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Endogenous Mass and Energy Losses in Ruffed Grouse

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Calculating utilization efficiencies of foods in birds is confounded by the mixing of urinary wastes with the undigested food passing through the cloaca, as well as by the continual loss of mass and energy from the alimentary tract in the form of sloughed epithelial cells, microbes, and digestive secretions. These are often termed endogenous urinary (U_e) and metabolic fecal (F_m) losses, respectively (National Research Council 1981). However, in this paper we refer to them collectively as endogenous losses. In cases where these endogenous losses have not been quantified the apparent assimilable mass coefficient (AMC*), and the apparent metabolizable energy coefficient (MEC*) can be calculated using the relationships:

$$\text{AMC}^* = (Q_i - Q_e)/Q_i = 1 - (Q_e/Q_i) \quad (1)$$

and

$$\text{MEC}^* = (GE_iQ_i - GE_eQ_e)/GE_iQ_i$$
$$= 1 - GE_eQ_e/GE_iQ_i, \quad (2)$$

where Q_i and Q_e are the rates of food intake and excreta output in $g \cdot kg^{-1} \cdot day^{-1}$, respectively, and GE_i and GE_e are the energy content of the food and excreta respectively (Kendeigh et al. 1977, Karasov 1990). If the endogenous losses are known, however, the true values of AMC and MEC may be calculated using:

$$\text{AMC} = \text{AMC}^* + E_m/Q_i, \quad (3)$$

and

$$\text{MEC} = \text{MEC}^* + E_e/GE_iQ_i, \quad (4)$$

where E_m and E_e are the endogenous losses of mass ($g \cdot kg^{-1} \cdot day^{-1}$) and energy ($kJ \cdot kg^{-1} \cdot day^{-1}$), respectively (Sibbald 1976, Karasov 1990).

Utilization efficiencies are most often reported as apparent coefficients and, in cases where the experimental subjects are eating sufficient quantities to maintain body mass, AMC* and MEC* will differ from the true values by only a few percent (Miller and Reinecke 1984, Karasov 1990). However, it is evident from equations (3) and (4) that in cases where food intake (Q_i) is low, or where E_m and E_e are unusually high, the impact of endogenous losses may become significant. Thus, differences in apparent utilization efficiencies that are observed within a species (as a function of diet or season) or between species might simply be artifacts of the interaction between endogenous loss and level of intake, rather than differences in food digestion or metabolism.

We felt that a correction for endogenous losses might be necessary in our planned study of the nutritional ecology of Ruffed Grouse (*Bonasa umbellus*) due to the possibility that the grouse would not eat enough of some of the less palatable plant species over a three-day feeding trial to allow us to measure utilization efficiencies accurately. Therefore, we sought to quantify the endogenous mass and energy losses in Ruffed Grouse rather than rely on extrapolation from data on other species. Endogenous losses have been calculated only for chickens (*Gallus gallus*; Guillaume and Summers 1970, Sibbald 1975, 1976, 1981, Sibbald and Price 1978, Campbell et al. 1983), the domestic Embden goose (*Anser anser*; Storey and Allen 1982a, b), the domestic Muscovy (*Cairina moschata*; Mohamed et al. 1984), the domestic Mallard (*Anas platyrhynchos*; Miller and Reinecke 1984, Lu et al. 1985) and the mule duck (♂ *Cairina moschata* × ♀ *Anas platyrhychos*; Lu et al. 1985).

Ruffed Grouse were captured using clover-leaf traps during the fall dispersal period (15 August–15 October, 1989) in Sawyer County, Wisconsin (Dorney and Mattison 1956). Eight birds (4 male, 4 female; body mass $\bar{x}$ = 601.1 ± SE of 20.1 g) were housed individually in 44 × 60 cm galvanized steel-mesh cages, each with a 42 × 42 cm opaque plexiglass hiding box attached to one end. Cage sides were lined with nylon screening to prevent sample loss (Parrish and Saunders 1989), and taut plastic ceilings were installed to protect the grouse from head injuries. Grouse were maintained on the experimental diet (a 50:50 mixture of Purina gamebird maintenance chow and Purina horse chow 100, 33.3% neutral detergent fiber, 13.1% crude protein, gross energy content 17.9 ± 0.61 kJ/g dry mass; the diet of Servello et al. [1987], except without 2% corn oil) for six months prior to the feeding trials. All experiments were performed at room temperature (19°–21°C). The photoperiod was 9 h light and 15 h dark because subsequent experiments required a simulated winter light cycle. Water and grit were provided *ad libitum*. Grit was removed prior to and during feeding trials.

