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in

Ruiz R. (ed.), López-Francos A. (ed.), López Marco L. (ed.).
Innovation for sustainability in sheep and goats

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 123

2019
pages 411-414

Fluorescence spectroscopy coupled with factorial discriminant analysis as a tool to identify sheep milk from different feeding systems

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Abstract. The present study aimed to determine the potential of Front face fluorescence spectroscopy (FFFS) to discriminate between milk samples from Sicilo-Sarde ewes fed three different diets – named control, faba bean meal and soybean meal - throughout the lactation stage (11 weeks). Milk samples were classified by factorial discriminant analysis (FDA). Similar results were obtained by separately applying (FDA) to different target nutrients (aromatic amino acids and nucleic acids (AAA + NA), tryptophan, vitamin A and riboflavin). In a second step, concatenation technique was applied to FFFS spectra. Results showed a good discrimination between milk samples from different lactation periods and diet compositions. Spectroscopic techniques may provide useful fingerprints, and allow the identification of milk samples from ewes fed different diets throughout the lactation periods.

Keywords. Ewe's milk – Lactation – Soybean meal – Faba bean – Front Face Fluorescence Spectroscopy – Factorial discriminant analysis – Concatenation.

La Spectroscopie de Fluorescence Frontale couplée à l'analyse factorielle discriminante pour identifier le lait des brebis alimentées de différents régimes alimentaires

Résumé. *L'objet de la présente étude est de tester le potentiel de la spectroscopie de fluorescence frontale (SFF) à discriminer le lait des brebis Sicilo-Sarde selon la nature de la source azotée (Tourteau de Soja ou féverole) utilisée en complementation et ce durant les onze premières semaines de lactation. Afin de déterminer le pouvoir discriminant des données de fluorescence, des AFD (analyse factoriel discriminante) ont été réalisées sur de spectres des quatre sondes intrinsèques utilisées (Trp, AAA et AN, Ribolavine et vitamine A). Une meilleure discrimination des laits, selon la nature du concentré et le stade de lactation a été obtenue en étudiant conjointement les différentes régions spectrales. Cette analyse combinée a été réalisée en utilisant la technique de concatenation. Les résultats obtenus ont montré que les techniques spectroscopiques peuvent fournir des empreintes digitales utiles et permettre l'identification d'échantillons de lait selon la nature du concentré utilisé en complementation des brebis durant la période de lactation.*

Mots-clés. *Brebis – Lactation – Tourteau de Soja – Féverole – Spectroscopie de Fluorescence Frontale – Analyse Factorielle Discriminante – Concatenation.*

I – Introduction

In Tunisia, Sicilo-Sarde dairy sheep feeding is based on grass grazing and forage, as a basal diet, and concentrate complementation all through the year (Rouissi *et al*. 2008). However, during the last years, the worldwide overall economic situation resulted in an increase in the price of raw materials used for livestock concentrate formulation (corn and soya). In this scenario, the search for other alternatives such as local food resources (barley, faba bean, etc) is still imperative. Milk con-

tains several intrinsic fluorophores, which represent the most important area of fluorescence spectroscopy. In the last years several authors (Karoui *et al*., 2005; Boubellouta and Dufour, 2008) used the spectroscopy of fluorescence tool to discriminate between milk and between the dairy products of various origins and / or from sheep having received various treatments. The objective of this study was to evaluate the potential of Front face fluorescence spectroscopy (FFFS) coupled with chemometric tools to discriminate between milk samples from Sicilo-Sarde dairy ewes fed different diets during the milking period.

II – Materials and methods

1. Animals

Forty-five Sicilo - Sarde ewes were divided into three homogenous groups according to their weight (51.3 ± 4.9 kg for the control (C) ; 51.9 ± 4.9 kg for the soybean meal (S) and 52 ± 5.4 kg for the faba bean meal (F), the litter size :1.47 ± 0.5 for (C) ; 1.4 ± 0.5 for (S) and 1.5 ± 0.5 for (F) and the rank of lactation : 2.4 ± 0.9, 2.6 ± 0.9and 2.6 ± 0.8 for the control, soybean and faba bean groups, respectively).

