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Influence of the housing system on meat quality of double muscled Piemontese young bulls

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ABSTRACT

The effect of the housing system on qualitative characteristics of longissimus thoracis et lumborum (LTL), semitendinosus (St) and supraspinatus (Ss) muscles was studied in cattle of hypertrophied Piemontese breed. Thirty young bulls, fifteen tie-stalled and fifteen housed in pens (5 m^2/head of space allowance), were fed the same diet and were slaughtered at about 17 months of age and 560 kg live weight. Chemical analyses (pH_{24}, chemical composition, haem iron and hydroxyproline contents, collagen heat solubility) and physical analyses (colour, water holding capacity, Warner Bratzler shear test) were performed on the three muscles, whilst sensory analysis was carried out on LTL muscle by a trained panel. Housing in pens increased hydroxyproline content and collagen solubility ($P<0.01$), decreased lightness of the three muscles ($P=0.05$) and influenced the other meat characteristics in a muscle-dependent manner. The loose housing system increased pH24 of LTL ($P<0.05$), water content of LTL and St ($P<0.01$), iron content of LTL and Ss ($P<0.05$), redness and yellowness of Ss ($P<0.01$), whilst decreasing protein content and yellowness of LTL and St ($P<0.01$). No significant differences for organoleptic quality due to housing system were observed. On the whole, even if significant, the differences in chemical and physical properties of the meat due to housing system were limited. Therefore, in comparison with the tie-stall housing, the housing in pens might promote the ethical quality of the meat product, being more respectful of animal freedom of movement, without worsening the meat quality.

Introduction

The Piemontese breed is highly specialised for beef production due to double muscled phenotype, which depends on a specific mutation in myostatin gene (McPherron and Lee, 1997). This phenotype has been increasingly favoured by artificial selection since the beginning of the 20th century, so that at present the frequency of the responsible allele, mh, in the breed is 0.98 (ANABORAPI, 2004). The mh allele affects many traits, including growth rate, meat yield and quality. Double muscling involves both hyperplasia, i.e. an increase in the number of muscle fibres, and, to a lesser extent, hypertrophy, i.e. an enlargement of individual muscle fibres (Ngapo et al., 2002a). This condition is not generalised throughout the body but it is more marked in the hindlimbs than in the forelimbs and mainly affects peripheral muscles and those with a large surface area (Boccard, 1981; Taylor, 2004). Double muscled animals have a higher percentage of white fast contracting muscle fibres. Breeds as Belgian Blue and Piemontese have been shown to have more type IIB fibres and less type I fibres, which partly explains the overall increase in whiteness of meat from hypertrophied animals (Cullen et al., 1999). Thus, the double muscled animals have a higher proportion of fibres adapted for glycolytic metabolism (Boccard, 1981), which results in a faster rate of glycolysis and earlier post mortem rigor development, whereas ultimate pH values are generally not different from those of normal animals (De Smet, 2004). Concerning meat quality, the double muscled Piemontese compared with normal animals have higher water and protein content (Barge et al. 1993); the intramuscular fat content is usually about 1% or lower (Barge et al., 1993) and consequently the triacilglycerol content is greatly reduced, as a result of lower fat deposition, with a positive increase of the polyunsaturated/ saturated fatty acid ratio (Barge et al., 1993). The meat from double muscled Piemontese animals is also very tender, due to a large reduction in muscle collagen content (Destefanis et al., 1994).

