Effect of electrical stimulation and other genetic and environmental factors on colour of lamb meat

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ABSTRACT

The research aimed to assess the effect of electrical stimulation, breed, sex, age at slaughter and rearing system on some colorimetric characteristics measured on the muscles Longissimus dorsi (LD), Gluteobiceps (Gb), Semimembranosus (Sm) and Rectus femoris (RF) of lambs belonging to the genetic types Gentile di Puglia (GP), Ile de France (IF) and the cross-breeds F1, F2 and F3. The IF lambs provided meat with the highest value of hue and lightness and the lowest chroma and redness. The F1 lambs showed the highest values of redness while the F2 crossbreed significantly differed from the F1 for the lowest value of lightness, yellowness and chroma. The F3 crossbreed revealed similar behaviour to the paternal breed for lightness, yellowness and pH. No differences between sexes were observed. The lambs slaughtered at 56 days showed higher a* values, while b* and hue were on average higher in younger lambs (35 days). The lambs reared with maternal milk, in comparison with the artificially reared ones (reconstituted milk), provided meat with higher a*, b* and chroma. The electrical stimulation made it possible to achieve lower lightness and pH and higher b* and chroma values. Among the considered muscles, RF provided the lightest meat; Sm showed the highest values of b*, hue and chroma; LD provided the “darkest” meat and the lowest values of b* and hue; Gb produced lower a* value than muscles LD, RF and Sm.

Key words: Lambs, Meat, Electrical stimulation, Colour.

RIASSUNTO

EFFETTO DELL’ELETTROSTIMOLAZIONE E DI ALTRI FATTORI GENETICI E AMBIENTALI SULLE CARATTERISTICHE COLORIMETRICHE DELLA CARNE DI AGNELLO

Scopo del presente lavoro è stato quello di quantificare l’effetto dell’elettrostimolazione, del tipo genetico, del sesso, del tipo di allattamento e dell’età alla mattazione su alcune caratteristiche colorimetriche rilevate sui muscoli Longissimus dorsi (LD), Gluteobiceps (Gb), Semimembranosus (Sm) e Rectus femoris (RF) di agnelli appartenenti ai tipi genetici Ile de France (IF), Gentile di Puglia (GP) e derivati dall’incrocio di sostituzione di quest’ultima con arieti Ile de France (F1, F2 e F3).

Gli agnelli IF hanno fornito la carne con un valore più elevato di tinta e di luminosità e con un più basso valore dell’indice del rosso e del croma. La carne dei soggetti F1 ha presentato il più alto valore di a*, mentre quella ottenuta dagli agnelli F2 si differenzia da quella degli F1 per un più basso valore di L*, b* e croma. Gli agnelli F3 hanno mostrato una risposta cromatica simile a quella del tipo genetico paterno per L*, b* e pH.

Mediamente, non sono state osservate differenze di colore della carne ottenuta dai due sessi, anche se questo comportamento varia a seconda del tipo genetico per la presenza dell’interazione.

La carne prodotta dagli agnelli macellati a 56 giorni ha mostrato un più elevato valore di a* e uno più basso di b* e di...
Introduction

Meat colour is the first characteristic valued by the consumer in the choice of food, since it is associated with product freshness. It is common knowledge that the meat to be eaten needs an ageing period, during which several physical and enzymatic processes occur, producing the tenderness of muscles and the development of meat colour. In this connection a commonly used technique to shorten the ageing time is electrical stimulation of the carcass. The treatment induces a series of muscular contractions and relaxations until energy supplies are depleted or as long as the muscle preserves its functionality. The anaerobic consumption of muscular glycogen produces high levels of lactic acid that promote a reduction in pH and the resulting rate of enzymatic-proteolytic activity. Those processes are the basis for the greatest degradation and fragmentation of myofibrils (Sonaiya et al., 1982; Pearson and Dutson, 1985; Dransfield et al., 1992; Rhee et al., 2000; Hwang et al., 2003), which is a basic step in meat development. Therefore, the meat, besides needing a shorter ageing time, can be quickly chilled without the risk of cold shortening.