2. Diets

Ewes received oat hay at 1.5 kg dry matter (DM)/ewe/day, and each group of animals was supplemented with 500g / ewe / day of one of three iso – energetic and iso – proteic concentrates, named control, soybean meal and faba bean meal – diets, during the lactation period (11 weeks). Ingredients (%) and chemical composition (% DM) of concentrates and roughage are presented in Table 1.

Table 1. Ingredient proportions and chemical composition of aliments (% Dry Matter)

Concentrates	Control	Soy bean meal	Faba bean meal	Oat hay
Ingredients (%)				
Barley	35	82.5	71.5	–
Corn	30	–	–	–
Soybean meal	15	13.5	7	–
Faba bean meal	–	–	17.5	–
Wheat bran	15	–	–	–
VMC sheep*	5	4	4	–
Chemical composition				
Dry matter (%)	90	89	89	84
Organic matter	93.6	88.9	92.7	92.2
Crude protein	15.8	16.8	16.2	5.2
Crude fiber	5.1	9.4	7.6	39.7

* VCM: Vitamin-mineral complex.

3. Sampling and preservation of milk

Individual milk yield was recorded one day a week on one milking during the whole milking period (11 weeks). For each group, the milk samples collected from the different ewes (n = 15 per group) were mixed and an aliquot of 100 ml was taken and kept in a freezer at - 20 °C until analyses. Before each analysis, milk samples were thawed during one night at + 4 °C in a refrigerator. All the analyses were made in triplicate (n = 11 weeks x 3 repetitions = 33 analyses).

4. Fluorescence spectroscopy

Fluorescence spectra were recorded using a FluoroMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France). The incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation and depolarisation phenomena were minimised. For each milk sample, three spectra were recorded.

5. Mathematical analysis of data

In a first step, Principal Components Analysis (PCA) was applied to the normalised spectra to investigate differences between the samples. In a second step, Factorial Discriminant Analysis (FDA) was performed on the first 10 Principal Components (PCs) resulting from the PCA applied to the fluorescence spectral data. Finally, the first 10 PCs of the PCA performed on each data set were pooled into one matrix and this new table was analysed by FDA. Chemometric analyses were performed in MATLAB (The Mathworks Inc., Natic, MA).

III – Results and discussion

The emission spectra (400 – 640 nm) of riboflavin are depicted in Fig. 1. Interestingly, Fig. 1 showed two spectral regions, being the broad peak at about 520 nm due to riboflavin (Miquel Becker *et al.*, 2003). A difference in the fluorescence intensity at 520 nm was observed between control diet and treatments (milk from ewes fed soybean or faba bean meals). In addition, milk collected from ewes fed faba bean appeared to be less oxidised than that collected from ewes fed soybean meal. That might be due to the presence of antioxidants (e.g. tannins) present only in milk collected from ewes fed faba bean meal. The emission spectra of aromatic amino acids and nucleic acids (AAA + NA), and tryptophan recorded after excitation at 250 and 290 nm (data not shown) presented very similar shapes and can, therefore, hardly be distinguished visually.

