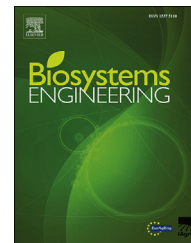


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## Research Paper

# Identification of beef cattle categories (cows and calves) and sex based on the near infrared reflectance spectroscopy of their tail hair



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Near infrared (NIR) reflectance spectroscopy combined with chemometrics was used to classify tail hair samples from animals of the same breed of cattle (Brahman) into cow or calf and into male or female animals. Tail hair samples ( $n = 74$ ) were scanned in the NIR region (680–2500 nm) using a fibre optic probe attached to an instrument operating in reflectance mode. Principal component analysis (PCA), and partial least squares discriminant analysis (PLS-DA) were then used to classify the samples according to their origin or sex. Full cross validation (leave-one-out) was used as the validation method when classification models were developed. Correct classification rates of 92% for cow and 100% for calf samples were obtained using PLS-DA. These results demonstrated the ability of NIR spectroscopy to discriminate between the animal categories and sex of animals. Further studies will be carried out to validate the methodology in various categories of beef cattle.

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## 1. Introduction

Near infrared reflectance (NIR) spectroscopy has been widely used as a tool to classify or discriminate different type of foods, plant parts, beverages and animal products (Cozzolino, 2014, 2015; Roberts & Cozzolino, 2016). Although, NIR spectroscopy has been used to analyse hair samples in humans to target metabolic and other health issues or forensic analysis (Kalasinsky, 2012), no such research has been reported in animals (Bortolot & Prater, 2009; Pringle, Roberts, Kohl, & Lekeux, 1999). The effect of the presence of hair on the reflectance spectra of different animal tissues when they were measured using NIR was also reported by Pringle et al. (1999).

Being able to discriminate species of animals, between animals within the same species or different categories within the same species, would allow the location and/or identification of animals in different production systems (Bortolot & Prater, 2009). These authors reported the use of hyper-spectral NIR spectroscopy to discriminate animal species based on a hair sample (Bortolot & Prater, 2009).

To date, relatively few studies have examined the spectral reflectance of hair from different animals (Hutchinson, Allen, & Spence, 1975; Gates, 1980; Wyatt, Anderson, Harsbarger, & Trivedi, 1984; Schwaller, Benninghoff, & Olson, 1984; Strong, Gilmer, & Brass, 1960; da Silva, La Scala, & Tonhati, 2003; Bortolot & Prater, 2009). The findings from these studies indicated that there is variability among species of mammals

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(Hutchinson et al., 1975; Gates, 1980; da Silva et al., 2003) and birds (Gates, 1980; Strong et al., 1960). These differences were observed in the NIR and shortwave infrared (SW-NIR) region of the spectrum (Gates, 1980; Strong et al., 1960). These authors also reported that different parts of the animal have different reflectance characteristics, and geometry of the sensor can also affect the measurements of optical properties as reported by other authors (Gates, 1980; Hutchinson et al., 1975).

Only one study specifically reported the use of reflectance spectra to discriminate among species, although three other studies have examined the ability to use spectral properties of animals to differentiate between different animal species (Wyatt et al., 1984; Schwaller et al., 1984; Strong et al., 1960). Therefore, classification or discriminant analysis based on the use of NIR spectroscopy combined with multivariate data techniques or chemometrics appears to be an alternative available to the beef industry as a tool to identify different categories of animals, or differences between animals associated with genetics, nutrition, and health. Given the crucial role that hair has played as a fitness trait in the evolution of cattle (O'Neill, Swain, & Kadarmideen, 2010) and, currently, as an adaptive phenotype in systems biology of livestock (Cole et al., 2013; Te Pas et al., 2017) the spectral analysis of hair samples has direct implications in the management of cattle in grazing conditions.

The objective of this paper is to report a feasibility study on the use of NIR to classify tail hair samples sourced from different categories or sex of animals.

