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Short communication

Application of near infrared reflectance spectroscopy (NIRS) on faecal samples from lactating dairy cows to assess two levels of concentrate supplementation during summer grazing in alpine pastures

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Abbreviations:
aNDF, neutral detergent fibre
DM, dry matter
LVs, latent variables
NIRS, near infrared reflectance spectroscopy
OPLS-DA, orthogonal partial least square discriminant analysis
OLVs, orthogonal latent variables
PCA, principal component analysis
PLS-DA, partial least square discriminant analysis
UFL, *unite fourragère lait*
VIP, variable importance for projection index

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ABSTRACT

The research aimed at developing a specific near infrared reflectance spectroscopy (NIRS) calibration using faecal spectra to assess two levels of concentrate supplementation provided to lactating dairy cows during summer grazing on alpine pastures. Faecal samples were used as analytical matrix since they are easy to be taken without being invasive for the animals. A total of 153 individual faecal samples were collected from lactating grazing cows in 28 alpine farms located in the highland of Asiago in the Veneto region of Italy during the summer of 2011 (80 samples from 10 farms) and 2012 (73 samples from 18 farms). The average amount of concentrate provided to the lactating cows was recorded in each farm and compared with the legal limit of 20% of the total energy requirement for maintenance and lactation set by the regional law 52/1978 on the use of woodlands and alpine pastures. Dried and ground faeces were subjected to chemical analysis and scanned by NIRS in the region between 1100 and 2500 nm. Faeces from cows supplemented with concentrate above 20% of their total requirement had lower ash ($P<0.001$), crude protein ($P<0.001$), and lignin content ($P<0.001$) but higher starch content ($P<0.001$) than those from animals supplemented in compliance with the legal limit. A partial least square discriminant analysis (PLS-DA) classification model for the two levels of concentrate supplementation built on NIRS faecal spectra showed variable importance for projection index (VIP) greater than 1 in the regions below 1400 nm, between 1700 and 1750 nm, around 2175 nm and between 2250 and 2300 nm. The model had a good fit for the calibration data with sensitivity and specificity >90%, but poor specificity (55.5%) for the validation samples. The orthogonal pre-processing of data (OPLS-DA) improved classification accuracy, with sensitivity and specificity values >90% also for the validation set. Therefore, this calibration can be proposed as a quick routinely tool to assess the two levels of concentrate supplementation during summer grazing in alpine pastures.

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1. Introduction

In the past decade, the structural and economic evolution of the dairy sector in Europe addressed many dairy producers operating in the mountains towards intensive farming systems. This intensification process with the introduction of high producing dairy breeds and the large use of concentrates to fulfil their nutrient requirements (Cozzi et al., 2009) has increased the abandon or the underuse of pastures grazing resulting in a loss of biodiversity and a decline of the mountain landscape (Luoto et al., 2003). In this scenario, the management of alpine pastures through cattle grazing is considered one of the best strategies to reduce landscape degradation (Sternberg et al., 2000; Bernués et al., 2011). To prevent the decay of the alpine pastures of public property, the law 52/1978 of the Veneto region (in the North of Italy) on the use of woodlands and alpine pastures set a maximum quantity for the concentrate provided as pasture supplement to grazing dairy cows equal to 20% of the total energy requirement of the animals. However, it is very complex for public authorities to verify the compliance of this nutritional constraint by the dairy herds under grazing conditions.

Faeces contain information about diet characteristics, because they represent the end-product of its digestive process. Lyons and Stuth (1992) examined the potential of the near infrared reflectance spectroscopy (NIRS) for predicting diet quality of free ranging cattle via faecal scans and more recently, faecal NIRS spectra confirmed to be a tool to accurately predict dry matter (DM) intake and digestibility in grazing cattle (Coates and Dixon, 2011; Decruyenaere et al., 2012). The present research aimed at developing a specific NIRS calibration using faecal samples in order to assess two levels of concentrate supplementation provided to dairy cows during summer grazing (*i.e.*, above or below 20% of the total energy requirement).

