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# True Metabolizable Energy of Submersed Aquatic Vegetation in Semi-Permanent Marshes for Dabbling Ducks in the Upper Midwest

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### **INTRODUCTION**

Many species of waterfowl depend on wetlands and vegetation communities produced therein for food and cover. Dabbling ducks (*Anatini*) consume vegetation, seeds, and invertebrates in different proportions depending upon factors such as species, age, sex, or time of year (Combs and Fredrickson 1996, Miller et al. 2000). During migration, wetlands in the upper Midwest provide resources that allow ducks to migrate and prepare for the energetically-demanding winter and breeding seasons (Straub et al. 2012, Davis et al. 2014, Hagy et al. 2014a). The amount of food and habitat available for waterfowl at migration stopovers has decreased as wetlands have been lost and converted to other land uses, and the quality of remaining wetlands has been degraded by myriad factors (Anteau and Afton 2009, Dahl 2011, Hagy et al. 2014b).

Waterfowl habitat destruction and degradation is a well-documented and pervasive problem within the Midwestern United States (Bellrose et al. 1983, Sparks 1995). Illinois has lost approximately 85% of historical wetlands in the last two hundred years (Dahl 1990). Draining and tiling wetlands for agriculture, dredging and channelizing rivers for navigation, and constructing dams and levees for flood control are among the primary anthropogenic activities that have historically and continue to remove wetlands from the landscape (Sparks 1995). Consequently, remaining wetlands are subject to increased sedimentation from altered hydrology and flow, decreased light penetration due to suspended sediment, and drastic changes in nutrient loads due to agricultural runoff and other point source pollution (Moore et al. 2010). All of these factors make it difficult for submersed aquatic vegetation (SAV) to thrive in wetlands, especially those with connections to large rivers with unnatural hydrology (Jackson and Pringle 2010, Moore et al. 2010). Stafford et al. (2010) found that the percentage of wetland area in the Illinois River Valley (IRV) covered in submersed aquatic and floating leaf vegetation declined from 26.1% during 1938–1942 to <0.1% during 2005–2006.

As wetland area and quality decreased, it has become imperative that wetland managers understand the resources necessary to sustain healthy populations of wetland-dependent organisms. Havera (1999) estimated that the Upper Mississippi River supports 36% of all continental migrating waterfowl, making ducks and geese significant consumers of wetland resources in the Midwest. The wetlands of the IRV provide approximately 7.5 million mallards (*Anas platyrhynchos*) use-days during fall migration each year (2012–2015, A. Yetter, Illinois Natural History Survey, unpublished data). Mallards are the most abundant duck species in North America, and are considered among the most generalist of dabbling duck species, both in habitat and diet selection (Baldassarre 2014). Mallards are opportunistic omnivores that utilize abundant food sources, even commonly feeding in crop fields containing corn, soybeans, and other grains (Anderson 1959, Combs and Fredrickson 1996, Dabbert and

Martin 2000). During autumn migration, the primary food of mallards is plant material e.g., seeds and leaves of aquatic vegetation; Anderson 1959, Fredrickson and Reid 1988, Callicutt et al. 2011). Hitchcock (2009) found that mallards consumed both invertebrates and seeds in proportion to their availability.

Current conservation planning for dabbling ducks in the Midwest is based on the assumption that food energy may be a limiting factor during migration. The basic principle of this food limitation hypothesis is that food availability during nonbreeding periods is related to demographic rates and by increasing food, survival, productivity, or other rates will increase (Williams et al. 2014). Biologists use daily ration models to determine the amount of habitat needed to support a target population of non-breeding waterfowl using daily ration models. Wetland managers need three pieces of information to determine the energetic carrying capacity of a wetland using a daily ration model, 1) the daily energetic requirement of ducks using a wetland, 2) the amount of food available in the wetland, and 3) the energetic value of the available foods for ducks (Soulliere et al. 2007). Daily energetic requirements are estimated using mass and resting metabolic rates from available allometric equations (Miller and Eadie 2006). Food availability within a wetland complex often is estimated by core and box sampling (Sychra and Adamek 2010, Hagy and Kaminski 2012). The energetic value an organism obtains from a food (i.e., true metabolizable energy) has become of great interest to wetland and waterfowl managers for its direct applicability in conservation planning.