## 2. Materials and methods

### 2.1. Experiment, animals and tail hair samples

Tail hair samples were obtained from Brahman (*Bos indicus*) cattle from Belmont Research Station, which is a 3260 ha property located in north-eastern Australia (150°13 E; 28° 8 S) on the Fitzroy River 30 km upstream of Rockhampton (Queensland, Australia). The research station has different paddocks combining native improved and legume-based pastures. Tail hair samples (approx. 20) were taken from both individual cows ( $n = 37$ ) and their corresponding calves ( $n = 37$ ). In addition, three cows had black hair and were removed from the data set used to develop the classification models. The range in age of the cows was 2–12 years and for the calves 18–29 weeks. The samples were collected in April 2016 following the protocol for collecting a tail-hair sample for DNA testing (Animal Genetics Laboratory, 2013).

### 2.2. Spectra analysis

The spectra of the tail hair samples were collected using a Thermo Scientific Antaris FT-NIR Analyzer (Thermo Fisher, Madison, USA) in the NIR region (680–2500 nm) using a fibre optic probe attached to an instrument operating in reflectance mode. The spectra were referenced using a ceramic disk. In order to evaluate the effect of the position of the fibre optic on the collection of the spectra, data were collected at the apical and basal position of the tail hair samples. After this experiment, no differences were observed and only one of the positions was used and compared in the following sections.

### 2.3. Chemometric analysis

Spectral data were saved in csv format and exported into The Unscrambler X (CAMO AS, version 10.3, Oslo, Norway) software for multivariate analysis. A primary evaluation of the data was carried out by means of unsupervised chemometric techniques such as principal component analysis (PCA), with the aim of exploring the relationship between samples and looking for potential outliers. PCA is a dimension reduction and display chemometric technique that transforms an original data matrix into a product of two matrices, one of which contains information about the variables (scores), and the other about the magnitude of the variables (loadings) (Brereton, 2003; 2007; Naes, Isaksson, Fearn, & Davies, 2002).

Discrimination models were developed using partial least squares discriminant analysis regression (PLS-DA). The PLS-DA regression technique is a variant of PLS regression, where, for each class, a model is developed [ $C = T \cdot q$ ], where T are the PLS scores obtained from the original data using the PLS algorithm, q is the vector and C is the class membership function (Brereton, 2003; 2007; Naes et al., 2002). This is obtained by PLS regression from an original C vector whose elements have values of 1 (cow samples) if an object is a member of a class and 2 (calf samples) otherwise, with a cut-off limit of 0.5. In this study, the PLS2 algorithm was used to develop the models. The main advantage of this algorithm is that it models all variables at the same time. Group membership of a new unknown sample is determined by its predicted value with PLS-DA (Brereton, 2003, 2007; Naes et al., 2002). A limit value is calculated according to the values predicted in the calibration step. Values above this threshold indicate that the sample belongs to the modelled class, whereas values below the limit values indicate that the sample does not belong to the modelled class (The Unscrambler, CAMO AS, version 10.3, Oslo, Norway). The threshold is estimated using Bayesian statistics and the available data in order to minimise total errors of classification. It is assumed that the predicted values follow a distribution similar to that expected to be observed for future samples (Brereton, 2003, 2007; Naes et al., 2002). The same protocol was applied to discriminate the samples according to sex. In this study, PCA and PLS-DA models were developed using full cross validation (leave one out) and defined by the PRESS (prediction residual error sum of squares) function in order to avoid overfitting the models (The Unscrambler, CAMO AS, version 10.3, Oslo, Norway).

The spectral data were processed using the second derivative (2nd derivative, 20 smoothing points and 2nd polynomial order) in order to remove and correct for baseline effects (Savitzky & Golay, 1964). The second derivative is a measure of the change in the slope of the curve ignoring the offset and is very effective in removing both baseline offset and slope from a spectrum (Savitzky & Golay, 1964).

## 3. Results and discussion

### 3.1. Spectra interpretation

Figure 1 shows the NIR spectra of tail hair samples sourced from the cow and calf samples after application of the second derivative as a pre-processing method. Absorption bands

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