Three-day feeding trials were conducted using the total collection method. Dropping pans were lined with 6-mil clear plastic. Uneaten food, fecal and cecal droppings were collected daily, frozen at −20°C, and lyophilized. Fecal and cecal droppings were combined for analysis. Grit was rarely encountered in fecal samples, and was removed as necessary. Samples from day 1 of the experiments were omitted from the analysis because the food passage rate is such that some excreta is a reflection of the intake level of the

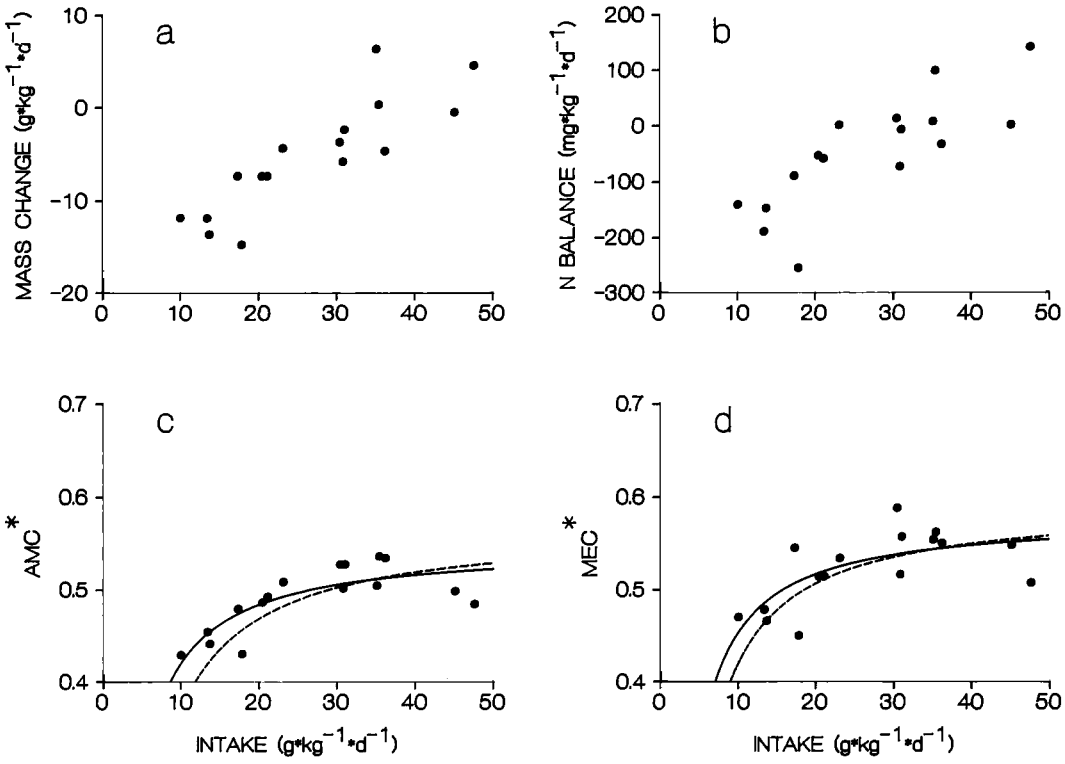

Fig. 1. (a) Mass change, (b) nitrogen (N) balance, and apparent utilization efficiencies of (c) mass and (d) energy of Ruffed Grouse existing on a diet at different levels of intake. Eight birds were each fed at two levels of intake. AMC* (apparent assimilable mass coefficient) and MEC* (apparent metabolizable energy coefficient) measured as described in text, and corrected to N balance using the data in (b). Solid lines in (c) and (d) are fits of data to equations (3) and (4), respectively. Dashed lines in (c) and (d) are fits of data to equations, but without correction to N balance. Notice that apparent utilization efficiencies are a decreasing function of food intake.

previous day (Gasaway et al. 1975, Guglielmo and Karasov unpubl. data). Body mass was measured daily.