True metabolizable energy (TME) values are commonly used in conservation planning because they account for fecal and urinary energy of non-food origin (Miller and Reinecke 1984). True metabolizable energy studies began in the 1970s with agricultural grains and domestic poultry (Sibbald 1976), and methods have since been adapted for wild waterfowl and natural foods to support conservation planning (Miller and Reinecke 1984, Hoffman and Bookhout 1985, Jorde and Owen 1988, Kaminski and Essig 1992). To date, nearly all available TME values for waterfowl are from moist-soil seeds and agricultural grains from a limited number of duck species (Checkett et al. 2002, Kaminski et al. 2003, Ballard et al. 2004). Seeds are considered relatively high in fat and low in ash, resulting in high digestibility (Fredrickson and Reid 1988, Ballard et al. 2004). True metabolizable energy values of moist-soil seeds across duck species range from 1.08 kcal/g for Pennsylvania smartweed (*Polygonum pensylvanicum*, Hoffman and Bookhout 1985) to 3.47 kcal/g for wild rice (*Zizania aquatica*, Sherfy 1999). The TME of vegetation is generally less than seeds because it is low in fat and high in fibrous, inorganic, and indigestible material (Hoffman 1983, Fredrickson and Reid 1988). Two previously assayed vegetation species (shoalgrass, *Halodule wrightii*, TME: 0.82 ± .03 kcal/g, Ballard et al. 2004; widgeon grass, *Ruppia maritima*, TME: 1.10 ± 0.14 kcal/g, Coluccy et al. 2014) have low TME values

relative to seeds. Until recently, SAV has been largely undervalued as a potentially significant source of energy for dabbling ducks and few comparisons have been made across species (Ballard et al. 2004, Straub 2008, Coluccy et al. 2014). Many dabbling duck species have omnivorous diets like mallards (Baldassarre 2014), but other species that are primarily herbivorous, especially gadwall (*Mareca strepera*), may differ in their digestive efficiency of SAV (Barnes and Thomas 1987).

Our primary objectives were to 1) estimate true metabolizable energy of common species of submersed aquatic vegetation in semi-permanent marsh habitats of the Upper Midwest for gadwall and mallards during autumns 2015–2017, and 2) use current and historic estimates of semi-permanent marsh vegetation communities during autumn within the IRV to document the net change in energetic carrying capacity for dabbling ducks and compare with habitat use by waterfowl using long-term aerial surveys of the Illinois Natural History Survey. We assayed seven species of SAV common in the Midwest that have been documented as waterfowl foods: coontail (Ceratophyllum demersum), wild celery (Vallisneria americana), Canadian waterweed (Elodea canadensis), southern naiad (Najas guadalupensis), Eurasian watermilfoil (Myriophyllum spicatum), widgeon grass (Ruppia maritima), and sago pondweed (Stuckenia pectinate; Anderson 1959, Stewart 1962, Bergman 1973, Havera 1999, Benedict and Hepp 2000, Hitchcock 2009, Baldassarre 2014). Understanding the energetic value of SAV for dabbling ducks will allow wetland managers to accurately evaluate wetland management practices and conservation planners to develop more accurate energetic carrying capacity models. We predicted that the energetic carrying capacity of semi-permanent marshes containing SAV will be slightly less than if the same wetlands were managed for moist-soil vegetation (Bowyer et al. 2005). We hypothesized that the TME of SAV per unit biomass will be less than that of moist-soil seeds and agricultural grains. Further, we hypothesized that the TME of SAV will be independent of sex and trial week.

### **METHODS**

### Capture, Aviary Design, and Husbandry

We captured wild mallards (August 2015) and gadwall (March 2016, November 2016, and March 2017) using rocket nets and swim-in traps baited with corn in central Illinois. We used wild ducks in trials because previous research found variation in TME values between game-farm mallards and wild ducks (Kaminski and Essig 1992). Each duck was banded with a numbered, removable, aluminum tarsal band for identification and the right primary feathers were clipped to prevent flight inside holding pens. We maintained birds in captivity and conducted all feeding trials at Forbes Biological Station near Havana, Illinois.

