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Original study

Post-thawing colour changes in meat of foals as affected by feeding level and post-thawing time

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Abstract

The aim of the present work was to investigate how chromatic properties of foal meat can vary after thawing out in relation to the feeding level of 11 months old horses and to the post-thawing time. Thirty-six Italian Heavy Draught Horse foals were used for the trial. They were subdivided in three groups according feeding level: FL150=150 %, FL180=180 % and FL200=200 % of the energy maintenance requirements. Two different surfaces were investigated for each sample: daily renewed cutting surface and not renewed cutting surface. Lightness fell on both surfaces with the increasing of the feeding level ($P<0.01$). The redness of both investigated surfaces increased with feeding level ($P<0.01$), while yellowness decreased ($P<0.05$). Consumers prefer to purchase meat that is red rather than brown in colour. So, from a chromatic perspective the thawed meat of Italian Heavy Draught Horse foals fed with a lower feeding level proved to be that which best meets the market requirements.

Keywords: foals meat, feeding level, meat colour, post-thawing

Abbreviations: DRCS: daily renewed cutting surface; GLM: General Linear Model; IHDH: Italian Heavy Draught Horse; LD: logissimus dorsi; NRCS: not-renewed cutting surface; PVC: polyvinyl chloride

Introduction

The characteristics of meat which can be visually evaluated by consumers are colour, texture and marbling. The meat market is subject to macroscopic evaluations of such elements as colour, which the consumer correlates with the freshness of the product (Smith *et al.* 2000). Although the quality perceived by consumers does not coincide with the objective quality,

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it can cause significant economic losses in the fresh meat market, in fact they prefer to purchase meat that is red rather than brown in colour (Jacob & Thomson 2011). The main characteristics of meat affecting its colour are: myoglobin concentration, chemical state, lipid oxidative status (Kannan *et al.* 2001), muscle structure (which depends on pH), marbling; Brewer *et al.* 2001), microbial growth (Stivarius *et al.* 2002), oxygen consumption rate (Wulf & Wise 1999) and drip losses (Choe *et al.* 2009). The factors affecting those characteristics are genetics, diet, age/weight at slaughter, pre-mortem handling, post-mortem conditions and packaging (Mancini & Hunt 2005). Although feeding is one of the most important factors affecting meat quality (Sami *et al.* 2004), very poor researches have been conducted on horse meat. Few reports have been published concerning the effect of slaughtering age, sex, meat ageing (Sarries & Beriain 2006) and breed (Lanza *et al.* 2009) on horse meat quality. In Italy, for many years efforts have been made to develop the national production of horse meat from both the qualitative and quantitative points of view. Today the production of horse meat is obtained from breeds such as the Italian Heavy Draught Horse (IHDH) that has been investigated also for milk production (Centoducati *et al.* 2012). Studies of the *in vivo* performance and of the quality of the carcasses of horses slaughtered at 11 months old (Tateo *et al.* 2005, 2008) have shown that animals of this breed are particularly suitable for meat production. The increased demand for horse meat on the Italian and European markets presents problems linked to ensuring continuous product availability during the year, and to welfare and health problems involved in the transport of the living animals. To bypass those problems, in the last years it is consolidated the practice of trading frozen horse carcasses. For these reasons it was decided to investigate how the colour of horse meat can vary after thawing in relation to the feeding level with which they were fed. The purpose of this study was to establish the best feeding techniques in IHDH foals slaughtered at 11 months in order to ensure meat with post-freezing colour acceptable to the consumer, also in relation to the post-thawing time.