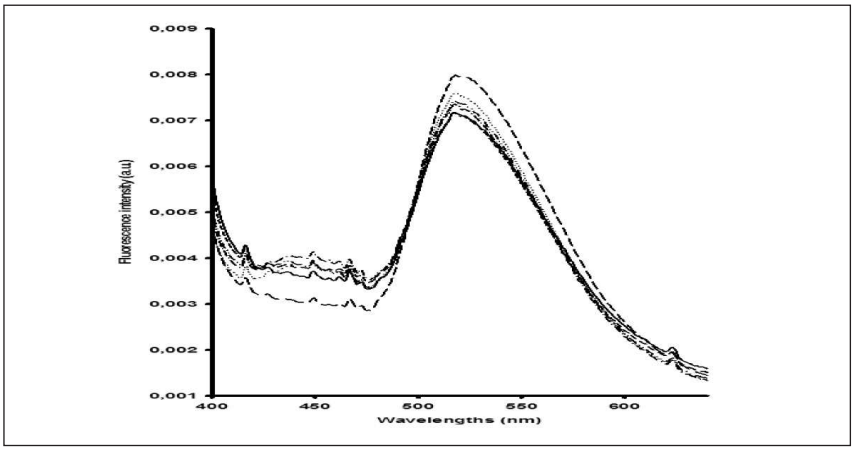

Fig. 1. Normalised fluorescence emission spectra of riboflavin recorded following excitation at 380 nm on Sicilo- Sarde ewe's milk fed faba bean after 1 (—) and 11 (...) weeks of lactation, soybean after 1 (- - -) and 11 (—..—..) weeks of lactation, and control diet after 1 (— —) and 11 (— ··· —) weeks of lactation.

In order to compare the results obtained from different intrinsic probes (target nutrients), concatenation technique was applied to AAA + NA, tryptophan, vitamin A and riboflavin spectra. The resultant similarity map is illustrated in Fig. 2. The similarity map allowed a good discrimination of milk samples according to the feeding system. Indeed, the three groups were well discriminated: milk from ewes fed the control diet gave negative scores according to the discriminant factor 1, and positive scores according to discriminant factor 2; milk samples from ewes fed faba bean meal had negative scores according to both discriminant factors 1 and 2; finally, milk samples from ewes fed soybean meal exhibited positive scores according to discriminant factor 1 and scores close to zero according to discriminant factor 2.

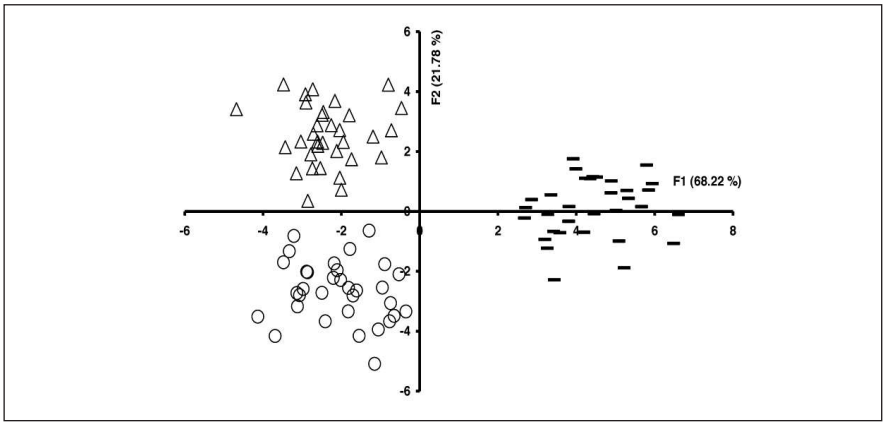

Fig. 2. Discriminant analysis similarity map determined by discriminant factors 1 (F1) and 2 (F2). Factorial Discriminant Analysis was performed on the 40 concatenated Principal Components corresponding to the Principal Components Analysis performed on the emission spectra of aromatic amino acids and nucleic acids, tryptophan, riboflavin and vitamin A of milk from Sicilo-Sarde ewes fed faba bean meal (o), soy bean meal (-) or a control diet (Δ).

IV – Conclusions

The results obtained in this study demonstrated that front-face fluorescence spectroscopy in combination with chemometrics can be considered as a fast, non-destructive and innovative technique to differentiate between milk samples from ewes fed different diets. The concatenation technique of the aromatic amino acids and nucleic acids (AAA + NA), tryptophan, vitamin A and riboflavin spectra allowed a good discrimination of milk from ewes fed different diets.

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