ estimates.

lection, the crop of each bird was removed along with the three longest tail feathers. The crop contents were removed in the field and stored in Whirlpak bags using 80% ethanol for preservation.

The tail feathers were allowed to air-dry in the lab for several days to one week. The feathers were then cleaned with 100% ethanol and prepared for chemical analysis. The calamus, which is the proximal and most recently synthesized portion of the feather, was prepared by removing the proximal one centimeter tip of the keratinous feather quill beginning one-half centimeter above the base of the feather. This sample was sliced and cut into small pieces using a sharp blade and stored in 1.5 mL micro-tubes.

The rachis, which is the distal and oldest synthesized portion of the feather, was prepared in a slightly different fashion. First, the feathery veins or colored portions of the feather, were removed using scissors or simply peeling the veins free from the shaft. The first and most distant five centimeters of the rachis were cut and discarded. The second five centimeters of keratin were used for the distal sample analysis. This portion was diced into pieces using scissors and then stored in 1.5 mL micro-tubes.

Small cuttings of keratin from the pheasant tail feathers were loaded in tin cups and weighed on a Cahn C-30 microbalance. These samples, with standards of known composition for $\delta^{13}C$, %N, and %C, were placed in an autosampler and introduced to a Carlo Erba NA 1500 elemental analyzer. The samples were fully combusted at 1030°C in a stream of pure oxygen and oxidative catalysts and reduced on pure copper wires at 650°C. Gases were separated on a chromatographic column and water was removed with a chemical trap. After peaks were detected and analyzed with a thermal conductivity detector, the gases, primarily CO_2, N_2, and the helium carrier gas, were passed to the sample inlet of a VG SIRA 10 dual inlet isotope ratio mass spectrometer through a stainless steel capillary. The CO_2 was cryogenically purified, admitted to the analyzer, and compared to a CO_2 reference gas of known isotopic composition to calculate an isotopic value. Stable carbon isotope ratios, $\delta^{13}C$, were measured relative to PDB, the Pee Dee Belemite scale.

RESULTS AND DISCUSSION

Pheasant tail feathers elongate as they grow which makes them useful timelines for studying dietary changes. Tail feathers are molted annually in the ring-necked pheasant, and like hair, the new feathers grow from the base not from the tip (Welty 1962). The calamus is the basal portion of the tail feather and contains the most recently synthesized keratin in the feather. The rachis is the distal portion of the feather near the tip and it represents the oldest part of the feather. The rachis in our mature tail feathers was most likely synthesized in early to mid-summer shortly after molting, while the calamus was synthesized in later summer or early fall. The condition of the feathers during sample collection in October indicated that tail feather growth had recently ceased in most of the birds. A few of the feathers still had an active blood supply, but most had already dried up, indicating the feather growth had ceased.

Crop content versus isotope ratio of feathers

The $\delta^{13}C$ of the feather calamus showed close correlation with the crop contents (Fig. 2). Birds with crops containing only C_4 plant material had a mean $\delta^{13}C$ value of –15.99 ‰. By contrast, birds with crops containing only C_3 plant material had significantly a lower mean $\delta^{13}C$ value of –20.99 ‰ ($p<0.05$). $\delta^{13}C$ ratios for modern corn, the predominate C_4 plant in the pheasant diet, in general are –11.2 ‰ (Tieszen and Fagre 1993). Typical $\delta^{13}C$ ratios for modern soybeans are not available, but an average value for C_3 plants is –27.1‰ (O'Leary 1988). Birds with crop contents containing a mixture of C_3 and C_4 food sources were intermediate between the other two groups, although with a mean $\delta^{13}C$ of -16.5 ‰ our analyses would suggest that these birds likely had been feeding on food sources heavily weighted towards C_4 plants. Although the crop contents on the day of sample collection contained significant C_3 material, the isotope data suggests that these birds may in fact have recently been feeding heavily on C_4 plant matter. This is one possible advantage of diet analysis us-

