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A half a century of measuring ungulate body condition using indices: is it time for a change?

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Abstract From a literature review of five wildlife ecology journals since 1937, we document how using indices to monitor ungulate body condition is common practice, with the kidney fat index (KFI = weight of fat around the kidneys/weight of kidneys without fat \times 100) as the favoured tool (82% of studies). In this context, we highlight the problems of using indices when underlying statistical assumptions are not met (isometry, parallel slopes between treatments). We show, with real and simulated data for two cervids with contrasting fat storage strategies, how results from analysis of variance of KFI values differ from analysis of covariance (ANCOVA) of raw data. We conclude that the KFI is affected by the restrictions typically associated with derived index values, and as a consequence, statistical analysis of the KFI could generate spurious results leading to erroneous interpretations concerning variation in body condition of ungulate populations. Thus, we recommend analysing fat weight as an untransformed variable in

ANCOVA (kidney weight as covariate) to describe body condition variation in ungulates.

Keywords ANCOVA · *Capreolus capreolus* · *Dama dama* · Kidney fat index · Ratios · Residuals · Proportions

Introduction

Mammals require energy from the environment to grow and maintain their tissues and organs, and to support the costs of biological activities. This energy can be stored through a metabolic process, and as a result, the weight of body tissues and organs may increase. The weight, size or appearance of these reserves is generally considered by wildlife biologists to describe “body condition”.

For more than half a century, ungulate body condition has been considered an important topic because most species are of some economic or recreational interest. Indeed, a search of the titles, abstracts and key words of papers published until 2007 in five wildlife orientated international journals [Journal of Wildlife Management (since 1937), European Journal of Wildlife Research previously named *Zeitschrift für Jagdwissenschaft* (since 1955), Wildlife Research (since 1956), South African Journal of Wildlife Research (since 1971) and Wildlife Biology (since 1995)] showed that at least one paper per year focussed on body condition in ungulates, covering 39 ungulate species over five continents (see the [Electronic supplementary material](#)).

In 101 articles focussed on body condition and published since 1937, we found that ungulate body condition has been evaluated 186 times by different indicators. In only 11 papers (6% of all reviewed papers), body condition was measured by visual inspection, just by looking at the animal’s appearance, or using qualitative attributes. Quantitative response variables,

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such as weights and fat depth, are more common and are either used as “raw variables” (raw variables are direct measurements or readings of specific attributes and have been used in 49% of all reviewed papers) or transformed into unit-free indices (e.g., organ weight/body weight, used in 45% of papers reviewed). When researchers conduct statistical analyses on these data, the effects of independent variables on the measure of body condition is frequently assessed using generalised linear models, notably ANOVA.

Even though the use of indices to monitor ungulate body condition is very common, their use has been severely criticised in other fields. The first paper to describe problems associated with ratios dates back to 1897 (Pearson 1897), but perhaps, one of the most relevant was published by Kronmal (1993) who explicitly stated: “A recommendation is made that the use of ratios in regression analyses be avoided”.

In zoological studies, criticism is mainly focussed on indices that attempt to correct for the effect of body size or weight on the variable of interest (Packard and Boardman 1988). Two simulation studies have emphasised the main limitations of using these kind of indices in nutritional studies (Raubenheimer and Simpson 1992; Packard and Boardman 1999). Simulation results differed depending on whether the dependent variable was non-transformed (e.g., organ weight) or converted to an index (e.g., organ weight/body weight). These authors concluded that analysis of variance (ANOVA) of index values may not necessarily detect small differences between treatments and, frequently,

may produce spurious significant results. They discussed the most important sources of error when using indices, which are summarised in the following:

