Prediction of heat stress status by infrared spectroscopy in dairy sheep

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Abstract

Advances in high-throughput phenotyping using Fourier-transform infrared spectroscopy (FTIR) offer the opportunity to efficiently measure new traits on a large scale that can be exploited in breeding programs as indicator traits. As new traits, the definition of phenotypes to improve the adaptation of animals to heat stress events is gaining interest within the context of Climate Change. Thus, in this work we evaluate the suitability of using FTIR to predict whether an animal has been exposed to a heat stress event. Milk samples from 305 ewes from the same flock were collected in two seasons: comfort (spring) and summer (hot season). Fourier-transform infrared spectra were collected on the same day as milk sampling and consisted of the transmittance values measured at 1,060 wavenumbers ranging from 5,011 to 925 (cm^{-1}). A quality control analysis using principal components analysis in the FTYR spectra was carried out in order to remove outliers. After QC, a PLS-DA analysis was conducted to evaluate if we were able to discriminate between both conditions, comfort and heat stress. The results showed a high discrimination capacity between ewes under comfort and ewes under heat stress. The variability observed within the group of samples analyzed under comfort was significantly lower than that observed for the group of samples analyzed under heat stress. This would indicate that while exposure to heat stress events produces physiological changes in the animal that are reflected in the composition of the milk, these changes are not the same in all animals. A detailed study of the regions of the spectra in which large variability among individuals was observed could provide insight into the metabolic pathways involved in the heat stress response. Also, the results open the possibility of considering the use of infrared spectroscopy as a breeding tool. For this, a more detailed study of the individual variability of spectra under heat stress would be necessary.

Keywords: mir spectra, heat stress, climate adaptation, breeding, sheep

Introduction

Exposure to HS events has negative consequences on milk production - both in quantity and quality – fertility, health and wellbeing (Baumgard *et al.* 2016). In response to the Climate Change (CC) challenge, breeding programs have been presented as very useful tools to improve the adaptation of animals to those negative consequences of CC that cannot be mitigated (Carabaño *et al.* 2017; Pryce *et al.* 2020). For this purpose, however, it is necessary to define phenotypes easy to measure on a large scale to characterize the thermotolerance of animals. In this regard, advances in high-throughput phenotyping using Fourier-transform infrared spectroscopy (FTIR) offer the opportunity to efficiently measure new traits on a large scale that can be exploited in breeding programs as indicator traits (Hammami *et al.* 2015). Mid-infrared (MIR) methodology is routinely used in quality assessment of milk samples. Focusing on adaptation to CC, FTIR spectra data could be used to the identification of animals suffering from HS and characterization of their thermotolerance as a way to improve adaptation to HS within breeding programs. With this background, the present work was designed as a preliminary study to examine the suitability of the use of FTIR spectra to discriminate between milks samples collected under comfort or heat stress (HS) and examine if they could be as thermotolerance indicator in dairy sheep.

Materials and methods

A total of 232 ewes belonging to a flock of the national association of breeders of the Manchega dairy sheep (AGRAMA) were sampled during two periods: (i) comfort season: April, May and September; and (ii) hot season: June to August. At each visit, individual milk quantity was recorded, and a milk sample was collected and sent to the laboratory to perform the quality analyses. At the lab, mid-infrared spectra were obtained individual milks samples over the spectral range of 4000 to 900 cm^{-1} using a Milk-Scan equipped with a Fourier transform infrared interferometer (FOSS electric A/S, Hillerod, Denmark).

In a first step, principal components analysis of the FTIR spectra data was carried out and the presence of outlier spectra was explored by calculating the Mahalanobis distance, so those spectra outside de range of ±3 standard deviations were removed from the data base. Milks samples were assigned to 2 groups based of the collection date: comfort (samples collected in April, May or September) and HS (samples collected in June, July or August) groups. Figure 1 shows average FTIR spectra for milk samples collected under comfort and HS and their differences. A partial least squares – discriminant analysis (PLSDA) was conducted to see whether it is possible to discriminate between samples collected under HS from those collected in comfort.

Results and discussion

Figure 1 shows average FTIR spectra for milk samples collected under comfort (top) and heat stress (centre), and the differences between both (bottom; comfort – heat stress). Differences were mainly in two regions of the spectra: around 1500 cm^{-1} and from 3500 to 3000 cm^{-1}. Some studies have reported that at these wavelength, carbon-oxygen and nitrogen-hydrogen bonds from fatty acids and proteins absorb being these regions used to predict fat and protein contents of milk (Nicolau *et al.* 2010). However, as this was an untargeted study, we do not have information to identify which milk components are responsible for the differences between samples collected under HS or Comfort conditions. Subsequent targeted studies will seek to deepen the study of the milk components that change due to exposure to heat stress through the use of metabolomics.