2. Materials and methods

2.1. Farms management, faecal sampling, and analysis

The research was carried out in the highland of Asiago, one of the main dairy areas of the Italian Alps, located in the Veneto region. This territory hosts 87 alpine farms, 77 of public property that are leased to private farmers who are in charge of their maintenance. The total pasture surface in the highland is 7775 hectares covering an altitude range of 700–2300 m a.s.l. The highland has a continental-temperate climate with summer temperatures from 8 to 20 °C and an average rainfall of 600 mm during the grazing season. In each farm, cattle graze on a free choice pasture and lactating cows receive cereal based concentrates (crude protein 100–180 g/kg DM; starch 350–500 g/kg DM; NDF 100–200 g/kg DM) as energy supplement according to their milk yield. The daily amount of concentrate is provided in two meals at each milking time.

With the aim of covering a wide range of concentrate supplementation levels, the faecal sampling protocol considered 28 alpine farms: 10 farms were sampled in the year 2011 and 18 farms in 2012. In each farm, the average amount of concentrate provided to the lactating cows was recorded and compared with the legal quantity set by the regional law as 20% of their total energy requirement (maintenance + lactation). Maintenance requirement was calculated using INRA (1988) equations based on cows average metabolic weight and this value, expressed as *unite fourragère lait* (UFL), was increased by +10%; 20%, or 30% as additional walking expense if pasture slope was <100 m; between 101 and 200 m or >200 m, respectively. Lactation requirement was calculated according to INRA (1988) considering the average 4% fat-corrected milk yield of the herd at the onset of the grazing season. A reference standard concentrate that provides 1 UFL/kg of DM was used to calculate the legal amount of concentrate. Faecal samples were collected on the first week of July after 30 days of grazing by grab sampling technique during evening milking. A minimum of 5 lactating cows were sampled in each farm collecting 80 samples in 2011 and 73 samples in 2012 and these two datasets were identified as Dataset 1 and 2. Faecal samples (from 500 to 1000 g of wet material per cow) were stored at −20 °C prior to their analysis. After thawing, samples chemical composition was determined by standard methods (AOAC, 2000), using the following procedures: DM-934.01, ash-942.05, crude protein-954.01, ether extract-920.39, and starch-996.11. Neutral detergent fibre (aNDF) was determined with heat-stable amylase according to Van Soest et al. (1991). Acid detergent fibre and lignin(sa) were determined as described by Robertson and Van Soest (1981). Above fibre analysis were performed using Ankom^{220} Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA). Prior to NIRS analysis, faecal samples were oven-dried at 60 °C for 48 h and then ground at 0.5 mm with Universal Cutting Mill Pulverisette19 (Fritsch GmbH, Idar-Oberstain, Germany). Ground samples were placed in a 50 mm diameter ring cups with quartz lens and scanned in duplicate in the region between 1100 and 2500 nm at 2 nm intervals using a NIRSystem MODEL5000 scanning NIR spectrometer (Silver Spring, MD, USA) in reflectance mode. Each spectrum is the average of 32 multiple scans. Reflectance (R) data were converted into absorbance (A) data through $A = \log(1/R)$. WinISI II version 1.5 (Infrasoft International LLC, State College, PA, USA) was used to acquire spectral data.

2.2. Statistical analysis

Chemical data of individual faecal samples were subjected to ANOVA using PROC-MIXED of SAS/STAT® 9.2. (SAS Inst. Inc., Cary, NC, USA). The model considered the fixed effects of concentrate supplementation (2 levels: ≤20% and >20% of total requirement) and year of sampling and the random effect of farm.

All statistical analysis of spectral data were performed either with the same package software or using MATLAB 8.0 and Statistics Toolbox 8.1 (The MathWorks, Inc., Natick, MA, USA) and *in-house* developed codes. As an exploratory tool, spectral data of samples belonging to Dataset 1 were subjected to a principal component analysis (PCA). Dataset 2 spectra were