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DIGESTION OF CHITIN BY NORTHERN BOBWHITES AND AMERICAN ROBINS[1]

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Abstract. Northern Bobwhites (*Colinus virginianus*) were acclimated to crickets, and food intake and excreta output measured. Their metabolizable energy coefficient (MEC* = 1 − energy excreted/energy ingested = 0.77) was significantly higher than that of American Robins (*Turdus migratorius*) that were unacclimated or fully acclimated to eating crickets. We measured apparent chitin digestibility (D_{chitin} = 1 − chitin excreted/chitin ingested) in both species and tested the predictions that (1) D_{chitin} would be higher in bobwhites than robins, and (2) D_{chitin} would be higher in robins after acclimation. D_{chitin} ranged from 0.07–0.14 with no significant difference between or within species, so both predictions were rejected. These apparent chitin digestibilities are low relative to values reported for seabirds that eat krill, and account for < 1% of the metabolizable energy in crickets.

Key words: insectivore, arthropod, cuticle, metabolizability, fat digestibility, Northern Bobwhite, American Robin, chitin.

Many birds rely on arthropods (including krill) as a food source. Up to 60% of arthropod dry mass can be cuticle and up to 30% can be chitin, the major cuticular component along with protein (Jeuniaux 1971, Welinder 1974, Hackman and Goldberg 1981, Bernays 1986). Whether the cuticle represents indigestible dietary bulk or a source of energy from either the protein or chitin components is practically unknown for wild avian insectivores (however see Jeuniaux and Cornelius 1978) . Knowing digestibility of cuticle and chitin would allow researchers to estimate energy intake of avian insectivores more accurately. Currently, the metabolizability of insects to birds (i.e., metabolizable energy coefficient, MEC* = 1 − energy excreted/energy ingested) is reported to vary considerably among species eating different insects (from 0.57 [Bryant and Bryant 1988] to 0.86 [Gibb 1957]), and even among species eating the same insect, the domestic cricket (*Acheta domestica*) (from 0.64 [Koenig 1991] to 0.83 [Robel et al. 1979]). How much of this variation is due to differences in chemical composition (i.e., the ratio of cuticle and chitin to the very digestible soluble proteins and fat of arthropods), to differences in ability to digest cuticle and chitin, or to differences in ability to digest the soluble protein and fat of arthropods?

We tested two hypotheses about the role of chitin as a source of variation in digestion of one type of arthropod within and among avian species: (1) we predicted that chitin digestion would be higher in American Robins (*Turdus migratorius*) after acclimation to a cricket diet than when first switched onto crickets, because their MEC* increased significantly ($P < 0.001$) from 0.58 during the first 3 days after a dietary switch from fruit to crickets to 0.71, 8 to 10 days after the switch (Levey and Karasov 1989). (2) We predicted that chitin digestion would be higher in Northern Bobwhites (*Colinus virginianus*) than in American Robins because the MEC* of Northern Bobwhites fed crickets is reported to be considerably higher (0.83; Robel et al. 1979) than in American Robins.

Although we previously had collected the data on American Robins (Levey and Karasov 1989), the data on Northern Bobwhites were collected in another laboratory. Therefore, we first repeated that experiment and confirmed the interspecies difference in MEC* on crickets. Then we performed gravimetric measurements of chitin content of food and excreta from the feeding trial with Northern Bobwhites and on stored samples from the previous feeding trial with American Robins. We calculated apparent chitin digestibilities and compared them within the American Robins and between the robins and bobwhites.

MATERIAL AND METHODS

Eight (5 males, 3 females) 30-day-old hatchery raised bobwhites were housed in separate cages (ca. 0.03 m^3) indoors at 24 ± 1°C and were kept on a 12 hr photoperiod; water was provided ad libitum. They received only crickets ad libitum for 3 weeks, then crickets supplemented with pheasant starter pellets (Hamre Feed Service, Deforest, WI) for 1 week because their mass gain was low relative to other bobwhites on pellets alone, and then only crickets for the final two weeks. On the last 5 days their food was rationed and body mass (mean ± SE = 146.5 ± 6.3 g) and excreta production were monitored.