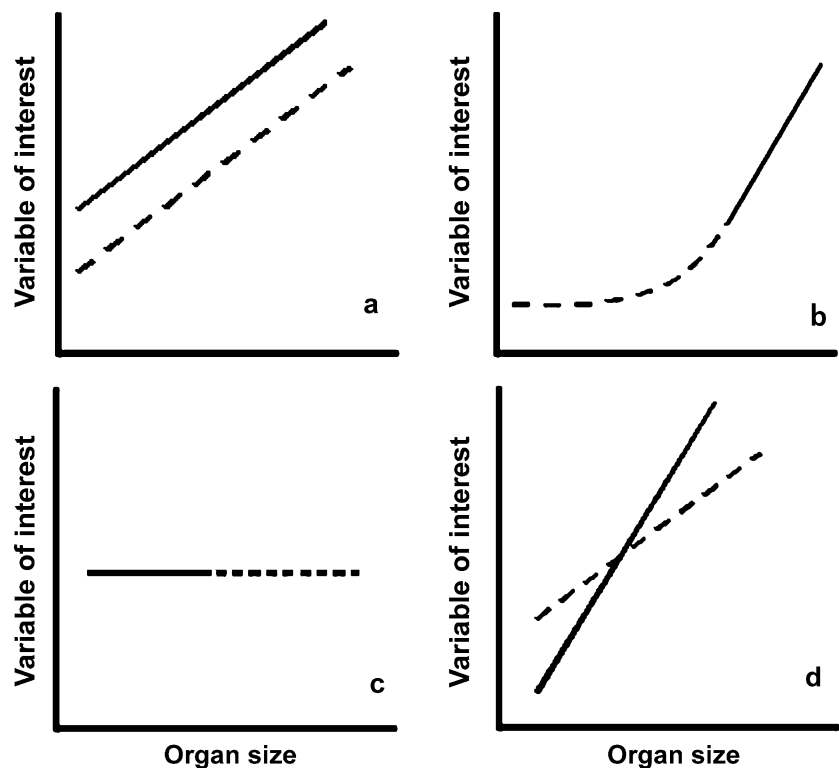
1. Relationship with a non-zero constant term or a non-linear form

When a measurement of body condition is standardised as an index, the main aim is to standardise the numerator (organ fat, organ weight, i.e., the variable of interest) for the effects of a denominator (often total body weight). However, this standardisation is possible only when the relationship between the numerator and the denominator is isometric. That is, the relationship between both weights is linear (e.g., organ weight = $B_1X^n + B_0$, where B_1 is a fixed coefficient, $n=1$ and $B_0=0$), and it passes through the origin (i.e., when the numerator=0 and the denominator also=0). However, when the constant, B_0 , differs from zero (Fig. 1a), simulations have shown that the F-ratio obtained from ANOVA is deflated, and as a result, the statistical power to detect small differences between treatments is reduced. On the other hand, if the numerator and the denominator are non-linearly related ($Y = B_1X^n + B_0$, where $n>1$, Fig. 1b), the F ratio is inflated and produces spurious significant results.

2. No covariance between denominator and numerator

The use of indices assumes not only a linear relationship between the two measured variables, but indeed, such a

Fig. 1 The most important sources of error when indices are used. **a** Allometric relationship due to a non-zero constant term, **b** allometric relationship due to a non-linear relationship, **c** no covariance between denominator and numerator and **d** non-parallel slopes among treatments (see text for explanations). The *solid* and *broken lines* represent two treatments (e.g., age classes, seasons, populations, etc.)



relationship exists. If there is no relationship or if it is very weak and the size measurement (denominator) differs between treatments (Fig. 1c), ANOVA will produce spurious differences simply due to the effect of variation in the denominator.

3. Non-parallel slopes among treatments

When indices are used and compared between treatments (i.e., sexes, age classes, seasons, populations or whatever), we lose information about the relationship between the size measurement (denominator) and the treatments. If the size measurement has a different relationship with the dependent variable between treatments (non-parallel slopes, Fig. 1d), an ANOVA based on index values will produce spurious significant results. In addition, we lose information about the different effect of the covariate between the treatments.

