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# Fat colour, a traceability parameter of grass feeding in lambs

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**SUMMARY –** Fifty Rasa Aragonesa light male lambs were submitted to one of the following four experimental treatments: Grazing (Gr), Grazing with supplement (Gr+S), Indoor (Ind) and Indoor-Grazing (Ind-Gr). The aim of this study was the use of fat colour to trace the different feeding systems, a specially grazing-fed *vs* indoors-fed. Muscle *L. thoracis* and perirenal fat colours in the CIELab space were measured with a spectrophotometer CM 2600d and the integral of the translated spectrum, as fat absolute value, was calculated. Data values were subjected to analysis of variance, a discriminant analysis, and a binary logistic regression. Meat from lambs reared under indoor systems showed a lighter colour and a sharp blooming 24 hours later. Fat from forage-fed lambs was lighter and more yellow. Discriminant analysis was able to classify them with 55% accuracy in the four treatments. However, it is confused when the classification must be done within feeding systems (Gr *vs* Gr+S and Ind *vs* Ind-Gr). A logistic regression classified 100% of lambs into the two feeding systems. Therefore, this regression can be used in on-line classification.

**Keywords:** Grazing, meat, fat, colour.

***RESUME –*** *"La couleur du gras, un paramètre de traçabilité chez les agneaux broutardes". Cinquante agneaux mâles de poids léger et de race Rasa Aragonesa ont été soumis à un des quatre traitements suivants: Pâturage (Gr), Pâturage supplémenté (Gr+S), Stabulation (Ind), et Stabulation-Pâturage (Ind-Gr). L'objectif de ce travail a été l'utilisation de la couleur de la graisse pour tracer les différents systèmes d'engraissement: Pâturage* vs *Concentré. La couleur du muscle* L. thoracis *et de la graisse périrénale a été mesurée au CIELab espace avec un spectrophotomètre CM 2600d, et on a calculé la valeur de l'intégrale du spectre transporté comme étant la valeur absolue de la graisse. Les données ont été traitées avec les analyses de variance et discriminante, et la régression logistique binaire. La viande d'agneaux élevés en stabulation était plus claire et avait une nette couleur rouge 24 heures après. La graisse des agneaux soumis au pâturage était plus lumineuse et jaune. L'analyse discriminante avait la capacité de différencier avec 54% de réussite entre les quatre traitements. Cependant, la classification a été confuse dans les systèmes d'alimentation (Gr* vs *Gr+S et Ind* vs *Ind-Gr). Une régression logistique arrive à classifier 100% des animaux par systèmes d'alimentation. Par conséquent, cette régression peut être utilisée pour la classification "on line".*

***Mots-clés :*** *Pâturage, viande, graisse, couleur.*

## Introduction

Meat from grass-fed lambs is different from concentrate-fed lambs in chemical composition, especially fatty acids (Wood *et al*., 2003), flavour (Sañudo *et al*., 2000), tenderness and colour (Coulon and Priolo, 2002). These differences are more pronounced according to the feed management before slaughtering (Priolo *et al*., 2002). Now, consumers demand increasing clear information about the feeding system and food supply used to rear meat animals, specially that included in "labelled category", as Organic meat or Protected Geographical Indications. The possibility to trace food supply is important to guarantee that producers respect the feeding conditions of the labelled meat sold at the market. Carotenoids are characteristic micronutrients of plants and their presence in animal tissue means green herbage intake. These pigments are stored in fat tissues and can be measured by colorimetric method according to Prache and Theriez (1999). Therefore, the content of this parameter can be a good marker to predict grass feeding.

The aim of this study is to measure the effect of green forage on meat and fat colour parameters as well as evaluate whether the colour of fat tissue can be used to discriminate between lamb meats obtained in different production systems, specially grazing-fed *vs* indoor-fed.