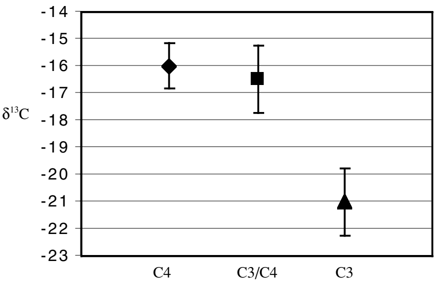

Figure 2. The relationship between the crop contents and mean $\delta^{13}C$ levels of the calamus (proximal feather portion). Those birds labeled with C_4 had only C_4 plant sources within their dissected crop. The birds labeled as C_3 had only C_3 plant sources in their respective crops and birds labeled C_3/C_4 had an unquantified mixture of both C_3 and C_4 plant material in their crops. The standard error bars show the standard error around the mean, calculated as the standard deviation divided by the square root of the sample size. A one-way ANOVA showed a significant difference between groups. A Tukey's HSD post-hoc test shows that at α=0.05, 3.954 $\delta^{13}C$ must separate samples in order for them to be significantly different. A difference was found between birds labeled C_4 and C_3 and also birds labeled C_3 and C_3/C_4. The difference between C_4 and C_3/C_4 was found to be insignificant.

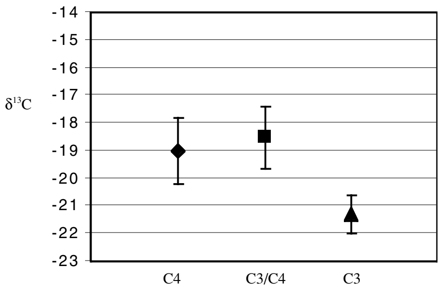

Figure 3. The relationship between the crop contents and mean $\delta^{13}C$ levels of the rachis (distal feather portion). Those birds labeled with C_4 had only C_4 plant sources within their dissected crop. The birds labeled as C_3 had only C_3 plant sources in their respective crops and birds labeled C_3/C_4 had an unquantified mixture of both C_3 and C_4 plant material in their crops. The standard error bars show the standard error around the mean, calculated as the standard deviation divided by the square root of the sample size. A one-way ANOVA gave a p-value of 0.17, indicating that no significant differences existed between groups. The insignificance suggests that changes in diet over longer periods of time make stable isotope analysis a more accurate option than crop contents for evaluating the pheasant diet.

ing stable isotope mass spectrometry. Though crop contents may indicate daily diet, they do not reflect the diet over a more extended period of time.

In contrast to data from the calamus, $\delta^{13}C$ from the older part of the feather (rachis) did not correlate as well with crop contents (Fig. 3). There were no significant differences in $\delta^{13}C$ among the three groups (p=0.17). This result was not unexpected since the rachis was synthesized in early to mid-summer. As such, its isotopic composition would be expected to reflect food sources ingested in June, not the Fall when our crops contents were analyzed. The fact that we obtained different statistical results when using $\delta^{13}C$ from the rachis versus calamus provides a hint that the pheasant diet may have shifted over the course of the growing season. A more direct examination of this possibility follows next.

Comparison of isotope ratios of the rachis and the calamus

Calamus samples had significantly higher $\delta^{13}C$ values than rachis samples, $p<0.001$ (Fig. 4). This difference was consistent across all four counties, although the effect was more pronounced in some areas than others. The increase in δ^{13}C in the younger, calamus portion of the feather is consistent with increased consumption of C_4 plants, such as corn, in late summer and early Fall when the calamus was actively growing. Earlier in the season, our isotope data provide evidence that the birds made greater use of C_3 food sources. The availability of mature corn kernels in the Fall may be the most significant factor contributing to the shift of isotope signature in the calamus (Fig. 4). It is well known that corn kernels are a significant food source for pheasants in the fall and winter (Trautman 1982). Thus it seems reasonable that pheasants might make use of a variety of food sources (C_3 and C_4) in early to mid-summer, but as agricultural crops ripened in the Fall, the pheasants would switch to a diet favoring corn, a C_4 plant.

As noted earlier, the seasonal shift in δ^{13}C was more pronounced in some counties than others (Fig. 4). These differences most likely are related to differences in plant and crop cover in the various counties. Birds from Jones County had the lowest δ^{13}C values, which is consistent with a higher concentration of C_3 plants in the diet. This is not surprising since Jones County does not support a lot of corn production. Land use in this county, located west of the Missouri River, is dominated by pasture lands with lesser amounts of row crop agriculture. By contrast, Moody and Spink Counties, located east of the Missouri, are dominated by row crops including significant amounts of corn. The elevated δ^{13}C values in calamus samples, so pronounced in Moody County birds may be explained by marked increases in corn consumption in the Fall. We do not have an explanation for the less dramatic increase in δ^{13}C in the calamus of birds from Spink Country.