From a practical point of view, a quick procedure to evaluate whether an index removes the effects of size is to assess the linear correlation between the denominator (e.g., body weight) and the index (index = kidney weight/body weight). If linear correlation exists, the index has not standardised the variable of interest (Packard and Boardman 1988). The authors concluded that when indices are used, we lose important information about how the relationship between the numerator and the denominator affects our ability to detect differences between treatments. This problem is even more acute if the denominator includes more than one variable.

There are three main solutions to control for the effects of body size: analysis of residuals, analysis of covariance (ANCOVA) and “shearing” when a multivariate description of body size is necessary (McCoy et al. 2006). Because body condition is commonly expressed in relation to a univariate descriptor of body size, analysis of residuals and ANCOVA are the most generally employed techniques. In analysis of residuals, the variable of interest is first regressed on the measure of body size, and the residuals from the regression are then compared across treatments using ANOVA. In ANCOVA, the body size variable is considered as a covariate and the variable of interest as the dependent variable. These two techniques differ in their statistical procedure and objectives (García-Berthou 2001), but ecologists generally prefer ANCOVA because the associated assumptions are less restrictive (Green 2001; Riggs et al. 2008).

Surprisingly, in the journals we reviewed, neither of these procedures has been used for estimating ungulate body condition over the last half century. Despite the limitations outlined above, which are well recognised in other research fields such as nutrition and physiology (Packard and Boardman 1988, 1999; Raubenheimer 1995), ANOVA of indices (as dependent variables) is still the favoured technique among wildlife biologists.

More precisely, when researchers study ungulate body condition, by far, the most commonly used measure is the kidney fat index (KFI = weight of fat around the kidneys/weight of kidneys without fat \times 100; Riney 1955). In our review, this was the chosen index in 82% of cases (number of studies where KFI was used/total number of cases where any kind of index was used; see [Electronic supplementary material](#)).

Why was the KFI created? Riney (1955) was the first to propose that the proportion of fat stored around the kidneys is a good indicator of total fat reserves in wild ungulates. In his classic paper, Riney’s conclusion was that the KFI was the most satisfactory index because it is easily estimated by weighing organs after animal death, it is highly variable (ranging from 0 to 300) and allows comparison of body condition across seasons and between animals of different size (Riney 1955, p. 436).

However, the KFI has been criticised by wildlife biologists (Batcheler and Clarke 1970; Dauphiné 1975; van Vuren and Coblenz 1985). These authors identified the fact that temporal changes in KFI values could be affected by changes in the weight of the kidney. Subsequently, Finger et al. (1981) suggested that the potential bias of the KFI due to oscillations in kidney weight may only be a minor problem because these oscillations are associated with major changes in body composition. Discussion of this topic among wildlife biologists seems to have died down, and the KFI is currently widely employed for indexing ungulate body condition. Furthermore, within the wildlife ecology community, the consequences of using derived variables (as defined by Sokal and Rohlf, 1995, Chapter 2, “derived variables are based on two or more independently measured variables whose relations are expressed in a certain way, i.e., ratios, percentages, indices or rates”), and the associated problems of interpreting variation in body condition are largely ignored.

Our aim is to focus the attention of wildlife ecologists on the risks in the analyses of KFI by most conventional statistical procedures, notably ANOVA. We investigated whether the KFI estimated in two cervids with contrasting fat storage strategies (roe deer *Capreolus capreolus*—income breeder; fallow deer *Dama dama*—capital breeder) is affected by the typical characteristics of derived variables.

Fallow deer are highly sexually dimorphic (Feldhamer et al. 1988) and accumulate substantial amounts of fat that is stored until required for reproduction (“capital breeder” sensu Jönsson 1997). Indeed, body reserves show strong seasonal variations, for example, Ninov (2003) reported that adult stags lose up to 22% of their body reserves during the rut. In contrast, roe deer are only slightly sexually dimorphic and rely on adjustment in food intake to offset the costs of reproduction (“income breeder”, sensu Jönsson 1997; see Andersen et al. 2000). As a result, their body

reserves vary only weakly between seasons (Hewison et al. 1996). These characteristics affect the quantity of fat stored around the kidneys; e.g., in our sample, fat weighed two to three times the weight of the kidney for adult fallow deer (fat/kidney: 133 ± 30 g/ $45 \text{ g} \pm 5.1$ for females and $103 \text{ g} \pm 43/63 \text{ g} \pm 4.8$ for males), but the weight of the fat and the kidney were similar for adult roe deer (fat/kidney: 29.47 ± 16.28 g/ 36.09 ± 9.2 g for females and 27.66 ± 13.29 g/ 40.39 ± 7.1 g for males).