## Material and methods

Fifty Rasa Aragonesa male lambs were used in this study. Lambs were randomly allocated to one of four experimental treatments: (i) Grazing (Gr), ewes and lambs (n = 13) remained at alfalfa meadows and lambs did not have access to concentrate; (ii) Grazing with supplement (Gr+S), ewes and lambs (n = 12) remained at alfalfa meadows and lambs had *ad libitum* access to concentrate; (iii) Indoor (Ind), ewes were permanently inside with *ad libitum* access to a total mixed ration and lambs (n = 13) fed with ewe milk and concentrate *ad libitum*; and (iv) Indoor-Grazing (Ind-Gr), ewes grazing at grass meadows from 08:00 h to 16:30 h and remaining indoors with lambs (n = 12) the remaining time. Lambs were fed with ewe milk and concentrate *ad libitum*. Lambs from Ind and Ind-Gr treatment were weaned at 41±3.96 days old, whereas lambs from Gr+S and Gr lots remained with their dams in meadows until slaughtering. Concentrate offered had 175 g CP/kg DM and 1.36 UFL/kg DM. Lambs were slaughtered when they reached 22-24 kg live-weight according to the specifications of Ternasco de Aragón Protected Geographical Indication (Regulation (EC) No. 1107/96). Carcasses were chilled 24 hours at 6ºC and then pH was measured at *M. Longissimus lumborum* ($4^{th}$ vertebral region) with pH-meter equipped with a Crison 507 penetrating electrode.

Samples of *M. Longissimus thoracis* were taken from the 11-13 ribs to measure the muscle colour at cutting time and after 24 h of air exposure. These samples were overwrapped with oxygen-permeable film without contact with meat surface, and stored at 4ºC in absolute darkness. Values were recorded on two locations randomly selected from the cranial surface of each piece to obtain a representative mean value. Colour was measured using a spectrophotometer Minolta CM-2600d in the CIELAB space (CIE, Commission Internationale de L'Eclairage, 1976) with 8 mm measured area diameter, specular component included and 0% UV, standard illuminant D65 that simulates day light (colour temperature 6504 K), angle 10º and zero observer and white calibration. The lightness (L*), redness (a*) and yellowness (b*) were recorded, and hue angle (h*) and saturation index (C*) were calculated as h* = arctan (b*/a*)*57.29 and $C^* = \sqrt{a^{*2} + b^{*2}}$ (Albertí *et al.*, 2005; Boccard *et al.*, 1981). Higher saturation indexes represent a more vivid colour and denote lack of greyness (Miltenburg *et al.*, 1992) and hue angle refers to the gradation of pure colour from red through yellow, green and blue, determined by the predominant wavelength of light. The spectral difference between the reflection values at 630 nm and 580 nm ($R_{630-580}$) was used as an index of oxymyoglobin content on the meat surface (Renerre, 1982) and its relation with meat visual acceptability (Renerre and Mazuel, 1985) with greater values indicating a more desirable red colour.

Fat colour was determined in perirenal (PR) area 24 hours *post-mortem*. Each colour values were recorded at three locations randomly selected avoiding blood blots, discolorations, and less covered areas. Besides trichromatic coordinates, the proportion of reflected light every 10 nm between 450 and 510 nm was collected and the absolute value from the integral of the translated spectrum (Sum) was calculated according to Priolo *et al*. (2002).

Data values were subjected to analysis of variance using the GLM procedure of SAS (2005) to evaluate the effect of management system, time of oxygenation and its interactions. A forward stepwise discriminant was made for perirenal fat with L*, a*, b*, C*, h* and Sum as evaluated variables. The same variables of perirenal fat were included in a forward stepwise binary logistic regression.

## Results and discussion

Values of pH were not affected by system management, with 5.5±0.08 mean values. System management only had a significant effect (P<0.05) on muscle *L. thoracis* on lightness (L*), while there was no effect on redness, yellowness, chroma, hue and $R_{630-580}$ (P>0.05) (Table 1). Oxygenation time had an effect of increasing values in most of the colour parameters, like L* and h* (Fig. 1), except for a* values in Gr (from 11.71 to 11.42) and Gr+S (from 11.22 to 11.94), while Ind and Ind-Gr increased meat redness from 9.61 to 12.05 and from 9.38 to 12.31, respectively.

As Fig. 1 shows, the L* values of both indoor treatments (Ind and Ind-Gr) were always greater than the grazing groups (Gr and Gr+S), at each time of measurement. Twenty-four hours after cutting time, L* and h* values were higher than at cutting, the behaviour being similar between main groups of treatment, (feeding system grazing *vs* indoor).

Table 1. Effects of management system and oxygenation time and their interaction on lamb *Longissimus dorsi* colour (*F* values)

| | Lightness (L*) | Redness (a*) | Yellowness (b*) | Chroma (C*) | Hue (h*) | $R_{630-580}$ |
|---|---|---|---|---|---|---|
| Management system | 6.12** | 1.79ns | 0.07ns | 0.76ns | 0.67ns | 0.26* |
| Time | 126.02*** | 35.40*** | 156.46*** | 117.67*** | 73.84*** | 0.20ns |
| System x time | 0.13ns | 9.54*** | 1.45ns | 4.27* | 1.40ns | 0.04* |

ns: no significant; *:P<0.05; **P<0.01; ***P<0.001.

