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ORIGINAL ARTICLE

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## Does choice of estimators influence conclusions from true metabolizable energy feeding trials?

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**Abstract** True metabolizable energy (TME) is a measure of avian dietary quality that accounts for metabolic fecal and endogenous urinary energy losses (EL) of non-dietary origin. The TME is calculated using a bird fed the test diet and an estimate of EL derived from another bird (Paired Bird Correction), the same bird (Self Correction), or several other birds (Group Mean Correction). We evaluated precision of these estimators by using each to calculate TME of three seed diets in blue-winged teal (*Anas discors*). The TME varied by <2% among estimators for all three diets, and Self Correction produced the least variable TMEs for each. The TME did not differ between estimators in nine paired comparisons within diets, but variation between estimators within individual birds was sufficient to be of practical consequence. Although differences in precision among methods were slight, Self Correction required the lowest sample size to achieve a given precision. Feeding trial methods that minimize variation among individuals have several desirable properties, including higher precision of TME estimates and more rigorous experimental control. Consequently, we believe that Self Correction is most likely to accurately represent nutritional value of food items and should be considered the standard method for TME feeding trials.

**Keywords** *Anas discors* · Avian dietary quality · Energy loss · Feeding trial · True metabolizable energy

### Introduction

True metabolizable energy (TME) is a measure of avian diet quality that accounts for metabolic fecal and endogenous urinary losses (hereafter, “endogenous losses” or “EL”), defined as excretory energy (EE) of non-dietary origin (Sibbald 1976; Miller and Reinecke 1984). The TME is estimated in feeding trials by correcting the EE of fed birds for EL, which is measured in control birds from which food is withheld. Sibbald (1976) described a TME bioassay for studies of poultry nutrition that has also been used to quantify diet quality for several species of wild waterfowl (Hoffman and Bookhout 1985; Jorde and Owen 1988; Kaminski and Essig 1992; Petrie et al. 1997, 1998; Sherfy et al. 2001; Kaminski et al. 2003; Ballard et al. 2004). The bioassay employs separate experimental and control birds that are starved for 24 h to clear digestive tracts, after which experimental birds are force-fed a known amount of the test diet, and control birds remain without food.

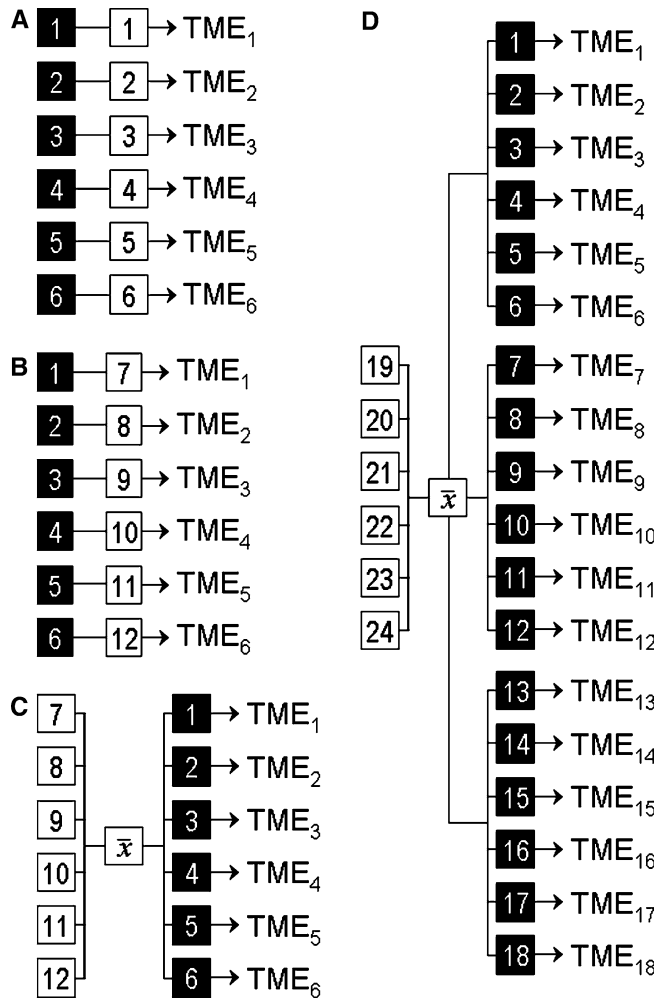
Sibbald (1976) recommended pairing experimental and control birds based on similarity in mass (hereafter, “Paired Bird Correction”). Using this estimator of TME, one observation requires data from two separate birds (Fig. 1B). Shires et al. (1979) concurred that birds should be paired by mass to reduce variation in TME estimates. Sibbald and Price (1980) note that precision of mean TME estimates and accuracy of TME estimates for individual birds depend on how closely the EL of control birds approximates the true EL of experimental birds. Thus, inter-individual variation would be minimized by a Self Correction approach, in which each bird serves as its own control (i.e., EE and EL are measured for each bird; Fig. 1A). A third method, Group Mean Correction, was once considered the standard approach