We maintained ducks in a large (1.22 m wide  $\times$  6.1 m long  $\times$  0.91 m tall or 1.68 m wide  $\times$  9.75 m long  $\times$  0.91 tall) aviary elevated approximately 1 m above the ground. During feeding trials, we placed ducks in two adjacent trial pens consisting of a similarly constructed aviary (2.44 m wide  $\times$  3.05 m long  $\times$  0.61 m tall; Fig. 1) with individual compartments (2 ducks/compartment; 1.07 m wide  $\times$  1.22 m long). Trial pens and aviaries were constructed with 2.5-cm vinyl-coated hardware cloth with up to two-thirds of each pen covered in outdoor carpet. Aviary pens contained a basin of water (0.6 m  $\times$  0.91 m) that was continually refreshed and trough-style feeder that was monitored daily. Each trial pen compartment contained a bowl of water (35 cm diameter  $\times$  9 cm depth) that was continually replenished with fresh water (Fig. 2). Trial pens did not contain a feeder because trial ducks were limited to a known ration of SAV vegetation (see below) while on trial and were fasted beforehand. All pens were rinsed with fresh water daily during trial season.

Apart from trial, we maintained ducks on a commercial poultry ration (Purina Game Bird Finisher 19% protein, 2% fat, 12% fiber; Checkett et al. 2002, Kaminski et al. 2003, Ballard et al. 2004, Dugger et al. 2007, Coluccy et al. 2014). We also provided ducks access to SAV (largely coontail and Eurasian watermilfoil) at least once weekly throughout the fall migration period (September–December) using freshly-collected vegetation when available and laboratory-maintained thereafter. Poultry ration provides a low-protein, balanced maintenance diet and helps prevent physiological issues (e.g., weight loss, angel wing) resulting from maintaining birds in captivity. We added SAV to diets to acclimate gut morphology to processing vegetation efficiently during feeding trials (Miller 1975, Checkett et al. 2002).

### **Collection and Handling of SAV Vegetation**

We collected SAV from Emiquon Preserve in Fulton County, Illinois, Sue and Wes Dixon Waterfowl Refuge at Hennepin and Hopper Lakes in Putnam County, Illinois, and Big Basin of the Des Plaines River in Will County, Illinois. Vegetation was collected by hand prior to feeding trials and transported to the Forbes Biological Station. We used fresh vegetation in feeding trials when available, and vegetation was stored in a laboratory refrigerator at  $2.2^{\circ}$  C to prevent decay until fed. We stored vegetation  $\leq 2$  weeks before discarding and recollecting. When needed, we kept vegetation in aquaria in the climate-controlled laboratory. We maintained aquaria water temperatures near  $28^{\circ}$ C using in-tank water heaters and photoperiod at 14 hours of light daily (0600–2000 hrs) using 40-watt T12 fluorescent bulbs. We retained vegetation in aquaria  $\leq 4$  weeks and used this vegetation in feeding trials only when natural vegetation senesced and could no longer be collected; typically, due to unseasonably cold temperatures. We confirmed that freshly collected and tank-maintained vegetation, when used within 4-weeks, provided commensurate energy content by conducting proximate analysis.

### **Feeding Trials**

We randomly assigned trial pairs (i.e., one male, one female) and housed each pair together in a separate compartment during trials. We removed individuals from the aviaries and weighed using an Ohaus balance (1 g; Ohaus Corporation, Parsippany, NJ) before the fasting period. Feeding trials began with a 48-hour fasting period to clear the digestive tracts of trial birds. Throughout the fasting period, birds had access to water ad libitum. After 48 hours, we reweighed each duck and precision fed a quantity of SAV approximately 1% of their body mass. Precision feeding consisted of balling up a small amount of vegetation (<1 cm diameter) and pressing it down the esophagus of each bird using a gloved finger. A typical feeding consisted of 3–10 replications of this process and took approximately 5 minutes.