As we indicate above, the aim of this preliminary study was to see if FTIR spectra data was useful to identify when and animal was under HS or not. Figure 2 shows the

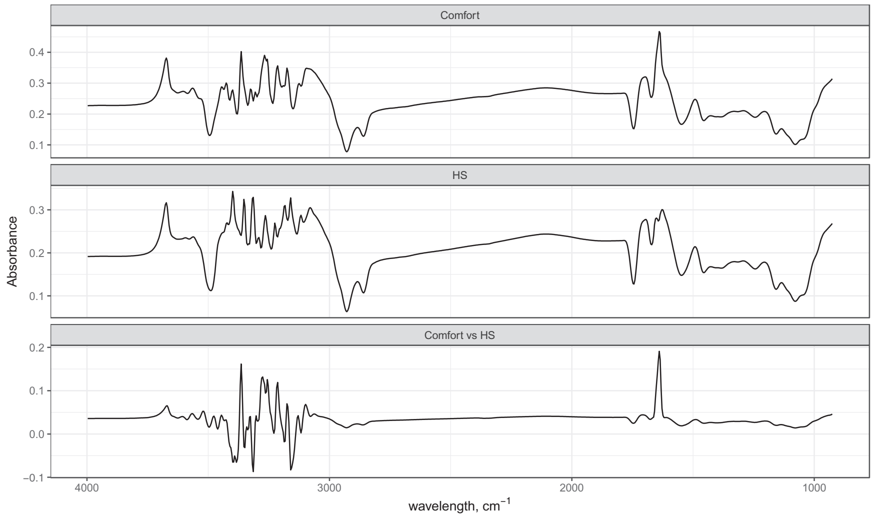

Figure 1. Average FTIR spectra of ewe milks samples collected under comfort and HS conditions and their differences

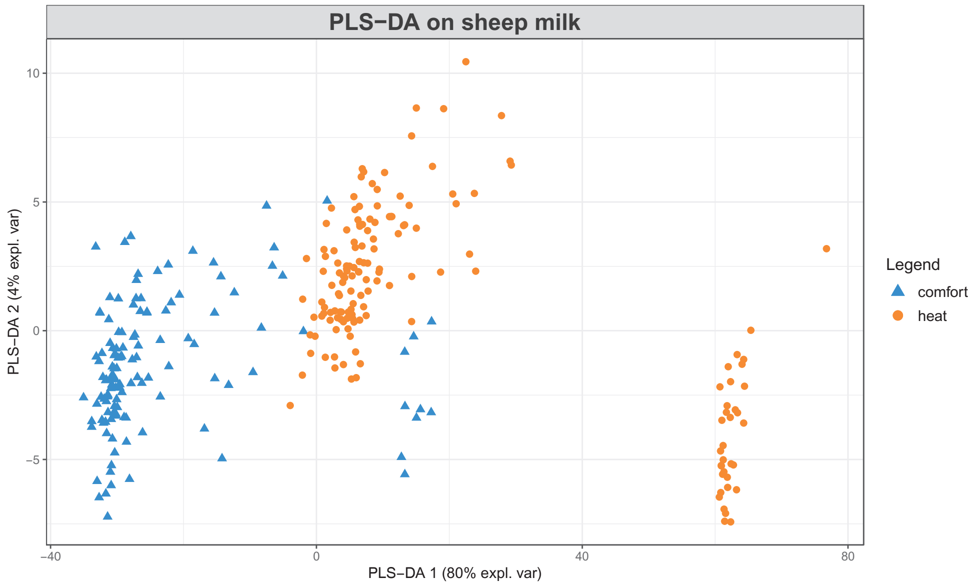

Figure 2. Classification of ewe milk samples of ewes based on PLSDA outcomes using FTIR spectra as predictor. Milk samples collected under comfort (blue triangles) and HS (oranges circles) are represented.

how mils samples were classified based on PLSDA outcomes using FTIR spectra as predictor. In general, the PLSDA procedure was able to discriminate between samples collected under comfort from those collected under HS. For the latter, two groups were clearly differentiated. Although not indicated in this paper, as was not an objective in this preliminary study, these two subgroups of milk samples under HS represent primiparous (the group of orange circles close to the group of samples collected under comfort) and multiparous (the group of orange circles further away from the group of samples collected under comfort) ewes. The same two groups existed for the milk samples collected under comfort, but no differences were observed between them.

Conclusions

The objective of this preliminary work was to examine whether FTIR spectra data from milk samples could be use identify if an animal was suffered from heat stress and its suitability as a HS phenotype to be use in breeding programs in order to select for animals more adapted to heat stress. Some regions of the spectra have clearly differed between samples collected under comfort or HS conditions, although it has not been possible to identify the milk components to which these differences are due. Predictions of status of the samples (comfort vs. HS) by PLSDA methodology appear promising to discriminate between both type of samples.

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