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**Fig. 1** Arrangement of fed (black boxes) and starved (white boxes) birds for the Self Correction (A), Paired Bird Correction (B), and Group Mean Correction (C) estimators of true metabolizable energy (TME), as well as a common application of Group Mean Correction in which a single mean EL is applied to groups of birds fed different test diets (D). Each estimator has different properties with respect to the EL component of TME, causing each estimate of TME for a given bird (e.g., TME<sub>1</sub>) to represent different sources of variation for each

to estimating TME (Sibbald and Price 1980). This method consists of applying the mean EL of a group of control birds to each experimental bird (Fig. 1C). Group Mean Correction is favored because its costs are low relative to Paired Bird Correction, which requires a unique control bird for each fed bird, and Self Correction, which requires independent measurements of EL and EE in each bird.

Several studies have evaluated performance of these estimators in domestic poultry. For example, Sibbald and Price (1980) found that Self Correction produced the most precise TME estimates in six of eight trials. Similarly, Ostrowski-Meissner (1984) found that TME estimates generated using Self Correction were more precise than those generated using Group Mean Correction. Lastly, Muztar and Slinger (1980) showed that the difference between EL values of two different birds is

generally higher than the difference between two repeated measures of EL on the same bird. They concluded that Self Correction was the only reasonable method of ensuring the accuracy of TME assays, and advocated using the mean of multiple EL measures for each individual in long-term studies. These studies illustrate desirable properties of some estimators, but similar comparative studies of estimator performance in wild birds are lacking.

Despite the apparent advantages of the Self Correction Method, it has not been widely implemented in studies of either wild or domestic birds. Self Correction has been used in 3 of 12 published TME studies of wild birds (Kaminski and Essig 1992; Sherfy et al. 2001; Kaminski et al. 2003), whereas the remainder relied on Group Mean Correction (Hoffman and Bookhout 1985; Petrie et al. 1997, 1998; Checkett et al. 2002; Ballard et al. 2004), Paired Bird Correction (Buckley 1989), estimates based on the linear relationship between intake energy (IE) and EE (Bennett and Hart 1993), or have not explicitly stated how EL was measured (Jorde and Owen 1988; Reinecke et al. 1989). Similarly, we could not identify any published TME studies of domestic poultry in which Self Correction was used. Rather, the favored approach is Group Mean Correction, which is often implemented with one mean value of EL being applied to multiple treatment groups of birds fed different test diets (Fig. 1D; King et al. 1997; Ragland et al. 1997, 1999; Villamide and San Juan 1998; Farran et al. 2001; Hong et al. 2001; Yaghobfar 2001; Yaghobfar and Boldaji 2002; Song et al. 2003). Consequently, the standard that has emerged in the literature on both domestic and wild species is inconsistent, with the available empirical evidence suggesting that Self Correction should be favored. Further, this evidence comes exclusively from studies of domestic poultry and there has been no complimentary evaluation of performance for TME estimators in wild birds fed natural seed diets. Consequently, we used data from Sherfy et al. (2001) to evaluate precision of three TME estimators for seed diets in wild-strain blue-winged teal (*Anas discors*).