Ngapo et al. (2002b) reported that the intramuscular collagen content in meat from double muscled animals was as much as 40% less than in normal animals. This fact is ascribed to the thinner network of perimysial connective tissue (De Smet, 2004). On the contrary, there is little difference in the nature of collagen crosslinks and collagen solubility in meat from double muscled and normal animals (Ngapo et al., 2002a; De Smet, 2004). These findings are in agreement with earlier data of Destefanis et al. (1997), who observed no differences in collagen solubility in hypertrophied and normal animals of Piemontese breed. Differences have been observed also for colour, which is paler compared with the normal animals (Destefanis et al., 1994), and for drip and cooking loss, which are higher, probably for the faster post mortem pH fall (Barge et al., 1996). As reported by Gariépy et al. (1999), the higher percentage of white muscle fibres could explain the paler appearance and reduced water holding capacity. In the area of production of the Piemontese breed the animals are usually reared in small farms and kept tie-stalled. Even if the loose housing accommodation system is increasingly used, it is estimated that almost half of the farms still adopt the tie-stall system (COALVI, 2006) for reasons linked to traditional rearing habits, and also for the empirical belief of the butchers that the meat of tie-stall animals has better qualitative characteristics, mainly the lighter colour. However, the tie-stall accommodation is criticised, as thetered animals have restricted movements and cannot walk or take exercise for long periods or express normal behaviour in relation to social interactions (EU-SCAHAW, 2001). These aspects are not conducive to good welfare and are in contrast especially with two out of five freedom points, i.e. freedom from discomfort and freedom to express normal behaviour (Phillips, 2002). Moreover, the EU Council Directive 98/58/EC. (1998) established that the freedom of movement of the animal kept for farming purposes must not be limited, and the program of rural development of Regione Piemonte. (2007) provides for change of the housing system from tie-stall to loose housing accommodation, in order to promote rearing system more respectful of freedom of movement of the animals. Besides, the choice of loose housing could give the product an added value, because some consumers' categories are more and more interested in beef production system related to animal welfare. Previous studies on rearing system and meat quality of young bulls mainly focussed on the effect of indoor and outdoor extensive raising. In general, it has been observed that the physical activity induces a fast to slow-twitch fibre transformation (Jurie et al., 1998; Vestergaard et al., 2000; Gondret et al., 2005), it has no effect on chemical composition, water holding capacity and shear force (Moloney et al., 2004; Dunne et al., 2008), whilst increasing collagen content (Jurie et al., 1998). Concerning colour, the results are inconsistent, but indicate that the effects, if any, are muscle dependent, because they are related to the anatomical location (Muir et al., 1998; Vestergaard et al., 2000; Moloney et al., 2004; Dunne et al., 2005; Dunne et al., 2008). However, few studies exist for hypertrophied breeds, in which the allele responsible for the double-muscled phenotype could interact with the accommodation system for its well-known effect on meat quality. Therefore, we carried out this study in order to compare tie-stall and loose housing systems for the effects on chemical, physical and organoleptic characteristics of the meat of hypertrophied Piemontese young bulls.

Materials and methods

Thirty hypertrophied Piemontese male calves aged 7 months were randomly assigned to two groups of fifteen animals. The animals in one group were tie-stalled (TS), housed on concrete floor with straw bedding; the space allowance of each tie-stall was 1×1.7 m. Those in the other group were loose housed (LH) in three pens with deep bedding (five animals each; 5 m2/head). The choice of 5 m2/ head space allowance was based on the rules of EU Council regulation 1804/1999 (1999). The live and slaughtering performances of the animals were reported by Biagini and Lazzaroni (2003). In brief, all the animals were fed the same diet consisting of hay (0.55 UFV/kg) at 2 kg/day and concentrate (0.95 UFV/kg) in varying