Material and methods

Animals

A total of 36 males IHDH breed foals were employed in the study, all of them were born on the same farm. At birth they were subdivided at random in three groups according to feeding level. The foals assumed colostrums and were suckled naturally and, from their second day of life, they followed their dams to the grazing areas for almost six hours per day. The foals were weaned at four months old and then were kept in three indoor stalls (one for each experimental group) with a surface area of 6 m^2 per head. Each group received a ration subdivided in three daily meals. The composition of the feed administered was the same for all the experimental trials and it was composed with 35 % of oat hay and 65 % of commercial feed (Table 1). Three different feeding levels were calculated on the basis of maintenance requirements assessed in relation to the metabolic live weight (Martin-Rosset *et al.* 1994) recorded every two weeks. The amount of dry matter of the experimental ration administered to the different groups was calculated to ensure the following feeding levels: FL150=150 %, FL180=180 % and FL200=200 % of the maintenance requirements.

Table 1
Chemical composition (% on DM) of diet fed to horses

Item Chemical composition	Concentrate	Oat hay
Dry matter	87.6	88.4
Crude protein	13.5	11.6
Crude fibre	10.8	33.4
Ether extract	3.2	2.9
Ash	7.0	11.1
Neutral detergent fibre	27.9	54.5
Acid detergent fibre	13.2	40.9
Acid detergent lignin	2.5	7.2
Horse forage units, n/kg of dry matter[1]	0.82	0.50
Digestible protein, g/kg	104.2	21.5

[1]Feeding level 100 % represents a level equal to the maintenance requirements.

Slaughtering and treatment of the samples

The slaughtering operations were carried out in the national slaughterhouse. After slaughtering the carcasses were kept in a chilling room at 4 °C for 48 h. After this portions of *longissimus dorsi* (LD) muscle between 13th and 18th thoracic vertebra (of around 500 g and a parallelepiped shape) were taken for analysis. These samples were held at 4 °C during the transport to the laboratory where, within two hours, samples, vacuum packaged, were frozen to –20 °C and kept in that state for 30 days.

Thawing was carried out at 4 °C in 24 h. The samples were subsequently inserted in 5 cm long and 10 cm diameter PVC cylinders, in such a way that the muscle fibres ran parallel to the axis of the cylinder. On one side of the cylinder a fresh cut was made which was not renewed. The not-renewed cut surface (NRCS) was aligned to the edge of the pipe. On the surface of the other side of the cylinder a cut (1 cm thick) was repeated daily (daily renewed cut surface; DRCS) immediately before making the tests. To facilitate the cut, made with a sterile scalpel, the meat sample exceeded the edge of the cylinder by 5 cm on this side (Tateo *et al.* 2007).

Value of pH, instrumental colorimetry and haematin concentration

Immediately after thawing, a 5 g sample of meat was used to determine the acid haematin concentration, according to the spectrophotometric method suggested by Hornsey (1956). For the three days following thawing (that is the first day), pH values and instrumental colorimetry data were collected on. The pH was measured with a portable pH meter provided with a puncture electrode (Forlab pH 710, Taranto, Italy). The colorimetric parameters were measured in triplicate in three different places, after turning the sample by 90 °C, using a colorimeter (Minolta CR-300, Model CR-300, Minolta Co., Ltd, Osaka, Japan), with a 1 cm aperture, illuminant D65 and a viewing angle. The colour was expressed with the CIE Lab according to e system, measuring colour coordinates: lightness (L*), redness (a*) and yellowness (b*). The standard white plate used for calibration had L*=94.5, a*=1.0, b*=1.9. The arithmetic mean of the nine recordings obtained was subject to the further statistical analysis. The coordinates a* and b* were used for the determination of the Chroma $(C)=(a^2+b^2)^{1/2}$ as indicated by Mancini *et al.* (2004) and Little (1975).

The pH and instrumental colorimetry were collected daily for the four days following thawing, both on the NRCS (one side of the PVC pipe) and the DRCS. The colorimetric parameters of the freshly cut surface were recorded 30 min after the cut, to let blooming.

Statistical analysis

The data obtained were submitted for the analysis of the variance for repeated measures employing the SAS (SAS Institute Inc., Cary, NC, USA) General Linear Model (GLM) procedure considering, as independent variables the feeding level, post-thawing days and the binary interactions between these factors. For a comparison of the averages the post hoc Tukey test for repeated measures was used. All the results reported are expressed as least square means and standard errors considering each post-thawing day as repeated measure.