The present research aims to assess the effects of electrical stimulation on chromatic characteristics of lamb's meat, in order to propose this technique as an alternative to the customary ageing period of meat in cold store, favouring its optimal ageing, improving its aptitude for transformation and preservation. In addition, this study, which is part of a wider research programme, aims to quantify the effects of the genetic type, sex, rearing system and muscle on meat quality of lambs slaughtered at 35 and 56 days of age.

Material and methods

The research was carried out for 4 years on 261 single birth male and female lambs, reared in a rough grazing area on the experimental farm of Li Foy (Istituto Sperimentale per la Zootecnia, Potenza, Italy) located at 1250 m above sea level. Genetic types were Ile de France, Gentile di Puglia and the result of grading up of the latter with Ile de France rams (Table 1). The lambs born in the autumn of the first year and in the spring of the second year were suckled till 5 days old and then were maintained on multiple boxes and reared till the 35th day with reconstituted milk (diluted at 16-18%). From the 36th to 56th day this milk diet, termed “artificial,” was supplemented with 200 g/head/day compound feed (Table 2). The lambs born in the autumn of the 3rd year and in the spring of the 4th year remained with their mothers until day 56 (“naturally reared”), receiving the same supplement as the previous group from day 36 to 56. For each feeding group the animals were divided into two slaughtering groups: 35 days (11 ± 2.3 kg of live weight) and 56 days old (15.7 ± 2.7 kg of live weight).

Within 30 min from slaughter, the left side of each carcass was electrically stimulated (ES) with twenty pulses (2 sec on/ 4 sec off) by means of direct current (48 V, 1.3 A). Two electrodes were fixed at 10 cm depth, one in the neck region, parallel to the backbone, the other in the leg muscle close to the Triceps surae tendons. The left side was dissected into commercial cuts two hours after electrical stimulation while the right non-stimulated side (NES) was refrigerated for 48 hours at 3-4 °C, and then dissected. The cuts obtained from the two half carcasses were frozen at -25 °C for about 120 days, then thawed out in a freezer at 3-4 °C according to the EU method (Boccard et al., 1993).
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1981) in order to isolate the muscles: Rectus femoris (RF), Gluteobiceps (Gb), Semimembranosus (Sm) and Longissimus dorsi (LD). Samples were collected from the middle part of each muscle and the colorimetric characteristics were determined by means of a Macbeth MS-2000 spectrophotometer, after one hour of blooming at 4 °C, under the protective film (Domopak®, Comital Saiag, Frosinone, Italy). The A lamp (the light of a bulb at 2865 °K) was employed as illuminating and the values of lightness (L*), redness (a*), yellowness (b*), chroma and hue were detected according to the CIEL*a*b* system. The pH was measured with pHMeter 29 (Radiometer, Copenhagen) at the same time as colour analysis.

A statistical analysis of variance was performed using two models. Model 1 includes all the considered factors of variation (genetic type, sex, age at slaughter, rearing system, electrical stimulation and muscle) without F3 crossbreed, because it was not possible to artificially rear these subjects. Model 2, instead, includes F3 lambs but not the rearing system. The factors included in both models were considered fixed, and the effect of each factor was expressed as deviation from the overall mean μ:

$$y_{ijklmn} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \phi_m + \lambda_n + 1^\circ \text{order interactions} + \text{subject}(\alpha_i \times \beta_j \times \gamma_k \times \delta_l) + \epsilon_{ijklmn} \quad (\text{model 1})$$

$$y_{ijklmn} = \mu + \alpha_i + \beta_j + \gamma_k + \phi_m + \lambda_n + 1^\circ \text{order interactions} + \text{subject}(\alpha_i \times \beta_j \times \gamma_k) + \epsilon_{ijklmn} \quad (\text{model 2})$$