amount to meet the INRA requirements for 1.2 kg daily gain, as indicated for late maturing beef cattle. The initial average weight of the animals was 225 kg for TS group and 234 kg of LH group. The average length of the trial was 285 days. The slaughtering weight was significantly higher for LH group (573 kg vs 539 kg; Pb0.05), whilst the average daily gain was not significantly different (1.11 kg for TS group and 1.13 kg for LH group). No significant differences were found in carcass weight (TS group: 371 kg; LH group: 397 kg) and in hot dressing percentage (68.91% and 68.51% for TS and LH group, respectively). Carcass conformation (SEUROP, 1–18 point scale; 2.67 for TS group; 2.33 for T group) and fatness (1–15 point scale; 3.27 for TS group; 2.93 for LH group) were not significantly different (Biagini and Lazzaroni, unpublished data). The animals were slaughtered in four times, when they reached the same commercial fattening degree. After a transportation time of about 30 min to a commercial abattoir, the animals were slaughtered according to the guidelines of the EU Council Directive 93/119/EC. (1993). The carcasses were split and the sides were stored in a chilling room at 2 °C. Twenty four hours after slaughter, the pH was measured in the longissimus thoracis (at the 13th thoracic vertebra level), semitendinosus and supraspinatus muscles of the right side, by a Hanna pHmeter (Hanna HI9025) with an Ingold spear electrode and automatic temperature compensator. Seven days after slaughter, the portions of longissimus thoracis et lumborum (LTL) muscle between the 9th thoracic and 1st lumbar vertebra, and approximately the central part of semitendinosus (St) and supraspinatus (Ss) muscles were taken from each right side. These muscles were chosen taking into account that muscle type is one of the most determinant of fibre type (Taylor, 2004). The longissimus thoracis and semitendinosus are white glycolitic muscles being richer of type IIB fibres (Taylor, 2004). Besides, the longissimus thoracis is considered the reference muscle for the meat analyses (Boccard et al., 1981). The supraspinatus muscle is characterised by a higher number of red type I fibres (Kirchofer et al., 2002; Taylor, 2004). Moreover, according to Boccard (1981), among these muscles, only the semitendinosus muscle should be considered hypertrophic. Immediately after ageing, on these samples the following chemical and physical analyses were performed:

• water, protein and intramuscular fat (ether extraction) content (AOAC, 1970). Nitrogen was determined by the Kjeldhal method using a Büchi System apparatus (Büchi Labortechnik, Flawil, Switzerland); crude protein was calculated by multiplying N×6.25. Lipids were determined by the Soxhlet method using a Büchi extraction system (Büchi Labortechnik, Flawil, Switzerland);

• hydroxyproline content (ISO [International Organisation for Standardisation], 1978) and heat solubility of collagen (2 h at 80 °C; Sörensen, 1981);

• haem iron (Hudzik, 1990); • lightness (L*), redness (a*) and yellowness (b*), according to CIE system (1978), using a Minolta CR 331C Chroma Meter (Minolta Camera Co., Japan) calibrated on the C illuminant; the measures were carried out after 1 h of blooming on a 4 cm thick steak;

• drip losses, on a steak weighing about 80 g and 1.5 cm thick, kept for 48 h in a plastic container with a double bottom (Lundström and Malmfors, 1985);

• cooking losses, on a 4 cm thick steak, vacuum sealed in a polyetylene bag and heated on a water bath, preheated at 75 °C, to an internal temperature of 70 °C (Barton-Gade et al., 1993);

• shear force (N) on cylindrical cores 2.54 cm in diameter, taken parallel to muscle fibres and obtained from the steaks used to determine cooking losses; the shear force was measured by an Instron Universal Testing Machine (Model 1011, Instron Corp., USA) equipped with aWarner–Bratzler shear device and calibrated at speed of 50 mm/min.

The sensory analysis was carried out on the thoracic part of LTL by seven assessors, selected and trained for beef evaluation according to the guidelines of American Meat Science Association (Cross et al., 1978). An 8-point structured scale was adopted, where 1 and 8 were respectively the minimum and maximum scores. Sensory characteristics were: appearance of the raw meat and eating qualities of the cooked meat. Eating qualities included: tenderness (ease of penetration; friability; residue after chewing), initial and sustained juiciness and overall acceptability. The steaks were cooked on a grill, preheated at 250 °C to an internal temperature of 70 °C. Cooking temperature was monitored by an iron/constantan thermocouple. The data of chemical and physical analyses were analysed by a split-plot design, considering the housing system effect in the main plot and the muscle effect in the sub-plot, as well as their interaction (SPSS Inc., 1997). When a significant effect was detected, means were compared using the Fisher's LSD test. The data of sensory evaluation were analysed by ANOVA GLM procedure (SPSS Inc., 1997) considering the housing system as main factor.