## Methods

Our primary goal was to evaluate precision of TME estimates using the Paired Bird, Group Mean, and Self Correction Methods. We used data from a previous study of the effect of gastroliths on TME of three seed diets in blue-winged teal ( $n = 29$ ) (Sherfy et al. 2001). That study consisted of two feeding trials with each of three test diets (milo, millet, and smartweed). Within diets, half of the birds were randomly selected to be fed during the first trial, and the other half were denied food to derive estimates of EL. We then switched groups for the second trial, and this resulted in one measure of EE and EL for each bird and each diet. We weighed all birds before and after the initial 24-h starvation period during each trial. This experimental design allowed us to com-

pare TME calculated for each fed bird when paired with itself (within diets), with another bird (within trials), or with a group of birds (all control birds within trials). Further detail on facilities and feeding trial methods is available in Sherfy et al. (2001).

We calculated TME for each fed bird using each of the three methods, using the single measure of EE for that bird and a unique value of EL that varied among methods. The Paired Bird Method employs fed and starved birds that are paired on the basis of body mass (Sibbald 1976; Shires et al. 1979). Within trials, we ranked fed and starved birds separately by mass and used ranks to pair fed with control birds (i.e., the lightest fed bird was paired with the lightest starved bird; Fig. 1B). For the Group Mean Method, we calculated the mean EL for all starved birds in each trial, and used this mean value for calculating TME for all fed birds in that trial (Fig. 1C). The Self Correction Method requires that each bird serve as its own experimental control. Thus, for a given trial, we calculated TME for each fed bird using the EE from that trial and the EL from the same bird during the companion trial with the same diet (Fig. 1A).

For each of the three methods, we calculated mean TME within feeding trials following Sibbald (1976). The TME was calculated identically for each method, except that EL varied among methods. Two of the methods (i.e., Paired Bird and Group Mean) are intended for single trials with separate groups of fed and starved birds, whereas the Self Correction Method requires two trials to obtain data on each bird in both the fed and starved conditions. Given the limitations imposed by the Paired Bird and Group Mean Methods, we first calculated TMEs within trials, and then combined the two trials to generate a dataset for each diet. Because TME was calculated for half of the birds during each trial, the combined dataset contained a single measure of TME for each bird.

Because the goal of conducting feeding trials is to measure TME of test diets (and not to measure EL), we considered TME to be the response variable of greatest interest. Consequently, we used two approaches to evaluate sensitivity of TME of the three test diets to the calculation methods described above. First, we used mixed model analysis of variance (PROC MIXED; Littell et al. 1996) to test for variation in TME among methods. The model used to represent the response variable ( $TME_{ijk}$ ) of the  $i$ th bird using the  $j$ th method under the  $k$ th diet was:

$$TME_{ijk} = \mu + b_i + m_j + bm_{ij} + d_k + db_{ik} + dm_{jk} + e_{ijk}$$

where  $\mu$  is the overall mean TME,  $b_i$  the random effect of the  $i$ th bird,  $m_j$  the effect of the  $j$ th method,  $bm_{ij}$  the random effect of the  $i$ th bird using the  $j$ th method,  $d_k$  the effect of the  $k$ th diet,  $db_{ik}$  the random effect of the  $i$ th bird under the  $k$ th diet,  $dm_{jk}$  the interaction effect between method and diet, and  $e_{ijk}$  is residual error. Second, we followed recommendations of Bland and Altman (1986)