Results

On the DRCS the lightness was lower in meat obtained from FL200 horses in comparison with meat obtained from horses fed with the other two levels investigated in the present work ($P<0.01$). The NRCS of meat obtained from FL200 horses showed lower lightness than that obtained from lower feeding level horses ($P<0.01$) (Table 2). Daily renewed cutting surfaces revealed lower redness in meat obtained from FL150 horses ($P<0.01$). Redness of not daily renewed cutting surfaces in meat of FL150 horses was lower than that observed in meat obtained from FL 200 horses ($P<0.01$) and FL180 horses ($P<0.05$). There were no differences on b* values on the DRCS depending on the feeding level. On the contrary, b* values on the NRCS of FL150 horses meat were higher than those of FL180 horses meat ($P<0.05$). The chroma values on the DRCS of meat of FL150 horses were lower than those of the others ($P<0.01$). Moreover, FL200 horses' meat showed higher chroma values than FL180 horses' meat ($P<0.05$). The NRCS of meat obtained from FL200 horses showed higher chroma values than meat obtained from horses fed with other feeding levels considered ($P<0.01$). Meat of FL200 horses showed higher pH values ($P<0.01$). Meat of FL150 horses contained a lower quantity of haematin ($P<0.05$).

Table 2
Influence of the feeding level on colorimetric parameters of NRCS, DRCS, on pH and haematinic concentration (least square means±standard error)

	Feeding level 150 %[1]	Feeding level 180 %[1]	Feeding level 200 %[1]
Daily renewed cutting surface			
L*	40.27±0.76^{A}	38.34±0.62^{A}	35.30±0.79^{B}
a*	15.50±0.33^{A}	17.21±0.27^{B}	18.20±0.34^{B}
b*	1.88±0.28	1.22±0.23	1.77±0.30
C	15.80±0.31^{A}	17.37±0.25^{Ba}	18.37±0.32^{Bb}
Not renewed cutting surface			
L*	37.39±0.71^{A}	35.24±0.58	33.36±0.74^{B}
a*	12.54±0.38^{Aa}	13.97±0.31^{Ab}	15.89±0.40^{B}
b*	4.18±0.36^{a}	3.04±0.29^{b}	3.35±0.37
C	13.75±0.32^{A}	14.67±0.26^{A}	16.48±0.33^{B}
pH	5.58±0.01^{A}	5.56±0.01^{A}	5.74±0.02^{B}
Haematin	137.70±18.34^{a}	187.48±14.97^{b}	196.71±19.15^{b}

Different letters in the same line show statistical differences ($^{A,B}P<0.01$; $^{a,b}P<0.05$), [1]Feeding level 100 % represents a level equal to the maintenance requirements.

The L* values of the DRCS increased significantly ($P<0.05$) between the first and second and fourth post-thawing day, while significant modifications were not observed on NRCS lightness (Table 3). The redness on the DRCS didn't show modifications during the post-thawing time. The a* values on NRCS were higher in the first day in comparison to subsequent days ($P<0.01$). Moreover, the second day was higher in comparison to the third day ($P<0.05$). The Post-thawing time had no effect on the DRCS b* values. On the contrary, on the second and the third post-thawing day, the NRCS showed a higher yellowness than on the first and the fourth day ($P<0.01$). Chroma values on the first post-thawing day of the DRCS were higher than those of the last two post-thawing days ($P<0.05$). On the NRCS chroma values fell down until the third post-thawing day ($P<0.01$). On the fourth post-thawing day these values were lower than those showed on the first post-thawing day ($P<0.01$). This meat showed a higher pH on first and second post-thawing day ($P<0.01$).