Results and discussion

The results of the chemical and physical analyses on meat from Piemontese animals tie-stalled or loose housed showed that the housing system had significant effects on a limited number of variables (Table 1). Loose housing increased hydroxyproline content and collagen solubility ($P<0.01$) and decreased lightness ($P=0.05$) of the three muscles. The muscle effect was significant for all the variables, except collagen solubility (Table 1). The Ss differed from LTL and St for higher intramuscular fat and hydroxyproline content ($P<0.01$), whilst, compared with LTL and Ss, St showed higher values of lightness, drip losses, cooking losses and Warner–Bratzler shear force ($P<0.01$). These differences reflect the respective anatomical location and function of the three muscles types studied, which in turn reflect their different involvement in physical activity and differentiation (Keeton and Eddy, 2004; Lawrie and Ledward, 2006). There were several housing system x muscle interactions. Loose housing increased pH24 of LTL ($P<0.05$), water content of LTL and St ($P<0.01$), iron content of LTL and Ss ($P<0.05$), redness and yellowness of Ss ($P<0.01$), whilst it decreased protein content ($P<0.05$) and yellowness ($P<0.01$) of LTL and St. According to Klont et al. (1998) and Taylor (2004), muscle metabolic and contractile types are adaptable and may be modified in living animals by environmental conditions, especially exercise, which are particularly important in cattle due to the large variation in production systems. Undoubtedly, it is difficult to interpret these results in comparison with those reported in other studies, because of differences in experimental conditions involving the individual effects of muscle type, breed, feeding plan and stocking density, etc. or the effects of various combinations of these factors, e.g. animals subjected to confinement compared with counterparts reared free range or subjected to forced exercise. Regarding pH, Vestergaard et al. (2000) found a higher final pH in longissimus dorsi, but not in semitendinosus, of Friesian young bulls reared extensively in comparison with tie-stalled counterparts. We obtained similar results, which indicate that the level of physical activity achieved in loose pens, even if limited, could be sufficient to affect glycogen metabolism and thus to raise the final muscle pH. Nevertheless, the pH difference, although significant, was very small (0.06) and most probably could not have any noticeable implication in meat quality. The intramuscular fat content was not affected by the housing system, as observed in a similar study on the hypertrophied Charolais breed (Dunne et al., 2008). Also Moloney et al. (2004) reported no significant differences for intramuscular fat content in bulls with larger space allowance, although all indices of fatness were lower. Similarly in pigs, the rearing in semi plein air (semi-outdoor housing) or outdoor did not modify the fat content of semimembranosus and longissimus muscles, in comparison with a classic rearing system with a lower space allowance (Gandemer et al., 1990; Lebret et al., 1998). On the contrary, a lower intramuscular fat amount in longissimus thoracis of young bulls and veal calves non hypertrophied