for evaluating agreement between two measurements of a clinical parameter (in our case, TME) whose true value remains unknown. We examined all possible pairs ( $n = 179$ ) of TME estimates generated by two different methods for the same bird and diet. For each such pair, we calculated the mean of the two estimates and the difference ( $d$ ) between the estimates, and plotted differences against means within diets. We examined these plots visually for indications of non-independence between differences and means. Assuming a normal distribution of differences, 95% of the differences will lie in the range  $d \pm 1.96 \times SD$ , and the two measurements could be used interchangeably if this range is judged to be biologically insignificant (Bland and Altman 1986). Because we knew a priori that TME differed among our diets (Sherfy 1999; Sherfy et al. 2001), we used diet-specific standards to judge biological significance of this range of values. We also could not rule out variation in TME among estimators a priori, so the standard for each comparison was specific to the two estimators being compared. Thus, when comparing two methods for a given diet, we evaluated the mean difference between individuals relative to the mean TME for the two methods:

$$V_{ABk} = \frac{\sum_{i=1}^{n_{AB}} (TME_{iAk} - TME_{iBk}) / n_{AB}}{(\sum_{i=1}^{n_A} TME_{iAk}) + (\sum_{i=1}^{n_B} TME_{iBk}) / n_A + n_B}$$

where the numerator is  $d$ , subscripts  $A$  and  $B$  denote the two methods being compared,  $n_{AB}$  denotes number of birds for which TME data were available for both methods, and  $k$  denotes diet. We judged those values of  $V_{ABk}$  that exceeded an a priori standard of 20% to be biologically significant. We selected this standard based on the many published studies that have shown very high precision of TME estimates (Petrie et al. 1997, 1998; Sherfy et al. 2001; Checkett et al. 2002; Kaminski et al. 2003). This method is preferable to correlation analysis, which measures only the strength of association between two variables and not the agreement between them (Bland and Altman 1986).

To assess logistical implications of selecting a given method, we estimated the sample size required to measure TME for each diet and method as  $n = t_{\alpha/2}^2 \times s^2 / \delta^2$ , where  $\alpha = 0.05$ ,  $s$  the standard deviation of the mean TME, and  $\delta$  is desired precision of the mean TME estimate (Zar 1984). We used the observed standard deviation from each combination of diet and method as an estimate of  $s$ , and a range of percentages of the observed mean TME as  $\delta$ .

## Results

The three methods of TME calculation generated nearly identical measures of TME for all three diets; means within diets varied by  $< 2\%$  among methods (Table 1). Main effects of method ( $F_{2,50} = 0.08$ ,  $P = 0.921$ ) and diet  $\times$  method ( $F_{4,66} = 0.17$ ,  $P = 0.954$ ) were non-significant in the mixed model, suggesting that the methods did not

**Table 1** Mean true metabolizable energy (kcal/g) for three seed diets in blue-winged teal (*Anas discors*), calculated by three estimators that vary according to the manner of conducting the endogenous loss correction

Test diet	Trial	Self			Paired bird			Group mean		
		Mean	N	CV	Mean	n	CV	Mean	n	CV
Millet	1	2.59	10	8.8	2.77	9	11.6	2.68	10	10.7
	2	2.92	11	8.6	2.78	10	10.4	2.84	12	11.4
	Mean	2.76	21	10.5	2.77	19	10.7	2.77	22	11.2
Milo	1	3.61	10	4.8	3.40	11	8.3	3.44	11	4.8
	2	3.33	12	8.2	3.47	10	16.9	3.50	12	12.9
	Mean	3.46	22	7.7	3.43	21	12.9	3.47	23	9.8
Smartweed	1	2.69	12	27.7	2.73	9	29.8	2.59	12	30.4
	2	2.63	9	14.5	2.66	9	18.4	2.71	9	16.2
	Mean	2.66	21	22.6	2.69	18	24.2	2.64	21	24.6

vary in their estimation of TME among diets. Coefficients of variation (CVs) did not vary substantially among the three methods, but the Self Correction Method did consistently produce the lowest CV for each of the three diets (Table 1). This pattern was particularly evident when data were examined separately by trial, which tended to magnify differences in precision among methods. Self Correction produced the lowest CV in all six trials, and Paired Bird Correction produced the greatest CV in four of the six trials (Table 1). A greater degree of variation among means was also evident when trials were examined separately, with means varying by 3–7% among methods (Table 1). Despite this increased variation, no method consistently produced the highest or lowest TME within trials.