In particular, we highlight discrepancies between results obtained using standard statistical tools, ANOVA of derived index values (KFI as the dependent variable) and ANCOVA of untransformed fat weight (fat as the dependent variable with kidney weight as a covariate) when body condition was compared among sexes and age classes.

Materials and methods

Deer data

A total of 139 fallow deer (45 juvenile females, 37 juvenile males, 46 adult females and 11 adult males) and 52 roe deer (ten juvenile females, ten juvenile males, 16 adult females and 16 adult males) were shot during winter for management purposes in the south and north of France, respectively. The fallow deer were sampled in an enclosure of 130 ha situated in the Forêt Domaniale of Buzet northeast of Toulouse, southeast France ($43^{\circ}46'$ N, $1^{\circ}35'$ E). This forest belonged to the Southwest European lowland-coline to montane downy oak forests. The roe deer data comes from the Dourdan forest (860 ha) 40 km southwest of Paris ($48^{\circ}19'$ N, $2^{\circ}01'$ E). This forest was a closed habitat surrounded by crops, typical of mixed oak–horbeam forests from the oceanic regions of temperate Europe.

The two kidneys with associated fat were removed with no additional tissues, blotted dry with a paper towel and taken to the laboratory. Each kidney, together with its associated fat, was weighed to the nearest 0.1 g. Then, all the fat was removed by peeling the peritoneal membrane to weigh the fat-free kidney mass. The KFI was calculated according to Riney (1955). We calculated the KFI, pooling the two kidneys and their associated fat to compensate for differences in kidneys and fat mass between the left and right (Anderson et al. 1972).

Analysis with real and simulated data

In a first analysis and using kidney and fat weight of deer, we determined whether the KFI exhibited the typical restrictions described for derived variables (Fig. 1). The allometric relationship due to a non-zero constant term (fat = $B_1 \text{ kidney}^n + B_0$, where $B_0 \neq 0$), the non-linear relationship (fat = $B_1 \text{ kidney}^n + B_0$, where $n \neq 1$) and the lack of

covariance between denominator and numerator (when the slope describing the relationship between kidney fat and kidney weight does not differ from zero, Fig. 1c) were evaluated by the linear regression procedure. The non-parallel slopes among treatments were evaluated by the interaction term between the covariate (kidney weight) and the treatments (sex or age class) in an ANCOVA analysis.

In a second step, we conducted a simulation study. We studied the properties of the variables of interest (fat and kidney weights) and the sampling distribution of the KFI. We found that kidney and log-transformed fat weights were approximately normally distributed (Kolmogorov–Smirnov test, $P > 0.10$) and were quite closely correlated ($r > 0.50$, $P < 0.001$). The mean, the variance and the Pearson correlation coefficient between fat and kidney weights were estimated based on the observed weights of fat and kidneys in our roe deer and fallow deer data sets. These values were then used as baseline parameters to conduct a simulation study. Two approaches were used: ANOVA with KFI as the dependent variable and ANCOVA with untransformed fat weights as the dependent variable and kidney weight as the covariate. According to Packard and Boardman (1988, 1999), Raubenheimer and Simpson (1992), Raubenheimer (1995), Green (2001) and García-Berthou (2001), ANCOVA is considered a more robust method than ANOVA to correct for size.