Daily dry matter consumption (6.6 ± 0.4 g dry mass day^{-1}, n = 8 bobwhite) was calculated by subtracting

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the dry mass of uneaten crickets (orts) from the dry mass of crickets fed to the bobwhites. The dry matter content (% of wet mass = 26 ± 1%), as well as an estimate of cricket size (93 ± 0.6 mg dry mass individual^{-1}), were determined in 5 batches of 50 crickets each, drawn randomly each day. Feces and orts were collected from plastic sheets on cage floors that were changed each morning within 1.5 hr of lights turning on. These procedures were similar to those used for robins by Levey and Karasov (1989) who report the robins' body masses (77.8 ± 4.6 g) and intake rates.

Energy contents of food and excreta were determined on a Phillipson microbomb calorimeter (Gentry Instruments). Two or more replicates were run on each sample and coefficients of variation were < 3%. Cricket energy content was 25.7 ± 0.3 kJ (g dry mass)$^{-1}$ (n = 4). Apparent metabolizable energy coefficient (apparent because uncorrected for endogenous losses) was calculated as defined above (Karasov 1990).

Chitin content (% dry mass) in samples of food and excreta from this feeding trial and from the previous trial with American Robins (Levey and Karasov 1989) was determined by the "fritted glass crucible method" for measuring crude fiber in animal feed (Helrich 1990). The technique includes a preliminary soxlet fat extraction with ether, so crude fat also was measured. Two or three replicates of fat and crude fiber were run on each sample, and coefficients of variation were < 5%. Jackson et al. (1992) found that the crude fiber technique gave an estimate of chitin content similar to that measured using specific chitinase. We compared the technique to a specific spectrophotometric assay for chitin (Hackman and Goldberg 1981) and found that it gave similar mean values for two batches of crickets, but that it was easier and more repeatable (independent t-tests on the arcsine of the square root of proportion chitin for each batch; P-values for differences between the techniques were 0.27 and 0.14). We made one change in the crude fiber technique: samples of crickets, but not excreta, were filtered through a California buchner funnel rather than a crucible after acid treatment, and then filtered through crucibles after treatment with base. This modification prevented the proteinaceous cricket material from clogging the crucibles before it was fully dissolved in the base solution. One sample was filtered both ways and the mean chitin contents were 4% apart. Chitin contents (% of dry mass) of crickets fed in trials were 6.6 ± 0.1% (n = 4) for bobwhites and 7.5 ± 0.1% (n = 4) for robins (t_6 = 10.3, $P < 0.001$). Crude fat (% of dry mass) of crickets fed to bobwhites (25.8 ± 0.1%, n = 5) was higher than in crickets fed to robins (21.8 ± 0.1%, n = 5; t_8 = 21.3, $P < 0.001$), which explains the higher gross energy content of crickets reported above (25.7 ± 0.3 kJ g^{-1}) than reported by Levey and Karasov (1989; 23.2 ± 0.1 kJ g^{-1}). Apparent chitin or fat digestibility was calculated as 1 − [(excreta mass)(% chitin or fat in excreta)/(mass food)(% chitin or fat in food)].

Results are presented as means ± SE. Within species comparisons of chitin digestibility for American Robins during the two different 3-day periods were made by repeated measures analysis of variance (ANOVA) on the arcsine of the square root of the fractions. Comparisons between robins and bobwhites were made using one-factor ANOVA (factor = species).