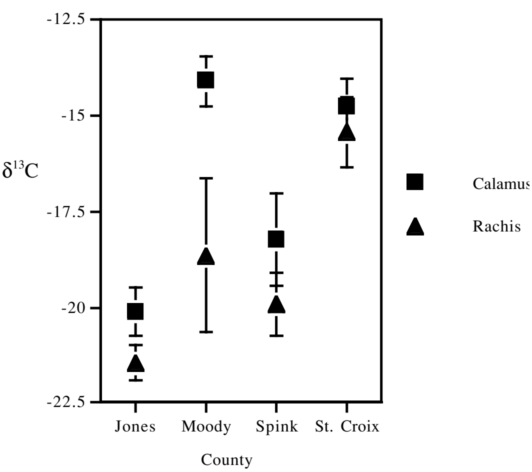

Figure 4. The differences between the averages of the δ^{13}C levels for the calamus (proximal portion) and the rachis (distal portion) between birds from the same county. Paired sample t-tests showed significant differences with a p-value of < 0.001 for all birds combined.

Isotope ratios of birds from St. Croix County, Wisconsin, were distinct from the South Dakota birds (Fig. 4). The mean δ^{13}C of rachis samples from Wisconsin was -15.43 ‰, which was elevated compared to rachis samples from South Dakota where mean values ranged from –21.44 ‰ in Jones County to –18.64‰ in Moody County (Fig. 4). Not only were the rachis values higher in Wisconsin birds, but there was no significant difference in δ^{13}C between rachis and calamus samples ($p=0.348$).

One explanation for the marked differences in $\delta^{13}C$ from the Wisconsin samples may relate to differences in plant cover. Our study areas in South Dakota lie in the prairie whereas St. Croix County, located in west central Wisconsin, is characterized by mixed forests and crop lands. Thus the corn fields in St. Croix County are not bordered by grasslands as in South Dakota, but rather by forests. In South Dakota, pheasants are frequently observed making considerable use of grassland habitat as well as crop lands. Pheasants are not generally associated with forest habitats, a view supported by our isotope data. Trees are C_3 plants with $\delta^{13}C$ values similar to other C_3 plants. The elevated $\delta^{13}C$ values in the Wisconsin birds (-15.43‰) provides strong evidence that these birds did not rely on trees or forest insects as food sources (Fig. 4). The lack of surrounding grasslands in Wisconsin may result in pheasants relying more on crop lands for food. Thus corn may have been the dominant food source for pheasants, not only in the Fall, but throughout the summer. Prior to ripening of the corn in the Fall, the pheasants may have fed directly on corn leaves and/or indirectly through consumption of herbivorous insects associated with corn. In either case, increased reliance on the C_4 corn plant as a food source throughout the growing season could explain the elevated $\delta^{13}C$ found in both the rachis and calamus of the St. Croix County pheasants, as well as the absence of a late season isotopic shift, so evident in the South Dakota birds (Fig. 4).

^{13}C Content in Pheasants as Related to Land Use

When available, pheasants appear to make heavy use of C_4 crops as a food source, as supported by our isotope and crop content data, personal observations in the field, as well as literature studies (Trautman 1982). The relationship between pheasants and C_4 crops was examined further by plotting the percentage of C_4 crop cover within a one-half mile radius (804.65m) around each of our study birds against the $\delta^{13}C$ levels from the birds (Fig. 5). Though all C_4 crops were included in the analysis, corn was the predominate C_4 food source seen during visual crop content evaluation. Only four birds had C_4 plant material in the crop other than corn.