For each of the four sex–age categories (male/female, juvenile/adult), a total of 10,000 random values of kidney weight and log-transformed fat weight were generated, assuming a bivariate normal distribution (S-Plus 2000 Professional Release 3 2000). Then, within each category, we sampled a number of individuals proportionate to that in the real data set (e.g., for fallow deer, 17% juvenile females, 24% juvenile males, 28% adult females and 38% adult males) to generate 100 new data sub-sets of 100 individual deer. For each sub-set, we performed ANOVA (of KFI values) and ANCOVA (of fat weights with kidney weight as the covariable), where sex and age class (and their interaction) were factors to be tested. Finally, we evaluated discrepancies in P values between the two types of analysis for each data sub-set, considering significant differences at P values < 0.05 .

Results

The KFI was affected by the restrictions typical of derived variables

Using the real data, the KFI did not completely remove the effect of kidney mass variation because there was a significant correlation between kidney mass and the KFI in both species ($r = 0.657$, $F_{1,51} = 37.28$, $P < 0.001$ for roe deer; $r = 0.543$, $F_{1,138} = 31.49$, $P < 0.001$ for fallow deer).

In fact, the estimation of KFI was affected mainly by both the lack of isometry due to a non-zero constant term (part of the first limitation, see “Introduction”) and non-parallel slopes (third limitation) in the relation kidney fat–kidney weight between sexes or age classes of both species.

Specifically, the constant term differed from zero in adult males of both cervids ($B_0 = -38.12$, $t = -3.53$, $P = 0.003$ for roe deer and $B_0 = 1.77$, $t = 2.42$, $P = 0.032$ for fallow deer, Fig. 2), and the slopes between kidney fat and kidney mass differed between sexes in juvenile fallow deer ($F_{1,83} = 6.18$, $P = 0.015$) and between age classes in male fallow deer ($F_{1,47} = 5.542$, $P = 0.022$, Fig. 2). However, the estimation of KFI was free of non-linearity (part of the first limitation) and lack of covariance (second limitation, see “Introduction”) between kidney fat and kidney weight in juvenile ($r = 0.75$, $F_{1,9} = 10.18$, $P = 0.013$ for females, $r = 0.77$, $F_{1,9} = 11.75$, $P = 0.009$ for males) or adult ($r = 0.76$, $F_{1,15} = 21.61$, $P < 0.001$ for females, $r = 0.81$, $F_{1,15} = 24.82$, $P < 0.001$ for males) roe deer, and in juvenile ($r = 0.65$, $F_{1,44} = 34.87$, $P < 0.001$ for females, $r = 0.62$, $F_{1,36} = 20.88$, $P < 0.001$ for males) or adult ($r = 0.45$, $F_{1,45} = 11.69$, $P = 0.001$ for females, $r = 0.77$, $F_{1,10} = 17.17$, $P = 0.032$ for males, Fig. 2) fallow deer.

There are discrepancies in results between ANOVA of KFI values and ANCOVA of fat weights in fallow deer

Despite the fact that the KFI can be affected by most of the limitations typically associated with index values described by Raubenheimer and Simpson (1992) and Packard and

Table 1 Number of times where a statistically significant difference was detected in 100 ANOVA where the KFI was the dependent variable, and ANCOVA, where fat weight was the dependent variable and kidney weight the covariable