Table 3
Influence of post-thawing days on colorimetric parameters of NRCS, DRCS and on pH (least square means and standard error)

	I	II	III	IV	S.E.
Daily renewed cutting surface					
L*	35.63^{a}	38.81^{b}	38.69	38.75^{b}	0.84
a*	17.87	16.90	16.53	16.59	0.36
b*	1.85	1.38	1.90	1.37	0.31
C	18.14^{a}	17.07	16.75^{b}	16.77^{b}	0.34
Not renewed cutting surface					
L*	35.63	35.83	35.39	34.47	0.79
a*	17.87^{A}	13.71^{Ba}	11.98^{Bb}	12.99^{B}	0.42
b*	1.85^{A}	4.97^{B}	4.71^{B}	2.56^{A}	0.39
C	18.14^{A}	14.80^{B}	13.20^{C}	13.74^{BC}	0.35
pH	5.62	5.64	5.63	5.62	0.02

Different letters in the same line show statistical differences ($^{A,B,C}P<0.01$; $^{a,b}P<0.05$).

The DRCS of meat obtained from FL200 horses revealed a higher redness on the second post-thawing day than meat obtained from FL150 horses ($P<0.01$). On the second post-thawing day, the chroma values on the DRCS in meat obtained from FL150 horses were lower than those showed by meat obtained from FL200 horses ($P<0.01$).

The NRCS of meat from FL150 horses on the first post-thawing day showed higher a* values than in the subsequent days ($P<0.01$). Meat obtained from FL180 horses revealed higher a* values on the first post-thawing day than on the other days ($P<0.01$). Moreover, on the second day, the redness was higher than on the third one ($P<0.05$). In meat obtained from FL200 horses, a* values on the first post-thawing day were higher than those of the second ($P<0.05$), third and fourth one ($P<0.01$). Only on the second post-thawing day, a* values of the NRCS of meat obtained from FL200 horses were higher in comparison to meat obtained from FL150 horses ($P<0.05$). The NRCS of meat obtained from FL150 horses showed a higher yellowness on the second post-thawing day than that showed on the first and fourth post-thawing day ($P<0.05$). In meat obtained from FL180 horses, b* values of the NRCS in the first post-thawing day were lower than those recorded on the second and the third post-thawing

day ($P<0.01$). Moreover, on the second post-thawing day, b* values were higher than those on the fourth post-thawing day ($P<0.05$). FL150 horses' meat showed higher chroma values on the first post-thawing day than in the following days on the NRCS ($P<0.01$). The NRCS of meat obtained from FL180 horses showed, on the second post-thawing day, higher chroma values than on the third, fourth ($P<0.01$) and first ($P<0.05$) day after thawing. Besides, on the second post-thawing day, chroma values were higher than on the third one ($P<0.01$). The NRCS of meat obtained from FL200 horses showed higher chroma values on the first post-thawing day than on the second ($P<0.05$), the third and the fourth ($P<0.01$) post-thawing day. On the second and the third post-thawing day, FL200 horses' meat showed higher chroma values than FL150 horses' meat ($P<0.05$). Moreover, these samples showed higher pH values in comparison to meat obtained from FL180 horses on the third ($P<0.01$) and fourth post-thawing day ($P<0.05$).

Discussion

Freezing permits food products to be consumed some months after production and is one of the most frequently used methods of conserving them (Haugen *et al.* 2006, Rajendran *et al.* 2006). In addition, this technique of conservation may contribute to the improvement of ageing and of the rheological characteristics of meat (Znamirowska & Stanislawczyk 2005).

The lightness of fresh meat is significantly influenced by its chemical composition, in particular by its water content and intramuscular lipid concentration (Mancini & Hunt 2005). On both the DRCS and NRCS, the meat of IHDH foals showed a falling trend with the increase of the energy density of the feed ration.

Because of the tendency of horses to concentrate adipogenesis in the subcutaneous district rather than in the intramuscular fat (Rossier & Berger 1988), horse meat has a lower intramuscular lipid content (Palenik *et al.* 1980, Robelin *et al.* 1984) and consequently lightness is less influenced by the intramuscular lipid component than by the water content. The reasons for this finding could be linked to the fact that lightness varies in relation not only to the quantity of intramuscular lipids, but also to their quality (Mancini & Hunt 2005). In fact, many authors, studying beef meat, have demonstrated that with the variation of the administered energy there is also a fatty acid composition modification in the intramuscular lipid tissue (Duckett *et al.* 1993, Huerta-Leidenz *et al.* 1993, Di Luccia *et al.* 2003), but there is a lack of in-depth studies in relation to equine species.