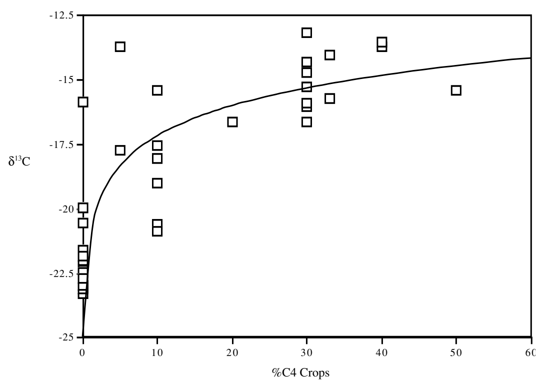

Figure 5. The relationship between the $\delta^{13}C$ levels from the calamus (proximal feather portion) and the percent of C_4 plant usage for a one-half mile (804.67 meters) radius around the sample. The relationship is significantly logarithmic as shown by a logarithmic transformation and regression analysis. The R^2 value for the relationship was found to be 0.64 and p<<0.01.

Pheasants harvested from areas without any corn cover had relatively low $\delta^{13}C$ levels in the calamus (Fig. 5). With increasing corn cover, $\delta^{13}C$ values increased (Fig. 5). The resulting logarithmic relationship was highly significant ($p<0.001$) and provides further evidence that pheasants make increasing use of corn in their diet as this C_4 plant becomes more available in the surrounding habitat.

Smith (1999) reported that the average home range for pheasants varies from as little as 35 hectares to 150 hectares. Each of our study area included 203.4 hectares, a large enough area to include the reported pheasant range. We are confident that the pheasants sampled were consistently feeding within our study range.

Though all C_4 crops were included in the study, corn was by far the most prevalent C_4 crop and will thus be used from now on to represent C_4 crop land use. The relationship between corn crop cover and pheasant isotope ratios shows evidence of saturation beginning at a level around 30-40% corn cover (Fig. 5). Thus when corn cover in the immediate vicinity of the birds reaches 30-40%, it appears that the pheasants reach a maximum for corn ingestion and assimilation. Extrapolating from our data, we would not expect a bird collected from an area supporting 100% corn cover to differ significantly in isotopic composition from pheasants living is an area with 50% corn cover.

According to the published data regarding $\delta^{13}C$, -11.2‰ is the typical $\delta^{13}C$ value for modern maize (Tieszen and Fagre 1993). Our data suggest that the maximum $\delta^{13}C$ for feathers from pheasants that are maximizing their intake of corn is only -14 or -15‰ (Fig. 5). Though no fractionation studies have been done on pheasants, it can be assumed that ^{13}C fractionation in the feather would be similar to birds with comparable diets. Hobson and Clark (1992) found that crows raised on a corn based diet had a +3.5‰ fractionation mean between the feather and diet. By assuming similar fractionation it can be reasoned that a pheasant feather with a $\delta^{13}C$ of -14‰ resulted from a diet with a –17.5‰ value. One possible explanation could be that pheasants, though they may prefer corn as a food source, may still mix in other foods (C_3 source) even when corn is highly available. This could also explain $\delta^{13}C$ ratios in pheasant feathers, which are more positive than what would be expected if they were feeding entirely on C_3 sources.

CONCLUSION AND RECOMMENDATIONS

The results presented show the numerous possibilities for stable isotope analysis to evaluate the pheasant diet and movement. Past studies evaluating the pheasant diet have relied on crop content analysis, requiring large scale destructive sampling techniques. Although destructive sampling techniques were used in this study, a nondestructive approach could feasibly be used in future studies. The combination of $\delta^{13}C$ stable isotope analysis with that of ^{15}N analysis would provide a deeper level of insight into the exact composition of the pheasant diet. This work would need to be strictly evaluated due to the less consistent nature of ^{15}N incorporation into tissue when other biotic stresses are prevalent (Hobson et al. 1993). A controlled experiment involving captive pheasants raised on diets with varying composition would help to establish a normal standard for the $\delta^{13}C$ expected in the tail feather as related to diet composition.

The usefulness and applicability of stable isotope data can be used in conjunction with other data to determine complex relationships between animals and their habitat. The importance of corn to the diet and overall fitness of snowgeese *(Chen caerulescens)* in relation to population has been evaluated by $\delta^{13}C$ analysis (Hobson 1999). Similar studies may be applicable to pheasants living in the varied habitat and bird population ranges of South Dakota. Isotope data could also be evaluated in conjunction with remote sensing to provide information on bird movement patterns and range.

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