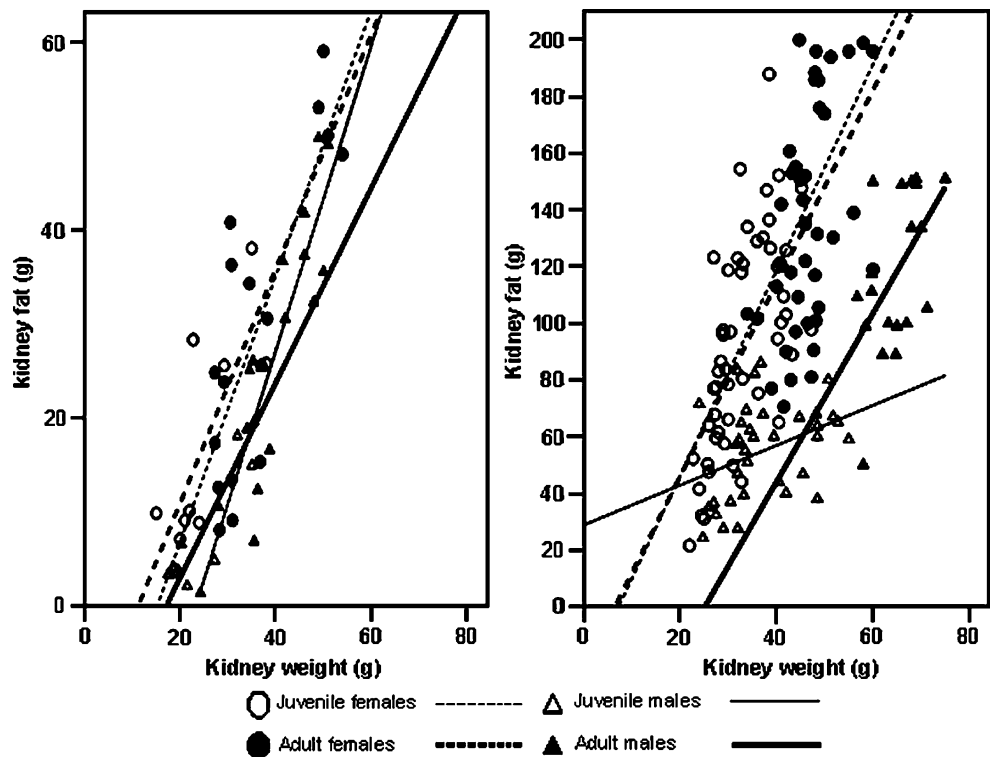
Species	Treatment	ANOVA of KFI	ANCOVA
Roe deer	Sex	7	6
	Age class	4	4
	Sex × age class	10	10
Fallow deer	Sex	100	39
	Age class	11	9
	Sex × age class	50	14

All analyses were carried out on 100 sub-sets of the same set of 10,000 simulated data.

Boardman (1999), our simulation analysis showed that, for roe deer, ANOVA of KFI values and ANCOVA of fat weights were robust enough to detect the same significant differences between sex–age classes (Table 1).

However, for fallow deer, ANOVA of KFI values generated many more statistically significant differences for the effects of age (two more significant results) and, particularly, sex (61 more significant results) and their interaction (36 more significant results) compared to ANCOVA (Table 1). Because of the significant interaction between the covariate (kidney weight) and the factor to be tested (sex) in juvenile and male fallow deer (see above), the estimation of the KFI in fallow deer is likely biased; hence, we suspect that these additional significant results are spurious (Raubenheimer and Simpson 1992).

Fig. 2 Linear relationships between kidney fat and kidney weight in juvenile, adult, female and male roe deer and fallow deer



Discussion

As our results show, calculation of the KFI does not correct for the effect of organ size on fat weight in either roe deer, which carries little fat, or fallow deer, where fat weighs twice that of the kidney. The estimation of the KFI may not respect some important assumptions concerning the proper use of indices, notably the requirement for isometry and the absence of an interaction between denominator and treatments. This led to discrepancies between the results of ANOVA and ANCOVA concerning the effects of treatments, but these were considerable only for fallow deer, probably because it stores much more fat around the kidney than roe deer. One limitation of our study was that we could not evaluate whether the observed differences in KFI or fat residuals represented real physiological differences between treatments (*sensu* Holand 1992). However, we suggest that wildlife ecologists always check the underlying assumptions associated with the use of the KFI before employing ANOVA. As a general rule, it is usually safe to analyse fat weight as the untransformed variable in ANCOVA with kidney weight as the covariate. However, we do agree completely with Riney's observations that the quantity of fat stored around the kidneys is an excellent indicator of body condition in ungulates, particularly for capital breeders.

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