Ecological Indicators 45 (2014) 522–528

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Near infrared reflectance spectroscopy (NIRS) for predicting glucocorticoid metabolites in lyophilised and oven-dried faeces of red deer

João P.V. Santos[a,b,*], Joaquín Vicente[b], Miriam Villamuelas[c], Elena Albanell[d], Emmanuel Serrano[a,c], João Carvalho[a], Carlos Fonseca[a,e], Christian Gortázar[b], Jorge Ramón López-Olvera[c]

[a] Departamento de Biologia & CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[b] Sanidad y Biotecnología (SaBio), Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13071 Ciudad Real, Spain
[c] Servei d' Ecopatologia de Fauna Salvatge (SEFaS), Departament de Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Barcelona, Spain
[d] Grup de Recerca en Remugants, Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Barcelona, Spain
[e] Universidade Lúrio, Campus de Marrere, Nampula, Mozambique

ARTICLE INFO

Article history:
Received 29 January 2014
Received in revised form 8 May 2014
Accepted 21 May 2014

Keywords:
Cervus elaphus
Faecal indicators
Glucocorticoid metabolites
NIRS
Stress

ABSTRACT

Interest in measuring faecal glucocorticoid metabolites (FGM) as indicators of physiological homeostasis and performance in wildlife is increasing. However, current reference techniques, specifically enzyme immunoassays (EIAs) and radioimmunoassays (RIAs), are expensive, time-consuming, reagent-based, and the samples are destroyed during their application. Conversely, near infrared reflectance spectroscopy (NIRS) is a rapid, reagent-free and non-destructive technique, which, once calibrated by standard laboratory methods, can be used at a low cost. The objectives of this study were to evaluate the feasibility of using NIRS to predict glucocorticoid metabolite concentrations in red deer (*Cervus elaphus*) faeces, as well as the effect of lyophilisation and oven drying on FGM quantification. Seventy-eight fresh faecal samples were collected directly from the rectum of hunter-harvested red deer and then divided into two equal portions; one portion of each individual sample was lyophilised and the other portion was oven-dried. After dehydration, all faecal samples were ground and then analysed by RIA (standard laboratory technique) and scanned with an NIR spectrophotometer. Modified partial least squares regression was used to generate NIRS calibration equations for both lyophilised and oven-dried samples and a cross-validation procedure was employed for their optimisation. Near infrared reflectance spectroscopy proved to be a feasible, acceptably accurate and reliable technique for predicting FGM concentrations in red deer faeces subjected either to lyophilisation or to oven drying. Calibration and cross-validation results indicated that predictive equations for lyophilised faeces were slightly more precise and robust than for the oven-dried ones (lyophilised: $R^2 = 0.90$, $r^2_{cv} = 0.81$, RPD = 2.72; oven-dried: $R^2 = 0.88$, $r^2_{cv} = 0.79$, RPD = 2.26; CV: cross-validation, RPD: ratio of performance to deviation). Nevertheless, oven-dried faeces may be used as an alternative to lyophilised ones to quantify FGM levels accurately, provided that an appropriate combination of dehydration time and temperature is used during the desiccation process. High degrees of association and statistically significant positive correlations ($p < 0.001$) were found between the lyophilised and oven-dried samples regarding their FGM content, both for RIA assays and NIRS analyses. This study provides a new approach for assessing stress levels in free-ranging populations and has practical implications concerning wildlife monitoring as it makes it possible to improve the efficiency and reduce the cost and time constraints of current analytical techniques.

* Corresponding author at: Departamento de Biologia & CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal. Tel.: +351 234370350; fax: +351 234372587.
E-mail address: joaovalente@ua.pt (J.P.V. Santos).

http://dx.doi.org/10.1016/j.ecolind.2014.05.021
1470-160X/

1. Introduction

Measuring stress in wildlife is increasingly relevant for conservation and management programmes, as it allows the assessment of the physiological status and performance of both individuals and populations (Busch and Hayward, 2009; Tarlow and Blumstein, 2007; Wikelski and Cooke, 2006). Glucocorticoids (*i.e.*, cortisol and corticosterone) and their metabolites have been used as biomarkers to monitor the physiological stress response in domestic animals (Morrow et al., 2002; Möstl and Palme, 2002; Palme et al., 2000) and wildlife (Azorit et al., 2012; Dalmau et al., 2007; Dehnhard et al., 2001; Huber et al., 2003; Zwijacz-Kozica et al., 2013). Glucocorticoids are steroid hormones secreted by the adrenal cortex and play a key role in helping organisms to overcome stressful situations, but chronic high glucocorticoid levels may produce deleterious effects on an individual's health and overall fitness (Pride, 2005; Reeder and Kramer, 2005; Sapolsky et al., 2000).