All grouse received two experimental treatments. In the first feeding trial, all grouse were fed the basal diet *ad libitum*. Then, after a six-day break, the grouse were randomly divided into four experimental pairs. Each pair was assigned one of the following levels of intake: 0.65, 0.55, 0.45, and 0.35 of each individual's *ad libitum* intake as measured in trial 1. Feeding trials were conducted as before.

Energy content of food and excreta were measured using a Phillipson microbomb calorimeter (Gentry Instruments) with a benzoic acid standard. Samples were dried overnight at 50°C before analysis. Total nitrogen was measured using the Kjeldahl technique by the University of Wisconsin Soil and Plant Analysis Lab. Means are reported ± standard errors.

Under *ad libitum* feeding conditions, dry matter intake averaged 36.45 ± 2.32 g·kg^{-1}·day^{-1}. Body mass change ($\bar{x}$ = −0.78 ± 1.53 g·kg^{-1}·day^{-1}) and nitrogen balance (total nitrogen intake minus total nitrogen excretion; $\bar{x}$ = 18.21 ± 24.6 mg·kg^{-1}·day^{-1}) were not significantly different from zero (P = 0.63 and 0.48, respectively). The ratio of excreta produced on day 3 to day 2 of a trial did not vary among treatments, indicating that there was no significant change in rate of digesta flow related to the reduction of food intake (F = 1.1, df = 3 and 4, P = 0.45). As food intake rate was reduced, the grouse lost body mass and went into negative nitrogen balance (Figs. 1a and 1b). Nitrogen intake during the food reduction trial was probably submaintenance (43–96% of that estimated for birds from Robbins 1993). Therefore, the grouse needed to utilize body protein to meet the daily nitrogen (i.e. amino acid) requirement. Amino acids not used efficiently in new protein synthesis along with any needed as fuel to meet the daily energy requirement were catabolized, resulting in an excess of nitrogen excreted relative to nitrogen ingested. If one assumes that all of the excess nitrogen was in the form of uric acid, a correction of mass and energy loss to nitrogen balance may be made by adding the product of the nitrogen balance and, respectively, 3 g/gN or 34.5 kJ/

gN, to the total fecal mass or energy in equations (2) or (3) (Parsons et al. 1982, Karasov 1990). These corrections have been recommended for determinations of utilization efficiencies and endogenous energy losses (Parsons et al. 1982, Sibbald and Morse 1983, Wolynetz and Sibbald 1984, Dale and Fuller 1986). The utilization efficiencies and endogenous losses we report are based on the nitrogen-corrected data.

The nitrogen-corrected apparent utilization efficiencies of mass and energy for the chow diet determined under the *ad libitum* feeding regime were 0.51 ± 0.006 and 0.55 ± 0.009, respectively. As expected, the reduction of food intake rate caused a significant drop in both AMC* ($P = 0.0047$) and MEC* ($P = 0.0137$). True AMC and MEC, and E_m and E_e were calculated by fitting the data of Figures 1c and 1d to equations (3) and (4), respectively (nonlinear curve fitting, Gaus-Newton algorithm in SYSTAT; Wilkinson 1990). The estimates and respective R^2 values were: AMC = 0.55 ± 0.013, E_m = 1.28 ± 0.25 $g \cdot kg^{-1} \cdot day^{-1}$ ($R^2 = 0.50$); MEC = 0.58 ± 0.017; E_e = 22.70 ± 6.03 $kJ \cdot kg^{-1} \cdot day^{-1}$ ($R^2 = 0.65$). Standard errors of these estimates were calculated from the curved regression.

