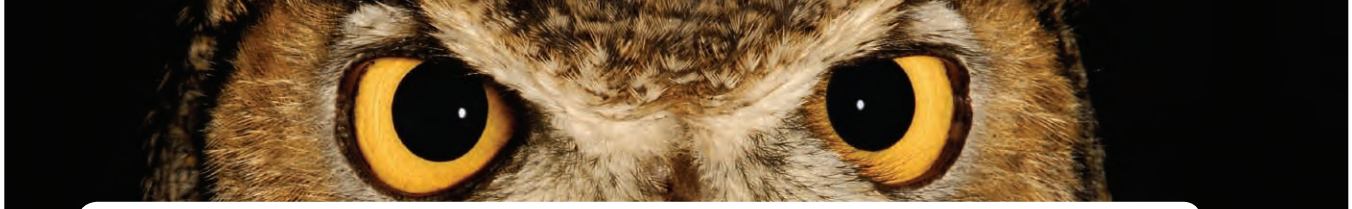

ONLINE INQUIRY & INVESTIGATION

Lipid Determination & Kidney Fat Index: An Experiment for Undergraduate Students in Wildlife Management

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Context

Our biology program is mainly centered on wildlife management and marine biology. Biochemistry and physiology are sciences that have held little interest for many of our students because most theoretical and laboratory textbooks for undergraduate students in these fields have an anthropocentric approach. To avoid this problem, we have adapted many of our biochemistry and physiology experiments to give examples linked to wildlife management or marine biology (Rioux & Blier, 1995a; Rioux & Blier, 1995b; Rioux et al., 1998; Diouf & Rioux, 1999; Diouf et al., 2000; Bolduc, Lamarre & Rioux, 2002). This approach allows us to increase the students' interest for these fields. The experiment proposed here combines a classic lipid extraction method (Bligh & Dyer, 1959) with a technique used by wildlife managers, the kidney fat index (KFI). The same approach can be used in laboratories that perform soxhlet extractions or that use a Goldfisch lipid extractor, which are methods also used for the assessment of the total carcass lipids in mammals (Havera, 1977; Martin, 1977; Whittaker & Thomas, 1983; Mawhinney & Millar, 1990; Prestrud & Nilssen, 1992; Virgl & Messier, 1992; Hanni & Millar, 1993; Winstanley, Saunders & Buttemer, 1998; Buck & Barnes, 1999).

Background on the Kidney Fat Index

Many biochemical and physical condition indices have been developed to evaluate the health status of wild mammals. The percentage of total carcass lipids is considered as one of the best. However, its determination in large wild mammals can be difficult because wildlife managers do not always have the equipment required for this assay (Huot, 1988). To avoid this problem, researchers and managers have used physical condition indices, such as the kidney fat index (KFI) (Riney, 1955), that are less expensive and less time consuming. The KFI is considered a good indicator of the abdominal lipid reserves and thus of the nutritional status or the physical condition of certain species. In animals having a large amount of fat, the concentrations of other fat reserves would be at adequate levels. In our experimental protocol, we present studies that have used the KFI in mammals found in Canada.

Objectives

In this experiment, students must answer the following question:

> Is the KFI, which measures only the perirenal fat, a reliable indicator of the total lipid content in the rabbit carcass?

The student must determinate the validity of using the KFI in this species by comparing it with a biochemical measure. For that purpose, we have determined both the KFI and the total lipid content in rabbits of different sizes.

Materials & Methods

Logistics

A minimum of seven hours of laboratory will need to be dedicated to completing the exercise.

A period of six continuous hours (or two periods of three hours) was necessary to complete this laboratory, with one additional hour scheduled the next day to finish the last part of the experiment. Students were paired into teams of two. Each team had a rabbit and completed all of the manipulations on its specimen. Class results were pooled and distributed to each team, which then had to analyze the

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results and write a scientific article as a laboratory report.

We used domestic rabbits They are easily available and inexpensive, and the KFI has already been successfully used in lagomorphs. The homogenization of these small mammals compared to cervids is relatively easy and does not require the acquisition of expensive equipment. Wild species of lagomorphs could also be used if samples were available.

Twelve carcasses of New Zealand rabbits (*Oryctolagus cuniculus*) of different sizes were bought from a local stock-breeder. Their masses ranged from 1.88 to 4.32 kg.

Kidney Fat Index Determination

The technique consists of removing the animal's kidneys with the perirenal fat. The fat is cut off perpendicularly at the ends of the two kidneys, then the rest of the fat is separated from the kidney. The kidney fat index is calculated by multiplying the fat mass: Kidney ratio by 100. For each rabbit, the final result is an average of the result obtained for each kidney. For more details on the dissection method, one may consult Harder and Kirkpatrick (1994).