where: $\mu$ = estimate of the overall least square means; $\alpha_i$ = fixed effect of the $i$th genetic type ($i = 1, 2...4$ in model 1 and $i=1, 2, ... 5$ in model 2); $\beta_j$ = fixed effect of the $j$th sex ($j = 1, 2$); $\gamma_k$ = fixed effect of the $k$th age at slaughter ($k = 1, 2$); $\delta_l$ = fixed effect of the $l$th rearing system ($l = 1, 2$); $\phi_m$ = fixed effect of the $m$th electrical treatment ($m = 1, 2$); $\lambda_n$ = fixed effect of the $n$th muscle ($n = 1, 4$); subject($\alpha_i \times \beta_j \times \gamma_k \times \delta_l$) = random effect of subject nested within.
genetic type, sex, age and rearing system; $\epsilon_{ijklmn} =$ random error; $\epsilon_{ijkmn} =$ random error.

The GLM procedure of the SAS (1997) statistical package was used. The subject effect was employed as error term to test genetic type, sex, age, rearing system and their interaction effects. The significance of the differences among the estimated means was tested using Student’s $t$ test.

Results and discussion

Almost all the variables were affected by one or more first order interactions. On the whole, the percentage of total variability explained by the model 1 ranged from 50-70 per cent for the colour characteristics to 80 per cent for pH (Table 3).

**Genetic type**

Among genetic types considered in model 1 (GP, IF, F1 and F2), IF lambs afforded a colour behaviour different from the other genetic types, providing meat with the highest value of hue and lightness, with lower chroma and $a^*$ value (Table 3). Therefore the meat obtained from IF, appearing brighter, results more pleasant to the consumer, although it results rheologically worse, since it shows higher values of hardness and adhesiveness (Zullo et al., 1993). It is important to underline that IF did not completely express its productive potential as it was less adapted to the 'harsh' environmental conditions of southern Italy in comparison with the Gentile di Puglia genetic type.

The F1 lambs furnished meat with higher values of $a^*$, chrome and hue than paternal breed but they did not differ from the maternal one; moreover, passing from F1 to F2 breed, it was possible to observe a significant decrease of $L^*$ and $b^*$ values. Comparing the five genetic types, the F3 lambs tended to resemble the paternal breed for pH and almost all of colorimetric characteristics, while GP, IF, F1 and F2 lambs confirmed the same values of model 1. In particular, the F3 subjects tended to show higher values of lightness, yellowness, chroma and pH (Table 4).

Statistical analysis showed that genetic type interacts with sex: the values of $a^*$ and chroma of meat obtained from females resulted significantly lower in IF than GP and F1, while the male lambs did not show differences among genetic type. Moreover, female lambs in comparison with males tended to show higher $a^*$ and chroma values in GP and F1 and lower in IF and F2 (Table 5). Therefore, F1 breed produced a value increase compared with paternal breed, until reaching that of maternal breed. This result was particularly clear in females that showed inheritance of colour characteristics probably tied to the sex, since the female lambs tended to be similar to the maternal breed.

The interaction between genetic type and rearing system, as far as pH is concerned, seems to be due to the higher value in GP male lambs compared with IF, while in the females the contrary is observed.

**Sex**

The meat obtained from male lambs showed similar chromatic behaviour to that of females (Table 3). The available literature offers conflicting information about the influence of sex on meat colour. According to some authors (Cosentino et al., 1986) the male Gentile di Puglia lambs tended to display brighter meat in comparison with females. Vice versa, Diaz et al. (2003) saw that Manchego female lambs show a significantly lower value of hue in comparison with male lambs, as far as the Rectus abdominis is concerned.

**Age at slaughter**

Age at slaughter is one of the factors which most affected the chromatic behaviour of meat: at 56 days higher $a^*$ values (Table 3) were noted, probably due to a higher myoglobin content of the muscle. The contents of this pigment increase with age and exercise of muscle (Lawrie, 1974). The hue and $b^*$ values were higher on average in animals slaughtered at 35 instead of 56 days of age, while the meat was less acid. As confirmed by other authors (Simoes and Ricardo, 2000; Santos-Silva et al., 2002; Diaz et al., 2003) the weight increase is often related to the reduction of $b^*$ values and, as a consequence, the meat of older and heavier subjects may appear darker.