E_m and E_e can also be estimated as the Y-intercept in a linear regression of excreted dry matter or energy against intake (Sibbald 1976). This procedure applied to our data gave values similar to those from nonlinear regression (E_m = 1.6 $g \cdot kg^{-1} \cdot day^{-1}$; E_e = 24.4 $kJ \cdot kg^{-1} \cdot day^{-1}$). We prefer the nonlinear regression technique and the plots in Figures 1c and 1d because they illustrate the significance of endogenous losses for determining utilization efficiencies, and linear regression requires extrapolation to zero food intake, well beyond the range of our data.

To assess the effect of the nitrogen correction on estimates of E_m, E_e, AMC, and MEC, we repeated our analysis using the uncorrected data. The resulting estimates were: AMC = 0.57 ± 0.016; MEC = 0.59 ± 0.019; E_m = 2.01 ± 0.32 $g \cdot kg^{-1} \cdot day^{-1}$; E_e = 30.98 ± 6.79 $kJ \cdot kg^{-1} \cdot day^{-1}$. Without the nitrogen correction the estimates are higher and have larger variances.

The preponderance of research on endogenous losses in birds has concentrated on domestic species because accurate measurements of metabolizable energy can directly translate into more cost effective feeding operations. Endogenous mass losses (E_m) reported for chickens range from 1.08 to 1.92 $g \cdot kg^{-1} \cdot day^{-1}$ with a mean of 1.52 $g \cdot kg^{-1} \cdot day^{-1}$ (Guillaume and Summers 1970, Sibbald 1975, Campbell et al. 1983). Estimates of endogenous energy losses (E_e) range from 10.33 to 38.07 $kJ \cdot kg^{-1} \cdot day^{-1}$, with a mean of 21.06 $kJ \cdot kg^{-1} \cdot day^{-1}$ (Guillaume and Summers 1970, Sibbald 1975, 1976, 1981, Sibbald and Price 1978, Campbell et al. 1983, Mohamed et al. 1984). Pooling these data for statistical comparison with our own is inappropriate due to the wide variety of techniques employed in these studies, and the fact that nitrogen corrections were not used consistently. However, our estimates of 1.28 $g \cdot kg^{-1} \cdot day^{-1}$ and 22.70 $kJ \cdot kg^{-1} \cdot day^{-1}$ fit well within the range reported for chickens. Values for the Mallard and Muscovy (body mass 1.0–1.2 kg and 2.0 kg, respectively) are similar to chickens and Ruffed Grouse at 21.9 $kJ \cdot kg^{-1} \cdot day^{-1}$ (mean of two studies; Miller and Reinecke 1984, Lu et al. 1985) and 24.7 $kJ \cdot kg^{-1} \cdot day^{-1}$ (Mohamed et al. 1984), respectively.

Endogenous losses in the Embden goose and the mule duck may be somewhat lower than those of chickens, Mallards, Muscovy and Ruffed Grouse. In the goose, E_m was 0.44 $g \cdot kg^{-1} \cdot day^{-1}$ and E_e ranged from 8.17 to 9.80 $kJ \cdot kg^{-1} \cdot day^{-1}$ (Storey and Allen 1982a, b; calculated from their data using body mass of 4.342 kg in 1982b and 4.6 kg in 1982a). In the Mule Duck (mean body mass 2.99 kg), E_e was 8.42 $kJ \cdot kg^{-1} \cdot day^{-1}$ (cf. Lu et al. 1985: Table 6). Some of the difference in endogenous loss per kilogram may relate to the greater mass of these animals. Data are too few to determine how those losses scale to body size.

It has sometimes been claimed that endogenous losses may be diet dependent. For example, Tenesaca and Sell (1978) concluded that endogenous losses were higher when silica gel was added to an experimental corn diet. However, thorough studies on this subject by Sibbald (1980, 1981) have shown that the addition of inert materials such as cellulose and even sand does not increase endogenous losses and that rates of endogenous loss do not vary with the level of food intake. Therefore, physical structure or roughness of a diet does not appear to affect endogenous losses significantly. Studies to date (including our own) have focused solely on cereal-based commercial rations. Under these conditions the endogenous losses measured can be viewed as minimum values that are essentially constant, and can be corrected for when food intake rate is low. One case where intake-independent endogenous losses may change may be where seasonal hypertrophy of the gut occurs, such as in many grouse species (Moss and Trenholm 1987). In this situation, endogenous losses could be somewhat higher due to the relative increase in gut size.