Direct comparison of methods within individual birds revealed a similar lack of bias attributable to methods. Values of  $d$  did not differ from zero for any of the nine between-method comparisons ( $|t| < 0.61$ ,  $P \geq 0.55$ ). The range of values for  $d$ , when expressed relative to the appropriate mean TME, exceeded our 20% standard for all nine comparisons (22.0–41.3%).

The Self Correction Method produced the lowest required sample size to obtain a given precision for all three diets (Fig. 2). Sample size curves revealed that obtaining 95% confidence limits equal to 10% of the mean TME estimate (a level of precision consistent with other studies (Sherfy et al. 2001)) would require 4, 2, and 19 birds using the Self Correction Method for millet, milo, and smartweed, respectively. These sample sizes were lower than for either of the other two methods (Fig. 2). There was no clear pattern among diets favoring either of the other two estimators. Paired Bird and Group Mean Correction had nearly identical sample size properties for smartweed, and each was preferable to the other for either milo or millet (Fig. 2).

## Discussion

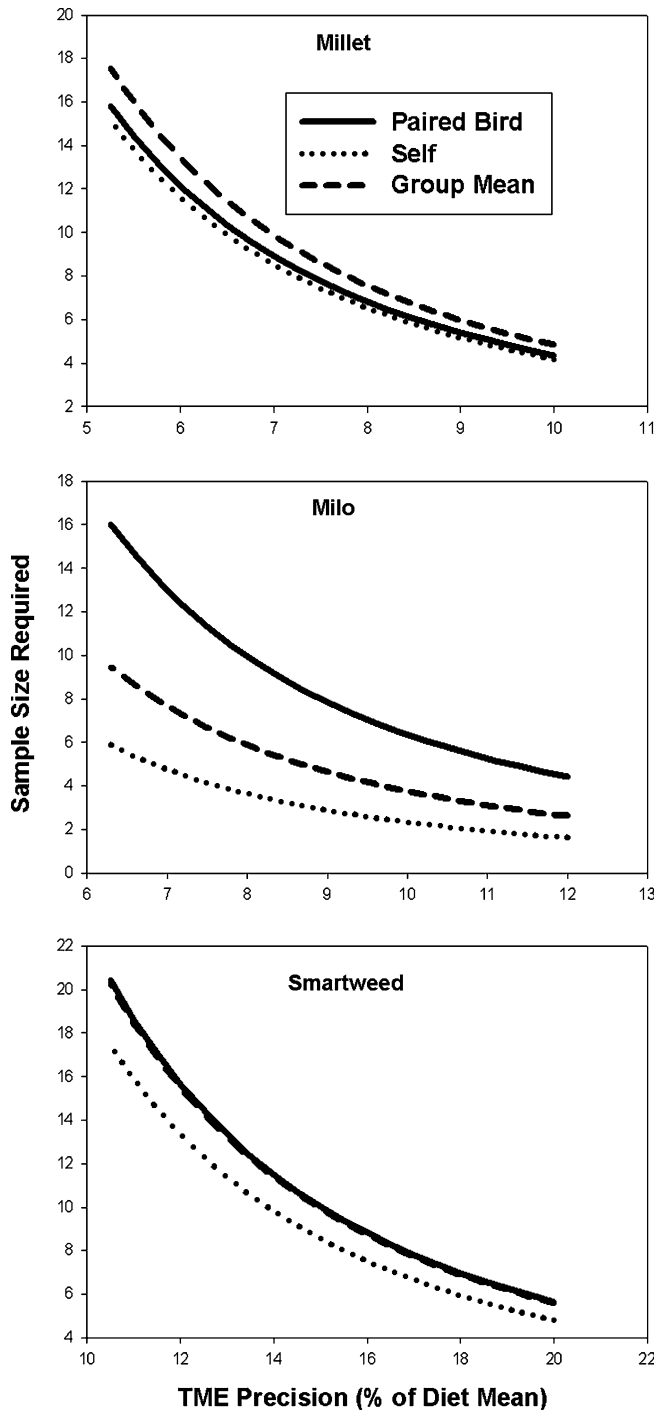
### Comparison of methods

The three calculation methods that we used provided mean estimates of TME that were not statistically