attributable to loose housing system compared with tying-type housing was reported by Andersen et al. (1991), Jensen and Oksama (1996), Andrighetto et al. (1999), Lee et al. (2008). Our data indicate that the rearing in pens decreased the protein content of LTL and St, possibly because during exercise the skeletal musculature exhibits a negative nitrogen balance and the rate of protein synthesis is decreased (Goldspink, 1991). Loose housing also increased water content in these two muscles, as already observed by Lee et al. (2008) in longissimus dorsi muscle of Hanwoo bulls. However, the differences between muscles observed in our study, although statistically significant, were negligible from a practical point of view. Compared with LTL and St muscles, no differences in Ss were observed for water and protein contents between animals tie-stalled and those housed in pens. In relation to the connective tissue, our investigation showed that the housing in pens raised hydroxyproline content, particularly in LTL muscle (+22%) and collagen solubility, particularly in Ss muscle (+25%). Also Jurie et al. (1998), in experimental conditions (6.5 m2/animal) comparable to those of our research, found in semitendinosus muscle of loose-housed young bulls of Salers and Limousin breeds a hydroxyproline content higher than that of animals tie-stalled, but no differences in collagen solubility. Gondret et al. (2005) reported an increased proportion of heat-soluble collagen in biceps femoris of male pigs following endurance training. On the whole these data are consistent with the long recognised evidence that the intramuscular connective tissue is dynamically remodelled and its synthesis and turnover are regulated by a range of factors, including exercise or immobilisation; thus, the exercise-induced increase in collagen synthesis results in muscles with less mature, and consequently more soluble collagen (Mc Cormick, 1992; Purslow, 1994, 2005). Most studies (Monin and Ouali, 1991; Jurie et al., 1998; Vestergaard et al., 2000) indicate that the exercise, whatever its intensity, increases the oxidative capacity of the muscle, and thus the total haem pigment content (Leiseigneur- Meynier and Gandemer, 1991). Consistently, in our study the animals reared in pens had a higher haem iron content in the LTL and Ss muscles; the absence of an effect on the St muscle could depend on the fact that muscles with different anatomical locations and functions may react differently to the form of stimuli induced by physical activity (Petersen et al., 1997, 1998; Vestergaard et al., 2000). Even if we did not analyse the muscle fibre types, it could be presumed a change in the metabolic characteristics of LTL and Ss muscles, to an increased oxidative activity of the muscle fibres. As for colour, Jensen and Oksama (1996) reported a lower lightness and a darker colour in longissimus thoracis of young bulls reared in pens, in comparison with young bulls tie stalled. Andersen et al. (1991) and Andrighetto et al. (1999) observed an increased redness and yellowness due to higher intensity of exercise of bulls and veal calves respectively, whilst Moloney et al. (2004) and Lee et al. (2008) did not find any effect of the exercise on colour parameters of longissimus thoracis. In particular, Moloney et al. (2004) attributed the lack of an effect on meat colour to the degree of exercise/ activity of the animals housed in pens (2.5 m^2 of space allowance) compared with animals reared at pasture and concluded that there might be a threshold space allowance above which no further effect of exercise will occur. Dunne et al. (2005) found that even a long-term daily exercise did not affect colour of longissimus dorsi and semitendinosus muscles and increased redness of extensor carpi radialis. Our results show that the housing in pens reduced the lightness of the three muscles, increased redness and yellowness of Ss and decreased yellowness of LTL and St muscles. Therefore, compared with LTL and St muscles, the Ss muscle colour seems to be influenced by housing system. According to Dunne et al. (2005), the anatomical position and function of the muscle and its involvement in movement seem important for colour variations. However, the colour variations of LTL instrumentally detected in our study did not correspond to by significant differences in meat sensory appearance between the two experimental groups of young bulls (Table 2). Regarding water holding capacity, housing system had no significant effect on muscle drip losses or cooking losses, as already reported by Dunne et al. (2008) and Lee et al. (2008). The higher hydroxyproline content and

collagen solubility in the young bulls housed in pens did not affect the tendernes of the three muscles, indirectly measured by theWarner–Bratzler shear test, or by the sensory trained panel in the LTLmuscle (Table 2). Other studies in cattle (Dunne et al., 2008; Lee et al., 2008) and in pigs (Petersen et al., 1997; Gentry et al., 2002) did not find differences in tenderness due to increased space allowance or increased level of physical activity. On the contrary, Jensen and Oksama (1996) reported an increased shear force of longissimus thoracis of Danish Friesian crossbred young bulls housed in deep bedding boxes, in comparison with tied up counterparts; however, no differences were observed when Danish Jersey was the dam breed. On the whole, the results indicate that the interactions observed between housing system and muscle seem to depend on fibre type composition of the three muscles. In fact, in comparison with glycolitic muscles (LTL and St), the oxidative Ss muscle reacted in a different way to loose housing accommodation, except for the iron content. The glycolitic muscles reacted in the same way to loose housing system, except for pH and iron content.

