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in

Acar Z. (ed.), López-Francos A. (ed.), Porqueddu C. (ed.).
New approaches for grassland research in a context of climate and socio-economic changes

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 102

2012
pages 457-460

Use of front face fluorescence spectroscopy to identify sheep milk from different feeding diets

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Abstract. The present study aimed to determine the potential of front face fluorescence spectroscopy (FFFS) to discriminate between milk samples belonging to Sicilo-Sarde ewes fed with three different feeding groups –named control, scotch bean meal and soybean meal– throughout the lactation stage (11 weeks). Milk samples were classified by factorial discriminant analysis. Similar results were obtained by separately applying factorial discriminant analysis (FDA) on each intrinsic probe [aromatic amino acids and nucleic acids (AAA + NA), tryptophan, vitamin A and riboflavin]. In a second step, concatenation technique was applied to FFF spectra. Results obtained showed a good discrimination among milk samples with regard to lactation periods and diet compositions. These results showed that spectroscopic techniques may provide useful fingerprints and allow the identification of milk samples according to the feeding systems given to the ewes throughout the lactation periods.

Keywords. Ewe's milk – Lactation – Soybean meal – Broad bean – Front face fluorescence spectroscopy – Factorial discriminant analysis – Concatenation.

Utilisation de la spectroscopie de fluorescence frontale 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 sja ou féverole) utilisée en complémentation et ce durant les onze premières semaines de lactation. Afin de déterminer le pouvoir discriminant des données de fluorescence, des AFD ont été réalisées sur de spectres des quatre sondes intrinsèques utilisées (Trp, AAA et AN, riboflavine et vitamine A). Une meilleure discrimination des laits, selon la nature du concentre 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 concaténation. 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 complémentation des brebis durant la période de lactation.*

Mots-clés. *Brebis – Lactation – Tourteau de Soja – Féverole – Spectroscopie de fluorescence frontale – Analyse factorielle discriminante – Concaténation.*

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 way, the search for other alternatives such as their entire or partial replacement by local food resources (barley, faba bean…) is still imperative. Milk contain several intrinsic fluorophores, which represent the most important area of fluorescence spectroscopy .These 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 having sudden various treatments. The objective of this study was to evaluate the effect of the partial substitution of faba bean for soya in concentrate formulation on milk production by Sicilo-Sarde dairy ewes during the suckling period using front face fluorescence spectroscopy coupled with chemometric tools.

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 scotch 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 scotch bean groups, respectively.

2. Diets

Ewes received a ration commun base (oat hay) at 1.5 kg DM/ewe/day and each group of animals was supplemented by a 500g / ewe / day of one of three iso-energetic and similar protein concentrates named: control, soybean rich and scotch bean rich diets during the lactation period (11 weeks). Ingredients (%) and chemical composition (% DM) of concentrates and roughage are presented inTable 1.

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

	Concentrates			Oat hay
	Control	Soy bean meal	Scotch bean meal	
Ingredients (%)				
Barley	35	82.5	71.5	-
Corn	30	-	-	-
Soybean meal	15	13.5	7	-
Scotch bean meal	-	-	17.5	-
Wheat bran	15	-	-	-
VMC sheep	5	4	4	-
Chemical composition				
DM (%)	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

3. Sampling and preservation of milk

Individual milk yield was recorded one day a week on one milking during the whole suckling 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 analyses, milk samples were thawed during one night at +4°C in a refrigerator. All the analyses were made in triplicate (n = 11 samples 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 Component 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 spectra are depicted in Fig. 1. Interestingly, Fig. 1 showed two spectral regions: the broad peak at about 520 nm is due to riboflavin as has been reported by Miquel Becker *et al.* (2003). A difference in the fluorescence intensity at 520 nm was observed among controls and treatments (ewe's milk fed with soybean or scotch bean meals). In addition, milks collected from ewes fed scotch bean appeared to be less oxidised than those collected from ewes fed soybean meal during the studied lactation periods. That could be due to the presence of antioxidants (e.g. tannins) present only in milk collected from ewes fed scotch bean meal. The emission spectra of AAA + NA and tryptophan recorded after excitation at 250 and 290 nm (data not shown). As most of the spectra presented very similar shapes and can, therefore, visually hardly be distinguished.

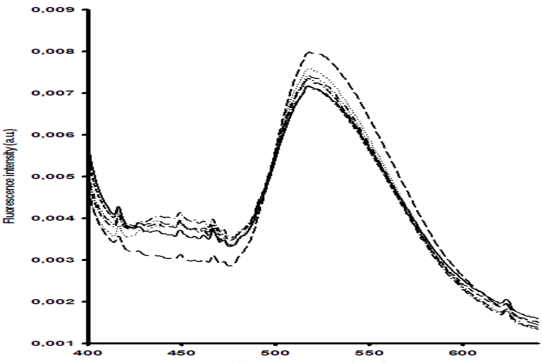

Fig. 1. Normalised fluorescence emission spectra of riboflavin fluorescence spectra recorded following excitation at 380 nm on Sicilo- Sarde ewe's milk fed scotch bean after 1 (—), 11 (…), soybean after 1 (– – –), 11 (—.·—..) or control after 1 (— —) and 11 (— ···—) weeks of lactation, respectively.

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

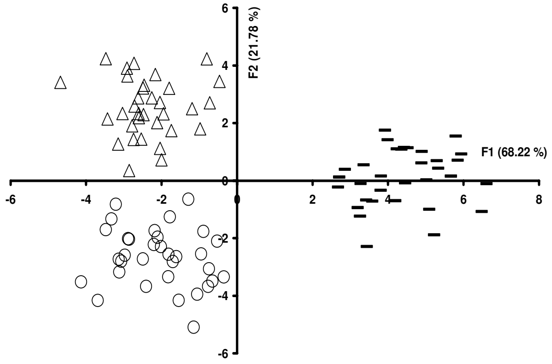

Fig. 2. Discriminant analysis similarity map determined by discriminant factors 1 (F1) and 2 (F2). (FDA) was performed on the 40 concatenated PCs corresponding to the PCA performed on the emission spectra of aromatic amino acids and nucleic acids, tryptophan fluorescence spectra , riboflavin and vitamin A fluorescence spectra of Sicilo-Sarde ewe's milk fed scotch bean meal (o), soy bean meal (–) or control (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 ewe's milk samples originating from different feeding systems. The concatenation technique of the aromatic amino acids and nucleic acids (AAA + NA), tryptophan, vitamin A and riboflavin acquired by using FFF technique allowed a good discrimination of ewe's milk from the different feeding system.

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