distinguishable among estimators, and that exhibited little (<2%) variation among means, suggesting that loss of accuracy is not a major consideration in selecting an estimator of TME. These results are similar to those of Sibbald and Price (1980), who found that mean TME of alfalfa meal in poultry varied by 1–5% among the same three methods. The pattern of precision in our data also was similar to that of Sibbald and Price (1980), in that variation was consistently lowest for the Self Correction Method. Our experimental design offered the opportunity to examine mean TMEs separately for trials within diets. Although the statistical effect of trials is not of particular interest (i.e., trials were a component of residual error in our mixed model), each trial does represent a potential outcome of a TME assay with a sample size more reflective of common practice than the overall diet means. When examined by trials, a moderate to substantial improvement in precision was consistently evident for Self Correction, with the magnitude of improvement being >25% for five of six trials and equaling 106% for the second milo trial (Table 1). Although less variation does not necessarily translate into improved accuracy, the Self Correction Method does eliminate among-bird sources of variation in individual TME estimates (Fig. 1), and therefore would be expected to provide the most accurate estimates.

The three estimators represent a continuum of control for individual variation, which reflects differences in assumptions regarding the principal source of variation in EL. Implicit to the Self Correction Method's high level of control for individual variation is the assumption that EL is a characteristic of individuals. In contrast, Group Mean Correction assumes that EL is characteristic of a species that is adequately modeled by averaging across individuals. Paired Bird Correction recognizes that EL varies among individuals, but assumes that this variation is largely a consequence of body mass. To further investigate dependence of TME on body mass, we conducted an a posteriori analysis using an approach that departs from assumptions of the Paired Bird Method. We randomly paired starved and fed birds within trials without regard to body mass, and calculated TME for each diet (millet: mean = 2.78, CV = 13.9; milo: mean = 3.52, CV = 10.5; smartweed:





**Fig. 2** Relationship between sample size (number of fed birds) and 95% confidence limits of TME estimates (as a percentage of the mean) for three seed diets and three methods of calculating TME in blue-winged teal (*Anas discors*). Curves for the Paired Bird and Group Mean methods are nearly identical for smartweed

mean = 2.70, CV = 24.4). Mean TMEs from random pairing varied by < 3% from TMEs generated by the other methods, and appreciably exceeded the CV for Paired Bird Correction only for millet (Table 1). This analysis demonstrates that there is little empirical basis for pairing birds on the basis of static measures of body

mass, at least with respect to reducing variation in mean TME estimates. Similarly, Sibbald and Price (1978) found that body mass explained only 23% of the variation in EL. Correlates of metabolic body size and energy expenditure, such as  $\text{mass}^{0.67}$  and  $\Delta \text{mass}^{0.67}$ , may be better predictors of variation in EL among individuals than body mass (Sherfy et al., unpublished data). However, controlling for body size variation did not fully remove variation among individuals in this study.

The Group Mean Correction Method offers the greatest degree of flexibility, because it does not inherently require an equal number of starved and fed birds and it allows simultaneous measurements of EL and EE in different birds. Further, Group Mean Correction allows TME to be calculated for multiple diets in one assay, although this approach does not maintain independence of observations because all TMEs are calculated with the same EL (Fig. 1D). Pesti et al. (1988) concluded that pooling of excreta samples from control birds (which is analogous to Group Mean Correction) did not compromise outcome of assays and appeared to be justified. Our data showed the opposite—that methods which fail to control for among-bird variation consistently lead to a minor decrease in measured precision of TME estimates. Poultry studies in particular have capitalized on the flexibility of this estimator with regard to sample size variation between starved and fed birds (e.g., Yaghobfar and Boldaji 2002) and the ability to apply a single group mean to multiple groups of fed birds (King et al. 1997; Ragland et al. 1997, 1999). However, little effort has gone toward determining how many birds are required to obtain an accurate mean estimate of EL, and few studies have acknowledged the consequences of using unreplicated control groups in Group Mean Correction (Fig. 1D). In the absence of empirical data addressing these factors, overall variation would be minimized by using Self Correction because each estimate contains information from one and only one bird (Fig. 1A).