Following feeding, we collected urinary and fecal excreta from birds for 48 hrs using a metabolic cage or Whirl-pak® collection bag (Adeola et al. 1997, Adeola 2006; McClain and Kenna, Illinois Natural History Survey, unpublished data). The metabolic cage method consisted of placing a bird in a wire cage  $(20 \times 20 \times 30 \text{ cm}, 1\text{-cm})$  within an open-top plastic tub that accumulated excreta (Fig. 3). The collection bag method consisted of collecting excreta in a Whirl-pak® bag positioned over the duck's cloaca with a semi-permanent custom harness (Adeola et al. 1997; Fig. 4). Our attachment deviated from that of Adeola et al. (1997) in that we attached the collection device sans sutures with cyanoacrylate glue and held in place with a backpack style harness (Dwyer et al. 1972). We then placed the bird in a plastic trial tub in case of collection bag failure. Trial tubs were similar in dimension to metabolic cages, but were plastic, and were vented to promote adequate air-flow. All trial birds, whether in metabolic cages or trial tubs, had access to water ad libitum. We replaced collection bags after 24 hrs and ducks were removed from trial 48 hrs after feeding and retuned to trial pens with access to food and water ad libitum. We collected a control sample from each bird to correct for energy related to fecal and urinary energy of non-food origin (Miller and Reinecke 1984). Individuals served as their own control to reduce effects of individual metabolism (Sherfy et al. 2005). Control trials were conducted as previously described except that individuals were fasted for 96 hrs and excreta was collected during the later 48hrs.

We collected excreta by rinsing contents of either the plastic tubs or collection bags into a 1-gal Ziploc<sup>®</sup> bag. In the laboratory, we removed from samples, feathers, regurgitated SAV, and any other debris by hand and froze samples until subsequent laboratory processing and analysis. We also froze fresh vegetation samples of each of six SAV species weekly for comparison with digested samples. Within 180 days of trials, we thawed and dried excreta and vegetation samples in an oven at  $60^{\circ}$  C. Once dry, we ground each sample into a fine powder with mortar and pestle, weighed material ( $\pm$  0.1

mg; Mettler Toledo, Columbus, OH), pressed material from each sample into pellets using a Parr pellet press (Parr Instrument Company, Moline, IL), and combusted pellets in a Parr 6050 compensated jacket calorimeter to determine gross energy of the sample. We then calculated TME (kcal/g) using the following equation:

$$TME = \underline{(GE_f \times W_f) - (EE_f - EE_c)}$$

$$W_f$$

where GE<sub>f</sub> is the gross energy of vegetation (kcal/g), W<sub>f</sub> is mass (g) of vegetation fed to each bird, EE<sub>f</sub> is the gross energy of excreta collected during feeding trial (kcal), and EE<sub>c</sub> is the gross energy of excreta collected during control trial (kcal; Sibbald 1976, Coluccy et al. 2014).

Additionally, we determined gross energy, percent water, ash, crude protein, fat, fiber, and nitrogen-free extract for each sample using proximate analysis and other techniques. Percent water and ash were analyzed according to standardized procedures (Association of Official Analytical Chemists (AOAC), 2006; methods 934.01 and 942.05). Crude protein was calculated from LECO (models FP2000 and TruMac; LECO Corp., St. Joseph, MI) and total nitrogen values (AOAC, 2006; method 992.15). Crude fat of each food was determined according to the methods of the Association of Official Analytical Chemists (2006; method 2003.06). Acid and neutral detergent fiber contents was determined by the method of Jeraci and Van Soest (1990). We calculated nitrogen free extract as (100% – [%water + %crude fiber + %ash + %fat + %crude protein]) where crude fiber is ADF x 0.80 (Petrie et al. 1998). Crude protein was also determined for all excreta samples, which we will apply using a nitrogen correction factor to account for energy of non-food origin, following the recommendation of Sibbald and Morse (1982).