The age at slaughter interacted with treatment of side for lightness and yellowness: at 35 days the ES side, compared with the NES one, showed a significant increase in $b^*$ and no difference in $L^*$ value; vice versa at 56 days the $b^*$ value
did not differ between treatments, while lightness was significantly lower in ES side.

### Rearing system

The lambs reared with maternal milk (natural) provided meat with higher values of yellowness, redness and chroma than the artificially reared ones (Table 3). These results agree with those obtained by Piasentier et al. (2000) on young goats and could be linked to higher live weight and better yield observed in lambs reared with maternal milk (Girolami et al., 1994). The meat obtained from lambs fed with artificial milk was more acidic (Table 3). This condition could be another reason for which the meat appears less pigmented, since a lower pH is the cause of a softer chromatic profile, due to a stronger denaturation of proteins (Lawrie, 1974).

The rearing system interacted with muscle for all colorimetric characteristics. Artificially reared lambs showed higher lightness in Sm and LD muscles than naturally reared ones, and lower a*, b* and chroma in all muscles except Sm (Table 6). Moreover, pH was influenced by interaction between rearing system and electrical stimulation: the ES caused a significant drop in pH of...
meat furnished by artificially reared lambs, while it did not influence the value of pH of naturally reared lambs.

Electrical stimulation

On average, the electrically stimulated side, in comparison with the unstimulated one, showed lower value of lightness and pH and higher b* and chroma values (Table 3). Electrical stimulation, indeed, did not act in the same way on the examined muscles as the two factors significantly interacted. The electrical stimulation made it possible to achieve: lower lightness value in LD and RF muscles together with higher hue; higher a* and chroma in RF and Gb and lower in LD; higher b* value in RF and Sm and lower in LD (Table 7). Although not all the Authors agree with the assertion that ES can modify the colour of meat, it seems almost assured that the electrical treatment is able to significantly influence the physical and chemical characteristics of muscle, and subsequently, the process of evolution of muscle into meat. Some Authors (Roeb et al., 2000; King et al., 2004) evidenced that ES determines an increase in colorimetric index according to our results, as far as the leg muscles are concerned. The different answer to the electrical stimulation of LD muscle could be due to the better bleeding of this muscle: the lower content of blood and haemoglobin could explain the lower chroma, a* and b* values.

Moreover, the literature suggests that the

| Genetic types | L* mean (%) | a* mean (%) | b* mean (%) | Chroma mean (%) | Hue mean (%) | pH mean |
|---------------|-------------|-------------|-------------|-----------------|-------------|--------|
| GP            | 51.48a      | 8           | 20.81a      | 11              | 15.41ac     | 11     | 5.81a  |
| IF            | 53.38b      | 7           | 19.87b      | 13              | 15.97b      | 10     | 5.89b  |
| F1            | 52.19ab     | 7           | 21.16a      | 10              | 15.52ab     | 10     | 5.86ab |
| F2            | 51.85ab     | 6           | 20.35ab     | 10              | 15.06ab     | 11     | 5.84ab |
| F3            | 53.18bc     | 9           | 20.88bc     | 11              | 15.71bc     | 10     | 5.88bc |

Different letters indicate significant differences between genetic types for P<0.05.