RESULTS AND DISCUSSION

The bobwhites were in slight negative mass balance (−0.68 ± 0.26% day^{-1}, n = 8), which can lead to a small underestimate of MEC* if uric acid from muscle catabolism is excreted (Karasov 1990). Even with this most conservative estimate, bobwhites still had significantly higher MEC* (0.77 ± 0.01, n = 8) than American Robins that were unacclimated (0.58 ± 0.01, n = 10) or fully acclimated (0.71 ± 0.01) to crickets ($F_{1,16}$ = 255 and 34, respectively, $P < 0.001$ in both cases). Despite this, apparent chitin digestibility for bobwhites (0.067 ± 0.02) was not higher than for American Robins either before acclimation of the latter to crickets (0.136 ± 0.027; $F_{1,16}$ = 2.7; $P > 0.1$) or after (0.078 ± 0.021; $F_{1,16}$ = 0.003; $P > 0.9$). Hence, we rejected prediction #2. Apparent chitin digestibility did not change significantly within American Robins before or after acclimation to crickets ($F_{1,9}$ = 2.0; $P > 0.1$), so we also rejected prediction #1.

Because differences in MEC* within robins, and between robins and bobwhites were significant (above), this suggests that increased chitin digestibility contributed little to increased MEC* within and between species. We explored this further by regressing the residuals for MEC* against the residuals for apparent chitin digestibility, but the relations were nonsignificant (for robins and bobwhites, respectively, t_8 = 0.57, $P > 0.5$ and t_6 = 1.67, $P > 0.1$). Thus, we rejected the predictions that higher chitin digestibility would correlate with higher MEC*s within and between species.

Presumably, the differences in MEC* are due to other digestive differences that determine the extraction xof protein and fat (Levey and Karasov 1992). Indeed, from our crude fat measures the apparent fat digestion of Northern Bobwhites (0.939 ± 0.002, n = 8) was significantly higher than for American Robins ($F_{1,16}$ = 44.3; $P < 0.001$), which did not differ significantly between the first period (0.907 ± 0.008) and second period (0.916 ± 0.003) ($F_{1,9}$ = 1.67; $P > 0.2$ by repeated measures ANOVA). Thus, differences in fat digestion may account somewhat for bobwhites' higher MEC* on crickets, but cannot account for the changes in MEC* during the robins' acclimation to crickets. Because fat accounted for about 40% of the crickets' energy (above), and the difference between species in fat digestibility (0.032) was smaller than the difference in MEC* (0.06), it is apparent that differences in crude protein digestibility probably account for a notable fraction of the difference between species in MEC*. More thorough digestion of both fat and protein could come about through longer retention of digesta (Levey and Karasov 1992), greater grinding action in the gizzard, or more enzyme activity in the proventriculus or small intestine.

The level of apparent chitin digestion was only about 10% in American Robins and Northern Bobwhites. Much higher apparent chitin digestibilities have been reported in some other birds, e.g., 57% of the chitin in mealworms by Japanese Nightingale *Liothrix lutea* (Jeuniaux and Cornelius 1978), and 39–

85% of the chitin in marine invertebrates by several species of seabirds (Jackson et al. 1992). Because all these studies estimated chitin contents by similar methodology (crude fiber), we suspect that the differences are real and not methodological artifacts. The suborganismal features that result in greater chitin degradation at the whole-animal level (e.g., longer digesta retention time, or higher chitinase activity by host, gut microbes, or even autocatalysis by prey) remain to be elucidated, as does the extent to which the apparently digested chitin is actually absorbed and metabolized.

For robins and bobwhites eating crickets, the energy potentially absorbed from chitin, estimated as 0.1×0.07 g chitin (g dry cricket)$^{-1} \times$ ca. 18 kJ (g chitin)$^{-1} = 0.126$ kJ g^{-1}, was less than 1% of the metabolizable energy in crickets (ca. 25 kJ g$^{-1} \times 0.75 = 19$ kJ g^{-1}; above). Even for seabirds with higher apparent chitin digestibilities, the energy released from chitin digestion is usually only a few percent of total metabolizable energy because the amount of chitin in arthropod prey is relatively small (Jackson et al. 1992). Hence, whereas birds may possess chitinase enzyme to break down the compound, its major benefit may be enhanced efficiency or rate of digestion of soft prey tissues within the exoskeleton (Jackson et al. 1992). We need more information on protein release from cuticle and the extent of the contribution of that protein to the energy and nitrogen economy of the animal.

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