We compared TME value (dependent variable) using general linear models in a mixed model framework (Proc Mixed in SAS v9.4) to examine variation from vegetation species, trial date, and sex (independent variables). We included body mass as a random effect to account for any variation in digestibility difference during the fall migration period. We built all-possible models using additive combinations of fixed effects and biologically-plausible interactions and used Akaike's Information Criteria (AIC) to determine the best model (AIC <2; Burnham and Anderson 2002). We examined residuals for normal distribution and use transformations as needed (Zar 2009). We calculated effect sizes from raw data or use back-transformations as appropriate. We model averaged parameter estimates or effects sizes as appropriate among competitive models (Burnham and Anderson 2002).

We used a daily ration model to estimate energetic carrying capacity of wetlands (i.e., duck energy days [DED]) within the IRV using a combination of published data and those generated in our analyses:

# DED = $\sum$ (Food available (g dry weight) x TME of food (kcal/g dry weight)) Species specific daily energy requirement (kcal/day)

(Checkett et al. 2002, Kaminski et al. 2003, Dugger et al. 2007, Williams et al. 2014). We used 358 kcal/day as the daily energy requirement of mallards as estimated by Souilliere et al. (2007).

### **RESULTS AND DISCUSSION**

From October–December 2016, we conducted 16 sets of trials, 7 for mallard and 9 for gadwall, resulting in 113 individual samples. We collected 61 samples from 16 mallards (8 females and 8 males) including Eurasian watermilfoil (n = 1), coontail (n = 1), sago pondweed (n = 5), southern naiad (n = 2), Canadian waterweed (n = 3), wild celery (n = 3), control (n = 22), and widgeon grass (n = 24). We collected 72 samples from 21 gadwall (12 males and 9 females) including sago pondweed (n = 12), coontail (n = 4), Eurasian watermilfoil (n = 3), Canadian waterweed (n = 8), southern naiad (n = 13), control (n = 16), and widgeon grass (n = 16). We were unable to collect TME values of wild celery for gadwall due to unpredicted early senescence in 2016. Combining samples from 2015–2016, we obtained our goal of  $\geq$  15 samples of each SAV species for mallards but not gadwall due to early senescence of SAV in 2016. We have additional trials for gadwall planned for fall 2017–2018 to obtain desired sample size.

For mallards, mean ( $\pm$  SE) TME (kcal/g) was greatest for Canadian waterweed (1.69  $\pm$  0.33), followed by southern naiad (1.40  $\pm$  0.43), coontail (1.24  $\pm$  0.42), sago pondweed (0.34  $\pm$  0.23), widgeon grass (0.28  $\pm$  0.39), wild celery ( $-0.08 \pm$  0.54), and Eurasian watermilfoil, ( $-0.53 \pm$  0.51). We are awaiting nutrient analysis for 2016 gadwall samples from the University of Illinois at Urbana-Champaign to adjust raw TME values for energy of non-food origin in samples. The TME of tested SAV for mallards is lower than most seeds, except Canadian waterweed, which has slightly greater TME than paspalum (*Paspalum laeve*) and smartweeds (*Polygonum lapathifolium* and *P. pensylvanicum*; Kaminski et al. 2003). However, several SAV species had greater TME than most invertebrates and several previously reported species of SAV (Sherfy 1999, Ballard et al. 2004, Dugger et al. 2007). Moving forward, we will report corrected TME values of all SAV species for mallard gadwall as results from nutrient analysis become available. We are conducting additional trials for gadwall in autumn 2017 to reach adequate sample sizes. Thereafter, we will report results pertaining to our hypotheses and predictions. Lastly, to satisfy our second objective, we will model energetic carrying capacity of wetlands in the IRV when final TME values are complied, using current and historic estimates of semi-permanent marsh vegetation communities. We will document the net change

in energetic carrying capacity for dabbling ducks and compare with habitat use by waterfowl using longterm aerial surveys of the Illinois Natural History Survey.

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Figure 1. Trial pens with feeders located at Forbes Biological Station in Havana, IL during autumn 2016.



Figure 2. Trial pen compartment at Forbes Biological Station in Havana, IL during autumn 2016.



Figure 3. Metabolism cage and tubs used during trials at Forbes Biological Station in Havana, IL during autumn 2016.



Figure 4. (a) Custom backpack harness and (b) Whirl-pak® used for excreta collection during true metabolizable energy trials at Forbes Biological Station in Havana, IL during autumn 2016.

a.



b.



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