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Traceability of pasture feeding using some fatty acids and spectrophotometric parameters in milk

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ABSTRACT – Seventy-three cow milk individual samples deriving from seven farms with different feeding system (pasture, P *vs.* stall, S), were collected with the aim to trace pasture feeding from different milk characteristics. Samples were analyzed for: linoleic and linolenic acids, colour parameters (L^* , b^* , a^* C and H) using a Minolta CM-2002 spectrophotometer; the integral value of reflectance spectrum between 530 and 450 nm was also calculated. The linoleic/linolenic acids ratio resulted significantly lower in P group ($P < 0.001$), whereas yellowness (b^*) and the absolute integral value resulted significantly higher in P group ($P < 0.001$). Linoleic/linolenic acids ratio allowed the complete discrimination between feeding groups, whereas the integral value and yellowness allowed only a partial discrimination (respectively 90.4% and 79.5% of cases). However, as milk fatty acid composition is strongly dependent on fat intake, any additional fat inclusion in the ration can modify it, apart from pasture presence in the diet. On the contrary integral value and yellowness are strongly related to carotenoids pigments, recognized as biomarkers of fresh herbage in the diet. For these reasons, integrating linoleic/linolenic ratio and colour parameters in a principal components analysis, a complete discrimination between pasture and stall feeding was obtained, with a greater reliability of results due to the combination of milk characteristics associated to different sources of variation (fats intake and carotenoids intake).

Key words: Cow milk, Carotenoids, Fatty acid, Tracer techniques.

Introduction – It has been recognized that the possibility to discriminate between products deriving from pasture or stall-fed animals can increase the consumers awareness regarding food “healthiness” (Dian *et al.*, 2007). Fatty acid composition could represent a useful tool to trace grass feeding in animal products. In particular linoleic to linolenic acids ratio results lower in products deriving from animals fed with grass than with concentrate based diets (French *et al.*, 2000; Wood and Enser, 1997). Carotenoids can be considered biomarkers of pasture in the diet of ruminants: Prache e Theriez (1999) used the effect of carotenoid pigments on reflectance spectrum of adipose tissue to discriminate between carcasses deriving from pasture or stall fed lambs. Then, Prache *et al.* (2002) and Priolo *et al.* (2003) respectively on bovine and ovine milk, used this method also considering the colour parameters (CIEL* a^* b^*). The aim of our study was to test if a combination of different parameters such as linoleic to linolenic acids ratio, reflectance spectrum and colour parameters can discriminate milk deriving from two different feeding systems (pasture *vs.* stall).

Material and methods - The trial was conducted collecting 73 milk individual samples deriving from 7 bovine Sicilian farms. In three farms (31 cows) the animals were fed at pasture for 3-4 hours per day and supplemented with hay, straw and concentrate (P group). In the other four farms (42

cows) the animals received a total mixed ration based on maize silage, hay, straw and concentrate (S group). Milk fatty acid composition was determined by gas chromatographic analysis (Chouinard *et al.*, 1999). The reflectance spectrum of all milk samples was measured at wavelengths between 700 and 400 nm, using a MINOLTA CM-2002 spectrophotometer (D65 illuminant, observer angle 10°); moreover the following color coordinates were measured: lightness (L*), redness (a*) and yellowness (b*) in the CIELAB uniform color space (CIE, 1986). Hue angle (H*) and chroma (C*) were also recorded. The reflectance spectrum (R) between 530 and 450 nm was translated to make reflectance value at 530 nm equal to zero (TR) according to Prache and Theriez (1999), modified by Prache *et al.* (2002) for milk samples. On the translated spectrum, the integral value was calculated as follows: $I_{450-530, TR.nm} = [(TR450/2) + TR460 + TR470 + TR480 + TR490 + TR500 + TR510 + TR520 + (TR 530/2)] \times 10$. All parameters underwent to a one-way analysis of variance to test the feeding system effect. A principal component analysis and a canonical discriminant analysis were performed to highlight if a combination of different parameters can discriminate milk deriving from the two different feeding systems.

Results and conclusions – The linoleic/linolenic acids ratio resulted significantly lower in P group (2.54 ± 0.66 vs. 7.19 ± 1.24 ; $P < 0.001$), as previously found by other authors (White *et al.*, 2001; Boken *et al.*, 2005). Indeed, this ratio allowed the complete discrimination of cases between feeding groups. However, taking into account that milk fatty acid composition is strongly dependent on fat intake, any protected fat inclusion in the ration can modify its value, apart from

pasture presence in the diet. For this reason this ratio, alone, seems to be unsuitable to recognize the pasture presence in the diet from milk samples obtained in different feeding conditions.