In the course of the post-thawing days the lightness is probably affected by the water content of the tissues. On the DRCS the lightness increases in the first 24 h and then remains constant, probably because only at the start there is a breakdown of the muscle fibres with the passage of water from the intercellular region to the extracellular region (Yu *et al.* 2005, Mortensen *et al.* 2006). On the NRCS, on the other hand, as the conditions of relative humidity remain virtually stable during storage, L* does not vary (Ramirez & Cava 2007).

The medium and highest feeding levels coincide with meat that is richer in haematin. This determines higher redness and chroma indexes (Gil *et al.* 2001, Vestergaard *et al.* 2002).

During the post-thawing time, the values of the redness and chroma indexes tended to fall on the NRCS. This is probably due to biochemical phenomena which occur when the myoglobin pigment is exposed to the air (Xiong *et al.* 2007). In fact the myoglobin oxidisation

causes a darkening of the meat (Livingston & Brown 1982, Wallace *et al.* 1982). The absence of redness and chroma variation on the DRCS is probably due to its exposition to the air for less time. Therefore, the DRCS was less affected by the partial pressure of oxygen and is also less liable to undergo oxidising processes (Tang *et al.* 2006, Tateo *et al.* 2006).

Only on the NRCS, the yellowness index is influenced by the feeding level and the post-thawing time. As yellowness is closely linked to the composition of intramuscular fat (Mancini and Hunt, 2005), its variations may be an indirect indication of a qualitative modification of the intramuscular lipids in relation to feeding level and post-thawing time (Realini *et al.* 2004). Moreover, the yellowness index on the NRCS increases in the first 24 h and then remains constant. This is probably because of the development of lipid oxidisation processes which are verified mainly in the first 24 h following the liberation of lipolytic enzymes in the intercellular interstices and with oxide-reducing activities within the fibres (Motilva *et al.* 1993, Tang *et al.* 2006). Similar results were obtained by Wulf & Wise (1999) in bovine meat and by Tateo *et al.* (2007) in buffalo meat.

Many authors observed no effects of feeding intensity on pH of meat in different species. French *et al.* (2000), Sami *et al.* (2004), and Fiems *et al.* (1999) on beef, Madruga *et al.* (2008) and Kannan *et al.* (2006) on goat meat, Leheska *et al.* (2006) on pork observed how intramuscular pH was not affected by the feeding system. Vestergaard *et al.* (2002) affirmed that a lower glycogen level, that post-mortem is converted in lactate and H+, in beef could be associated to a lower dietary energy intake. Sarries & Beriain (2005) observed no effects of the feeding system on the pH of horse meat. Our results seem discordant from that what has been recorded by other authors, but only from a statistical point of view. In fact, pH values recorded in the present work are similar to those reported by other authors (Gill 2005, Sarries & Beriain 2005, Lanza *et al.* 2009). The statistical significance of the differences of pH mean values recorded in the present paper is due to the very low variability of the data set and to the high sensibility of the statistical test adopted, although these differences have a range between the experimental thesis of maximum 3.8 %, not significant from a meat quality point of view.

In conclusion, the feeding level affected only chromatic parameters of the NRCS and not on the DRCS. Colour is an important visual cue denoting freshness and quality to consumers who prefer to purchase meat that is red rather than brown in colour. Considering this, probably, from a chromatic perspective, the thawed meat of IHDH foals slaughtered at 11 months of age and fed with a feeding level of 150 % is that which best meets the consumers' requirements. Since the qualitative aspects of the freeze-stored meat can not only be explained by chromatic parameters, further studies on the post-thawing quality of horse meat are necessary.

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