Total Carcass Lipid Determination

Once the KFI is determined, the carcasses (including the kidneys and perirenal fat removed for the KFI determination) were homogenized in a Hobard® commercial meat grinder for the determination of total lipid content using the well-known and simple method described (Bligh & Dyer, 1959).

Lipids are very soluble in organic solvents. In this technique, lipids contained in a homogenate are extracted with a Waring® blender using a mix of chloroform and methanol. Water is then added to help the phase separation: The methanol (more miscible with water than chloroform) and water form a phase that can be clearly separated from the lipid-chloroform phase. The lipid–chloroform phase is then collected in a flask, the solvent evaporated, and the fat recovered.

Our experimental protocol contains a review of the classic techniques for lipid content determination: extraction by methanol-chloroform, extraction by soxhlet, and the colorimetric method based on the sulphophospho-vanillin reaction.

Protocol

A sample of the whole body homogenate weighing 30 to 50 g (recorded as M_S, sample mass) was placed in a Waring blender containing 100 ml of methanol; 50 ml of chloroform was then added under a fume hood. The blender was tightly closed and the mixture homogenized for two minutes. Fifty ml of chloroform was then added and mixed again for 30 to 60 seconds. This step was repeated, but this time with 50 ml of water. The mixture was separated by vacuum filtration with a Whatman #1 filter. Residues were rinsed with 25 ml of chloroform.

The filtrate was transferred to an empty 500 ml separation funnel. The container was rinsed with 5 to 10 ml of chloroform and this volume also transferred to the flask. To obtain a clear phase separation, 5 ml of water was added and the extraction was allowed to proceed overnight.

Whatman #1 and #4 Filters (15 cm) were folded and the #4 Filter then placed inside the #1. This double filter was placed in a filling funnel and filled to 3/4 of its capacity with anhydrous sodium sulfate. A 500 ml evaporating flask was weighed (and its mass recorded as M_F1). The filling funnel was then placed in the flask. The separation funnel was placed at about 1 cm above the anhydrous sodium sulfate. Gently and slowly, the chloroform-lipid phase was recuperated. The anhydrous sodium sulfate was rinsed with 25 to 50 ml of chloroform. The chloroform-lipid phase was evaporated at 40°C with a rotary evaporator. Traces of chloroform were eliminated by leaving the flask open under a fume hood for at least four hours before weighing it again. This time, the weight includes the mass of the fat (M_F2).

Calculation of the lipid content percentage:

$$\text{Lipids (\%)} = [M_F2 - M_F1) / M_S] \times 100$$

Where:

(M_F2) = mass of the flask containing the lipids

(M_F1) = mass of the empty flask

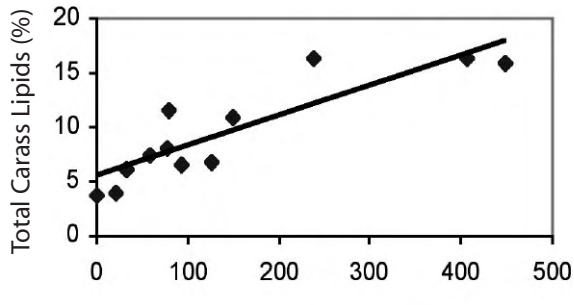
(M_S) = mass of the sample

Results & Discussion

The kidney fat index was highly significantly and positively correlated with the percentage of total carcass lipids in the rabbits ($F_{1,10} = 34.9$, $p<0.001$; $R^2 \sim 0.78$) (Figure 1). The KFI is found to be a simple index for predicting the total carcass lipid content. Statistically similar results, despite the 12 different teams of experimenters, show that the experiment is sufficiently robust to be used in an undergraduate class. Therefore we obtained a positive correlation between the two indices for two consecutive years.

We asked the students to discuss the results by consulting some studies that compared the two methods, for instance, in fox squirrel (Havera, 1977), red fox (Winstaley, Saunders & Buttemer, 1998) and white-tailed deer (Finger, Brisbin & Smith, 1981; Watkins et al., 1991).Suggested

Figure 1.
Relationship between the total carcass lipids (%) and the kidney fat index in rabbits ($R^2 = 0.78$; line equation: $y = 0.026x + 5.335$).



readings for students could include references that have used lagomorphs as the experimental subject in relation to the kidney or the abdominal fat index (Martin, 1977; Henke & Desmarais, 1990; Flux, 1971; Pépin, 1987).

This experiment illustrating lipid extraction has been found to be well suited to wildlife management undergraduate students and may be used in an experimental biochemistry course developed for these students.

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