The integral values ($I_{450-530}$) resulted always negative and therefore will be reported as absolute values. A higher absolute value would reveal a greater carotenoids concentration, as a consequence of the presence of fresh herbage in the diet. Milk from pasture feeding showed a higher integral value, compared to milk from stall feeding (191.5 ± 64.5 vs. 70.7 ± 36.1 ; $P < 0.001$) as reported by Prache *et al.* (2002) and Priolo *et al.* (2003) in bovine and ovine milk. Separating the integral values in classes reported in figure 1 it is possible to highlight that the 100% of data higher than 151 derives from pasture, whereas values lower than 151 derive mainly, but not completely, from stall feeding. Priolo *et al.* (2002) in ovine milk found a similar trend, but with a threshold value equal to 250, probably due to the different species studied.

The integral value correctly classified only the 90.4% of milk. Similarly, Prache and Theriez (1999) and Priolo *et al.* (2002) discriminated only, respectively 81% and 78.1% of their samples. Table 1 reports the colour parameters. Yellowness (b*) resulted significantly higher in P group according to Priolo *et al.* (2003) in ovine milk, as a consequence of a greater carotenoids concentration in milk. However, also in this case, b* did not allow a complete discrimination between the two feeding systems, correctly classifying only the 79.5% of samples. Similar results were obtained with the other colorimetric parameters. Combining the variables $I_{450-530}$, L*, a*, b*, C, H and linoleic/linolenic acids ratio through the analysis of the principal components (PC1 e PC2 explaining the 90% of total variability) we obtained a complete separation between milk samples from pasture and from stall feeding (Figure 2). Canonical discriminant analysis confirms this separation, resulting in a highly significant ($P < 0.001$) Mahalanobis distance between groups. In conclusion, the possibility to discriminate the feeding origin of a milk

Table 1. Colour parameters of milk from different feeding system (means \pm standard deviation).

		P	S	P
Lightness	L*	70.61 \pm 1.79	70.69 \pm 1.74	NS
Redness	a*	-1.89 \pm 0.49	-2.10 \pm 0.38	*
Yellowness	b*	2.84 \pm 1.38	0.77 \pm 1.15	**
Chroma	C	3.53 \pm 1.01	2.48 \pm 0.31	**
Hue angle	H	129.56 \pm 93.39	161.70 \pm 123.13	**

NS=not significant; *= $P < 0.05$; **= $P < 0.001$.

Figure 1. Distribution in the classes of absolute value of the integral of the translated reflectance spectrum of milk

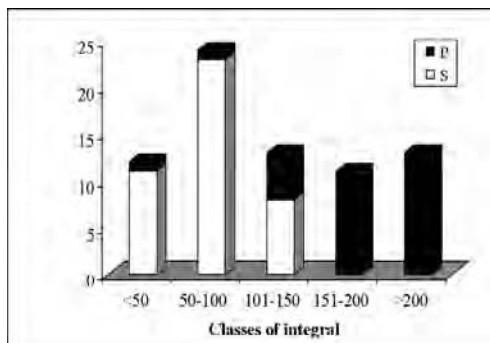
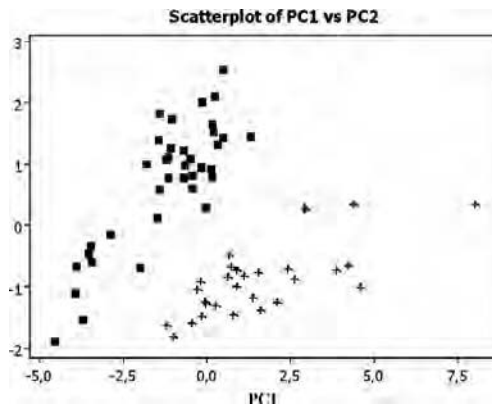


Figure 2. Scatter plot between the two Principal Component (PC1 and PC2) for feeding groups: S (■), P (+).



sample seems to be improved by a combination of parameters such as linoleic to linolenic ratio, colour parameters and reflectance spectrum. The robustness of these parameters integration in recognizing the pasture presence in the diet from milk samples, derives from the different discriminating causes (feeds fat composition and intake or carotenoid pigments intake) that render the system more generalizable than a system based only on a single parameter.

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