For wild foods that contain chemical defenses, endogenous losses may be both diet and intake rate dependent. For example, plant secondary metabolites often are conjugated to compounds of endogenous origin, such as glucuronic acid or ornithine before being excreted. In our studies of Ruffed Grouse eating male quaking aspen (*Populus tremuloides*) flower buds, combined losses of these two compounds were as high as 4 $g \cdot kg^{-1} \cdot day^{-1}$ (unpubl. data). Also, in addition to decreasing utilization of plant protein (Robbins et al. 1987), tannins may form indigestible complexes with endogenous proteins, enzymes and amino acids, effectively increasing endogenous losses. These and other as yet unknown effects of plant chemistry may significantly complicate attempts to fully understand the dynamics of the digestion of wild plants.

However, changes in endogenous losses that are diet dependent (i.e. due to chemical composition) will

inevitably be intake dependent as well. At zero intake there will be no effect, and endogenous losses will be the same as those measured on undefended foods. Therefore, these diet or intake-dependent losses should be considered as real losses associated with a particular food and no correction should be applied.

In light of their limitations, what is the utility of measuring endogenous losses in wild species? First, in balance trials where the palatability of an experimental diet is low due to factors such as aversive chemicals (in plants and perhaps insects), food intake rate may be low enough to cause apparent utilization efficiencies to deviate substantially from true values (Jonsson and McNab 1983). Used judiciously, values of E_m and E_c determined in other experiments with more palatable foods may be used to obtain estimates of true AMC and MEC. Alternatively, using our method, one may be able to examine the relationship between intake rate of the food of interest and apparent nitrogen-corrected utilization efficiency (even over a range of low intakes) to obtain estimates of true utilization efficiencies. Second, true MEC values of wild foods are recommended for use in foraging models that use multiples of basal metabolic rate (BMR) to estimate daily energy budgets (DEB; Miller and Reinecke 1984). The ability to measure true MEC may improve such models.

The method we have employed to measure true utilization efficiencies and endogenous losses offers several advantages in studies with wild species over the common bioassay applied in poultry: (1) The standard technique requires an initial fasting period followed by a pulse feeding (usually force fed), and an extended period of excreta collection and continued fasting. This may not be appropriate for use with wild birds where stress may be an important consideration. (2) Fasting is associated with changes in gut structure and function. On the time scale of the traditional bioassay, changes in nutrient transport systems and microflora populations are possible that might alter utilization efficiencies (Karasov and Diamond 1983, Giesecke 1970). This may be particularly important in the study of species, such as grouse, where microbial fermentation is thought to be a significant digestive process. (3) Our technique allows one to measure endogenous losses in a trial using the food of interest over fairly natural levels of intake in birds that have been allowed long dietary acclimation. Thus, its results should be ecologically realistic.

Our approach does require more trials, however. Also, the regression technique assumes that true MEC is independent of intake level, whereas at high intake conceivably there is a decline in digestive efficiency and, hence, true MEC (Storey and Allen 1982a).

The large variances of our estimates of E_m and E_c may be due to regression analysis with small sample size. For practical purposes, however, these estimates are adequate. At the ecological level it is probably as important to know the relative magnitude of these losses as it would be to know the absolute values. We conclude that endogenous losses in the domestic chicken, Mallard, Muscovy, and Ruffed Grouse are very similar. It is possible that these estimates may be sufficient for use with other galliforms and some ducks. It appears that endogenous losses are lower in the Embden goose and mule duck, but this should be confirmed with related species along with a consideration of body size.

There are no data available on endogenous losses in other avian species. Future research should seek to provide information from a wider variety of taxa, especially passerines. In addition, different types of feeders might be studied such as carnivores, insectivores, and frugivores. The main question is whether any of these animals have endogenous losses notably different from those already measured in the herbivorous and granivorous nonpasserines studied so far.

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