Conclusions

The results indicate that the housing system (housing in pens vs tie-stall) affected some chemical and physical characteristics of meat of the hypertophied Piemontese young bulls, often in a muscle-dependent manner. However, the differences were of limited practical consequence and did not seem such as to modify the meat quality. Moreover, the noticeable increase in hydroxyproline content in loose housed animals was counterbalanced by the increase in collagen solubility. Consistently, the sensory evaluation of the LTL muscle did not highlight any difference due to housing system, both in the visual assessment and in the eating qualities. Having no negative effects on meat characteristics of young bulls, housing in pens could be widely adopted in the production of double muscled Piemontese young bulls, so promoting the ethical quality of a product being more respectful of animal welfare.

Conflict of interest statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcame.

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Table 1 Chemical and physical analyses of longissimus thoracis et lumborum (LTL), semitendinosus (St) and supraspinatus (Ss) muscles from Piemontese young bulls tie stalled and loose housed.

	Tie-stalled			Loose housed			SEM	Significance		
	LTL	St	Ss	LTL	St	Ss		Housing system	Muscle	Hs × M
pH_{24}	5.58^{a}	5.57^{a}	5.62^{bc}	5.64^{c}	5.60^{ab}	5.62^{bc}	0.012	n.s.	*	*
Water (%)	75.30^{a}	76.07^{c}	77.42^{e}	75.66^{b}	76.36^{d}	77.31^{e}	0.085	n.s.	**	**
Protein (%)	22.12^{d}	21.75^{c}	19.81^{a}	21.49^{bc}	21.32^{b}	19.83^{a}	0.115	**	**	*
Ether extract (%)	0.52	0.47	0.72	0.50	0.38	0.76	0.048	n.s.	**	n.s.
Hydroxyproline (μg/g)	418.92	628.93	779.50	510.62	688.15	898.74	33.638	**	**	n.s.
Collagen solubility (%)	17.93	16.13	15.52	19.33	18.79	19.41	0.838	**	n.s.	n.s.
Haem iron (μg/g)	9.66^{b}	7.99^{a}	13.88^{d}	10.71^{c}	8.06^{a}	15.44^{e}	0.260	n.s.	**	*
L^* (lightness)	42.02	50.23	42.11	40.68	48.64	40.66	0.367	*	**	n.s.
a^* (redness)	26.11^{a}	26.77^{a}	27.55^{b}	25.89^{a}	26.58^{a}	29.05^{c}	0.254	n.s.	**	**
b^* (yellowness)	11.72^{b}	15.14^{e}	12.16^{b}	11.06^{a}	14.44^{d}	12.88^{c}	0.178	n.s.	**	**
Drip losses (%)	2.82	4.94	2.88	2.75	4.24	2.96	0.200	n.s.	**	n.s.
Cooking losses (%)	17.93	30.03	24.43	17.41	26.65	24.40	1.104	n.s.	**	n.s.
WBs (Warner–Bratler shear force; N)	60.55	102.61	84.44	63.16	102.85	88.31	3.198	n.s.	**	n.s.

SEM: mean standard error.
a, b, c, d, e Within rows, means assigned different superscripts differ significantly ($P<0.05$).
n.s.: not significant; *$P<0.05$; **$P<0.01$.

Table 2 Sensory analysis on longissimus thoracis muscle.

	Housing system			Significance
	Tie-stalled	Loose housed	SEM	
Appearance*	6.58	6.65	0.06	n.s.
Ease of penetration*	6.44	6.50	0.07	n.s.
Friability*	6.98	6.17	0.08	n.s.
Residue after chewing*	5.61	5.82	0.08	n.s.
Initial juiciness*	5.90	5.71	0.08	n.s.
Sustained juiciness*	5.53	5.41	0.08	n.s.
Overall acceptability*	5.99	6.03	0.08	n.s.

*The scores were expressed using an 8-point structured scale, where 1 and 8 were respectively the minimum and maximum score.
SEM: mean standard error.
n.s.: not significant, P>0.05.