| Factors | Genetic types | GP mean | CV (%) | IF mean | CV (%) | F1 mean | CV (%) | F2 mean | CV (%) |
|---------|---------------|---------|--------|---------|--------|---------|--------|---------|--------|
| Redness (a*): Male | 20.25 | 12 | 19.8 | 13 | 20.17 | 12 | 20.59 | 10 |
| Female | 21.03a | 13 | 19.18b | 14 | 21.16a | 11 | 20.16b | 10 |
| Chroma: Male | 25.31 | 10 | 25.28 | 9 | 25.56 | 8 | 25.52 | 8 |
| Female | 26.16a | 10 | 24.73b | 9 | 26.30a | 8 | 25.15b | 8 |
| pH: Artificial | 5.75a | 2 | 5.69ab | 3 | 5.70ab | 2 | 5.65a | 3 |
| Natural | 5.81a | 2 | 5.87b | 2 | 5.85ab | 3 | 5.84ab | 2 |

Different letters within the factors indicate significant differences for P<0.05.
behaviour of muscles exposed to the electrical stimulation can be different, as the contraction capacity of each muscle and its function are also different. For example, Houlier et al. (1984) noticed that the slow contraction muscles mainly consisting of SO (Slow oxidative) fibres (i.e. leg muscles) were less affected by low voltage electrical stimulation than muscles with a high content of FG (Fast glycolytic) fibres (LD). Matassino et al. (1986) and Zullo et al. (1993) evidenced that the effect of ES on LD muscle is different than Sm, Gb and RF. In the latter, the electrical stimulation resulted in smaller fibres and more tender and acceptable meat, while in LD muscle the contrary was observed.

No interaction between muscle and side treatment was observed for pH which reached lower values in the electrically stimulated muscles ($P<0.01$; Table 2). This result agrees with other Authors’ works which showed that the technique induces faster conversion of glycogen into lactic acid (Sheridan, 1990; Moore and Young, 1991; Shaw et al., 1996; Simmons et al., 1997; Polidori et al., 1999; Geesink et al., 2001).

### Muscle

Muscle was a significant source of variation in chromatic characteristics and pH. The LD showed the lowest values of lightness, hue and $b^*$ in comparison with RF, Gb and Sm; the $a^*$ value was the same for LD, RF and Sm, and was higher than Gb (Table 3). The Sm muscle showed the deepest colours, registering the highest hue, $b^*$ and chroma values. Finally, RF showed the highest light-
ness and pH values. Hence muscle showed once again its pronounced individuality in agreement with the findings of other authors for different species (Chiofalo et al., 1983; Sañudo et al., 1998).

Conclusions

The Ile de France genetic type provided the brightest and least pigmented meat, able to satisfy the European market demand in terms of colour. In contrast with IF, the GP genetic type and the product of first generation F1 showed more pronounced colours (higher chroma and $a^*$ values), while F2 had very variable $L^*$, $a^*$ and chroma but intermediate between the two parental types IF and GP. The F3 subjects tended to show higher values of lightness, yellowness, chroma and pH. This result and the better yield at slaughter of F2 and F3 lambs (Giorlami et al., 1994) therefore justifies the use of the breed Ile de France in programmes of genetic improvement of native breeds, so as to enhance the meat production aptitude.

The rearing system resulted in considerable differences of colour and pH among the lambs belonging to the two feeding groups, demonstrating the strong connection between diet and meat quality. The subjects fed with reconstituted milk provided 'pinker' meat, with softer colours, therefore able to satisfy the consumer in terms of colour. Indeed, the consumer often links the soft and less pigmented colour of meat with higher tenderness and better flavour, especially with regard to ovine meat. The better acceptance together with the better myorheological characteristics of the meat provided by the artificially reared lambs (Zullo et al.,1993), is a valid reason to promote the use of this rearing system. The leg muscles showed a greater lightness, yellowness and hue than LD, probably due to the different functional exercise which these muscles are subjected (Lawrie, 1974).

The ES influenced the colour traits of muscles differently. Although significant, the differences between the two half carcasses (for $L^*$, $a^*$, $b^*$ and pH) evidently did not result wide. Therefore, since electrical stimulation produced the same effects as the usual ageing time in cold store (48 hours at 3-4 °C) on colorimetric characteristics, greater use of this technique should be advisable in slaughterhouses in order to reduce the times, and hence the costs of refrigeration, offering to the consumer a high quality product.

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