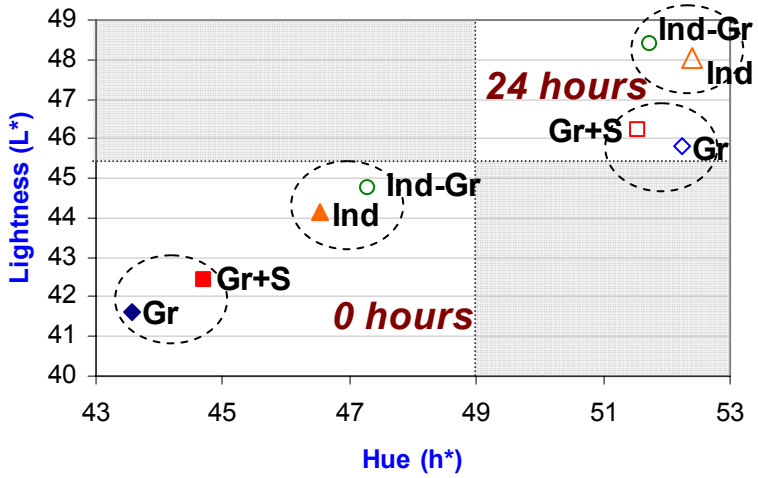


Fig. 1. Relation of lightness and hue values, at cutting time (0 hours) and 24h later, of *L. thoracis* from lambs submitted to four treatments: grazing (Gr), grazing and supplementation (Gr+S), indoor (Ind) and indoor with ewes grazing 8 hours (Ind-Gr).

Significant interactions (P<0.05) were found between time of oxygenation and management system for $R_{630-580}$ .The acceptability of *L. thoracis* at cutting time was similar in all treatments. However, 24 hours later, meat from Gr treatment had a significant lower value of $R_{630-580}$ (P<0.05) than Ind and Ind+Gr (Fig. 2). As in redness values, the acceptability of both indoor treatments increases with time, while Gr group decreases and Gr+S did not change with increasing time. The low values of $R_{630-580}$ in meat from grazing systems (Gr and Gr+S) may be due to lower conversion from myoglobin to oxymioglobin in grazing groups due to grass antioxidants.

Values of perirenal fat colour are showed in Table 2. Management system had an effect (P<0.05) on L* and C*. Yellowness (b*) and Sum values are significantly (P<0.001) greater in grazing systems. Gr and Gr+S had lighter, more yellow and saturated fat colour than Ind and Ind-Gr, although Gr had not significant differences with any group in L*. There was a significant effect of feeding management on Sum, with absolute values greater when lambs were grass-fed (Gr and Gr+S) than indoor-fed (Ind and Ind-Gr) as a result of the greater area included between the abscissas axis and the translated spectra (Fig. 3), which agrees with Priolo *et al.* (2002).

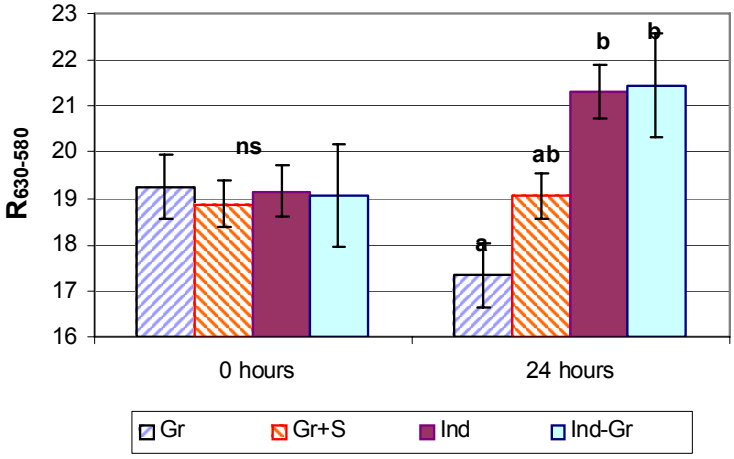


Fig. 2. Acceptability of *L. thoracis* from lambs submitted to four treatments: grazing (Gr), grazing and supplementation (Gr+S), indoor (Ind) and indoor with ewes grazing 8 hours (Ind-Gr).