### Sample size requirements

Our analyses revealed that sample size requirements to obtain a given level of precision were largely similar among the three methods, although they were lowest for Self Correction in all three diets. The reduced sample size benefit to Self Correction was trivial for millet, but of practical significance for milo and smartweed (Fig. 2). Sample size curves revealed that precise estimates of TME can be obtained with relatively few experimental birds for milo and millet, whereas smartweed required a substantially greater sample size (Fig. 2). This property of smartweed reflects the greater variance associated with its mean TME estimates, and is consistent with other studies that have demonstrated relatively low TME and digestive efficiency for smartweed diets fed to waterfowl (Petrie et al. 1997, 1998). These results illustrate the variable digestive responses of wild birds to

natural seed diets. We conclude that the Self-Correction Method maximizes control for undesirable sources of variation that are likely to arise under these experimental conditions.

### Conclusions and implications for research

Our results demonstrate that TME estimates are largely insensitive to the method of calculation used. However, for sample sizes of birds those are commonly employed in feeding trials, TME generally varied by 5–7% among estimators. Despite variation in responses and diets, Self Correction consistently provided greater precision of TME estimates, and therefore required the lowest sample size to achieve a fixed level of precision. Differences in precision were not of a magnitude that would negate the potential use of any particular estimator. Nonetheless, the ability of Self Correction to minimize the effect of spurious sources of variation is a desirable quality. We conclude that Self Correction is a more appropriate method because it offers the greatest degree of experimental control and does not introduce multiple sources of variation (i.e., birds) into single TME estimates. Group Mean Correction remains a tenable estimator in terms of precision, but its logistical benefits should be carefully considered against its statistical properties prior to implementation.

### Zusammenfassung

Beeinflusst die Art der Messung die Schlussfolgerungen aus Bestimmungen der wahren metabolisierbaren Energie?

Die wahre metabolisierbare Energie (true metabolizable energy, TME) ist ein Maß für die Qualität von Nahrung, die nicht nahrungsbedingte Energieverluste durch Kot- und Harnbildung (energy losses EL) berücksichtigt. Zur Bestimmung von TME werden verschiedene Methoden angewandt. Entweder bekommt ein Vogel ein Testdiät, oder die EL wird von einem anderen Vogel abgeleitet (Paired Bird Correction), vom selben Vogel (Self Correction) oder mehreren anderen Vögeln (Group Mean Correction). Wir untersuchten die Genauigkeit dieser verschiedenen Ansätze, indem wir mit jedem einzelnen Verfahren die wahre metabolisierbare Energie von drei Körnerdiäten für Blauflügelenten *Anas discors* bestimmten. TME unterschied sich um weniger als 2% zwischen den Verfahren bei allen drei Diäten. Dabei lieferte die Self Correction die am wenigsten variablen Werte. TME unterschied sich nicht zwischen den Verfahren bei neun paarweisen Vergleichen innerhalb der Diäten, aber die Unterschiede zwischen den Verfahren innerhalb einzelner Vögel waren groß genug, um von praktischer Bedeutung zu sein. Obwohl die Unterschiede in der Genauigkeit zwischen den verschiedenen Metho-

den nur gering waren, benötigte die Self Correction die kleinste Stichprobengröße, um eine angestrebte Genauigkeit zu erreichen. Um Unterschiede zwischen Individuen zu minimieren, bedarf es Methoden, die eine höhere Genauigkeit der TME Messung und strengere experimentelle Kontrolle ermöglichen. Die Self Correction Methode ist die genaueste Methode zur Bestimmung des Nährwertes eines bestimmten Futters und sollte deshalb als Standardmethode für TME Bestimmungen in Betracht gezogen werden.

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