Over the past years, the measurement of glucocorticoid concentrations in blood, saliva, urine, faeces, and more recently in hair and feathers, has been used to assess stress in wildlife (Sheriff et al., 2011). Faecal hormone monitoring has recently gained particular notoriety in wildlife research and conservation biology mainly due to the non-invasiveness and ease of sampling (Keay et al., 2006; Millspaugh and Washburn, 2004; von der Ohe and Servheen, 2002). Faecal glucocorticoids are previously metabolised by the liver and later by gut bacteria, and therefore the relative composition of the metabolites formed differ considerably among species (Möstl and Palme, 2002; Palme et al., 2005; Touma and Palme, 2005), and is also affected by individual factors such as gut microbiota, diet, sex and metabolic rate (Goymann, 2012). Enzyme immunoassays (EIAs) and radioimmunoassays (RIAs) have been widely used as reference methods to quantify glucocorticoid metabolites in faecal samples (Möstl and Palme, 2002; Sheriff et al., 2011). However, these techniques are expensive, require chemical reagents or radioactive substances (in the case of RIAs), and the samples are destroyed by analysis. Furthermore, they are time consuming since glucocorticoid extraction is needed before biochemical assays (Pappano et al., 2010; Sheriff et al., 2011).

During the last decades, near infrared reflectance spectroscopy (NIRS) has shown a huge potential for assessing the physicochemical properties of many substances and materials. It has the advantage of being a non-destructive and reagent-free technique that provides a rapid analysis of complex samples containing a wide range of components (Williams, 2001). In addition, the samples require little or no preparation before analysis (Roggo et al., 2007; Siesler et al., 2002). Near infrared reflectance spectroscopy has been widely and successfully applied in various research fields and industries, especially in those linked to agriculture and evaluation of food quality (Cen and He, 2007; Ozaki et al., 2007; Roberts et al., 2004; Williams and Norris, 2001), but also in soil science research (Ludwig et al., 2002; Vendrame et al., 2012) and physiological studies (Banaji et al., 2008; Obrig and Villringer, 2003). The analysis of faecal material by NIRS has also been extensively used to assess numerous aspects of nutrition, physiology and ecology, particularly of domestic and free-ranging herbivores (Dixon and Coates, 2009; Dryden, 2003; Foley et al., 1998; Gálvez-Cerón et al., 2013; Lyons and Stuth, 1992). However, to our best knowledge, no studies have been reported using NIRS to predict the concentrations of stress hormone metabolites in faeces. In fact, the use of NIRS as an analytical method in hormonal studies is still scarce (Fountain et al., 2003; Kinoshita et al., 2012; Pérez et al., 2004; Xia et al., 2007). Near infrared reflectance spectroscopy is a predictive (indirect) method, which requires the development of calibration models against appropriate standard laboratory methods. Hence, the performance of NIRS as a procedure for quantitative analysis will depend on the accuracy and precision of those reference methods (Cen and He, 2007).

In this paper, our intent was to explore cost-effective and timesaving methods for assessing stress in wild populations, in particular, that of free-ranging herbivores. The objectives of this study were two-fold. Firstly, we evaluated the feasibility of using NIRS to predict the concentration of faecal glucocorticoid metabolites (FGM) in red deer (*Cervus elaphus*) faeces. The red deer has a widespread distribution in the northern hemisphere (Wemmer, 1998) and is one of the most important game species in many European countries, where their populations are subjected to different management and exploitation regimes (Apollonio et al., 2010). In some cases, those populations are overabundant and intensively managed for hunting (Gortázar et al., 2006). This species can also suffer from various diseases, many of which are shared with livestock and some are zoonoses of importance for public health (Ferroglio et al., 2011; Gortázar et al., 2007). Considering the variety of potential stress-inducing factors to which red deer are exposed, monitoring stress in their wild populations is of general interest. Secondly, we investigated the effect of two drying methods (lyophilisation and oven drying) on the quantification of FGM and developed NIRS calibration models for predicting their concentrations for each drying treatment. Previous studies have shown that different drying procedures affect FGM concentrations and, until now, lyophilisation has been the preferred method used for drying faecal samples in stress research, as it allows for effective preservation/recovery of FGM (Terio et al., 2002). However, apart from the fact that lyophilisers are not always available in laboratories, this freeze-drying process can take up to three or four days to complete (Goymann et al., 1999; Millspaugh et al., 2001). On the other hand, oven-dried samples can be ready for analysis in half the time, but their use is generally not recommended since the oven drying process may cause FGM degradation (Terio et al., 2002). Therefore, we intended to verify how effective and reliable the oven drying method is in preserving FGM in relation to lyophilisation.

2. Materials and methods

2.1. Sample collection and storage

Fresh faecal samples ($n = 78$) were collected directly from the rectum of hunter-harvested red deer at eight different locations in the Iberian Peninsula during three consecutive regular game seasons (September to February 2010–11 to 2012–13). Young and adult male and female deer were sampled to take into account any potential variations in FGM concentrations due to age and sex (Goymann, 2012; Millspaugh and Washburn, 2004). Upon collection, the faecal samples were placed in individual appropriately identified plastic bags, and then transported in a cooler box with freeze gel packs to the laboratory, to prevent any degradation of the hormone metabolites (Palme, 2005; Sheriff et al., 2011). Once in the laboratory, the samples were stored in a freezer at −20 °C, without any chemical treatment (Millspaugh and Washburn, 2004), until processing.

2.2. Drying and preparation of faecal samples

Individual faecal samples were separated into two equal portions: one portion was lyophilised at −20 °C for 96 h, whereas the other portion was oven-dried at 60 °C for 48 h. After dehydration, all samples were ground in a cyclone-type mill to pass a 1 mm screen and stored in zip-lock bags in a cool, dry place, before the NIRS analysis.