Table 2. Perirenal fat parameters colour and value of the translated spectrum (Sum) integral

| | Gr | Gr+S | Ind | Ind-Gr | F value |
|---|---|---|---|---|---|
| L* | 75.38$^{ab}$ | 77.33$^{a}$ | 72.81$^{b}$ | 74.19$^{b}$ | 3.99* |
| a* | 3.02 | 3.29 | 2.61 | 2.57 | 0.85ns |
| b* | 13.29$^{a}$ | 13.48$^{a}$ | 10.51$^{b}$ | 10.59$^{b}$ | 7.46*** |
| C* | 13.68$^{a}$ | 13.92$^{a}$ | 10.88$^{b}$ | 10.93$^{b}$ | 6.89** |
| h* | 77.40 | 76.90 | 75.84 | 76.72 | 0.21ns |
| Sum | 253.28$^{a}$ | 257.00$^{a}$ | 110.28$^{b}$ | 118.91$^{b}$ | 23.86*** |

Different superscript in a row means significant differences.
ns: no significant; *P<0.05; **P<0.01; ***P<0.001.

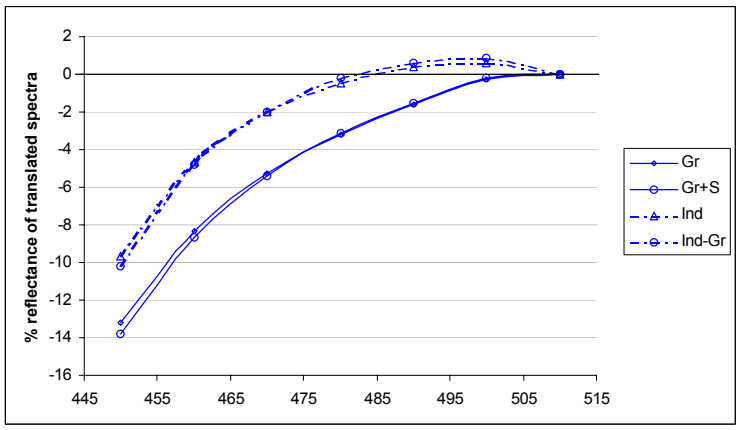


Fig. 3. Reflectance spectrum of perirenal fat at 24 hours *post mortem*.

When a discriminant analysis was made for fat variables, Sum and Chroma were selected to classify correctly 54% of the cases (Table 3). The analysis is able to discriminate between grass-fed

lambs (Gr and Gr+S) and indoor-fed ones (Ind and Ind-Gr). However, it is confuse when the classification must be done within one of the two main groups of treatments: grazing (Gr *vs.* Gr+S) and indoor (Ind *vs*. Ind-Gr). In addition, a low percentage of cases of grazing group are erroneously classified in Ind-Gr group, probably due to the influence of grazing on ewes in this treatment.

Table 3. Discriminant classification (%) for perirenal fat

| Group | Foreseen membership | | | |
|---|---|---|---|---|
| | Gr | Gr+S | Ind | Ind-Gr |
| Gr | 53.8 | 30.8 | 0.0 | 15.4 |
| Gr+S | 41.7 | 50.0 | 0.0 | 8.3 |
| Ind | 0.0 | 0.0 | 46.2 | 53.8 |
| Ind-Gr | 8.3 | 0.0 | 50.0 | 41.7 |

However, when grass-fed and indoor-fed were regrouped into alfalfa-fed (A) or non-alfalfa-fed (NA), and the following logistic regression was applied to determine the probability of a lamb to belong to a NA treatment (PNA), 100% of lambs were correctly classified.

$$PNA = \frac{e^{-590.3-2.8\cdot Sum+8.4\cdot L^*+37.5\cdot b^*}}{1+e^{-590.3-2.8\cdot Sum+8.4\cdot L^*+37.5\cdot b^*}}$$

The use of spectrophotometers complemented with this kind of mathematical expressions is an easy ready-made on-line tool to trace grass fed systems.

## Conclusions

Meat from lambs reared under indoor systems (Ind and Ind+Gr) presented a lighter colour in both cases and had a sharp blooming 24 hours later. Fat from grass-fed lambs (Gr and Gr+S) had a lighter colour, more yellow than that from indoor systems. Colour parameter is a good tool to classify animals from indoor-fed to grass-fed. However, it does not obtain a reliable classification within each of the two feeding systems (grazing-fed *vs* indoor-fed)

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