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SHORT COMMUNICATION

MC1R gene analysis applied to breed traceability of beef

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ABSTRACT

Since the breed of origin highly affects the beef price, reliable methods are needed to detect incorrect declarations. As most breeds are standardised for coat colour, the Melanocortin 1 Receptor gene (MC1R), involved in the regulation of eu/pheomelanins synthesis, has been suggested as marker for breed traceability of products of animal origin. The aim of this investigation is to characterise the main breeds reared in the Piedmont Region by MC1R locus and to apply the analysis of the locus to breed traceability of beef cuts purchased in different outlets of the Region. A total of 168 DNA samples of four cattle breeds (Piemontese, Blonde d'Aquitaine, Italian Friesian and Aosta Red Pied) were analysed for MC1R locus by PCR-RFLP. In addition, 28 DNA samples from beef with breed indication were tested. Piemontese and Blonde d'Aquitaine were monomorphic for the E^+ and *e* allele, respectively. In the Friesian breed the E^dE^d genotype was the most frequent, but E^de was also observed (2%). Aosta Red Pied was the most variable breed, with the presence of the three alleles and five genotypes out of six. The comparison of the genotypic distribution in the four breeds clearly indicates that it is possible to distinguish among Piemontese, Blonde d'Aquitaine and Friesian breeds, but the same is not true for Aosta Red Pied, which has genotypes in common with the other breeds. The results on beef samples revealed a high percentage of mislabelling (about 18%), which concerned Friesian breed and crossbreds. These results indicate that MC1R locus is an effective marker in breed traceability of beef, when the involved breeds are characterised by different genotypes. Moreover, compared to other genetic markers, it has the great advantage of not requiring DNA reference samples. This survey, though limited, has revealed a high percentage of incompatibilities. Therefore, the analysis of MC1R locus is recommended in the framework of product certification, at least for random controls within a system aimed at preventing fraud.

Key Words: Cattle, Beef, MC1R gene, Traceability.

RIASSUNTO

ANALISI DEL GENE *MC1R* PER LA RINTRACCIABILITÀ DELLA RAZZA DI ORIGINE DELLA CARNE

Per rispondere alla crescente richiesta di informazioni da parte dei consumatori, gli operatori della filiera carne hanno adottato un sistema di etichettatura volontaria, tramite il quale mettere a disposizione, oltre alle informazioni previste per legge, anche altri dati come, ad esempio, la razza dell'animale da cui proviene la carne. Poiché la maggior parte delle razze bovine sono ormai standardizzate per il mantello, il gene Melanocortin Receptor 1 (MC1R)*, coinvolto nella regolazione della sintesi di eu/feomelanine, è stato suggerito come marcatore per la rintracciabilità di razza dei prodotti di origine animale. L'obiettivo del lavoro è di valutare l'applicabilità dell'analisi del gene* MC1R *come strumento di verifica della razza dichiarata nell'etichetta che accompagna il taglio di carne. A tale scopo sono stati analizzati complessivamente 168 soggetti appartenenti alle razze più diffuse in Piemonte: Piemontese, Blonde d'Aquitaine, Frisona Italiana e Valdostana Pezzata Rossa, per caratterizzarne la struttura genetica nei riguardi del locus MC1R. Sono stati, quindi, analizzati 28 campioni di carne per i quali era indicata la razza di origine, prelevati in differenti punti vendita. Tutti i soggetti Blonde*

d'Aquitaine e Piemontesi sono risultati omozigoti per l'allele e ed E^+*, rispettivamente. Per la razza Frisona Italiana sono state confermate sia la netta prevalenza dell'allele* E^d*, sia la presenza dell'allele e, individuato allo stato eterozigote. La razza Valdostana Pezzata Rossa ha mostrato un'elevata eterogeneità, con la presenza di tutti e tre gli alleli e di cinque dei sei possibili genotipi. Il confronto delle distribuzioni genotipiche fra le quattro razze indica che è possibile distinguere l'una dall'altra le razze Piemontese, Blonde d'Aquitaine e Frisona Italiana, ma non la Valdostana Pezzata Rossa, la quale presenta genotipi in comune con le altre razze. Le analisi sui campioni di carne ha rivelato un'alta percentuale di errore (circa il 18%) rispetto a quanto riportato in etichetta. Nel complesso, il gene MC1R si è rivelato un utile marcatore per la rintracciabilità della razza di origine della carne, quando le razze interessate si caratterizzano per genotipi diversi. Inoltre, questo marcatore presenta il vantaggio di essere utilizzabile anche in assenza di una banca di DNA. Poiché questa indagine, se pur limitata, ha rivelato un'elevata percentuale di incompatibilità, si suggerisce l'impiego dell'analisi del locus MC1R nell'ambito della certificazione dei prodotti, almeno per controlli casuali mirati alla prevenzione di frodi.*

Parole chiave: *Bovini, Carne, Gene* MC1R*, Rintracciabilità.*

Introduction

In the framework of beef certification the current European Commission regulation (CE n. 1760/2000) provides for the possibility of giving additional information, including the breed of the subjects from which beef derives. Even though traceability systems based on electronic records (Nageotte *et al.*, 2000) have been developed, mislabelling of beef can occur, due to unintentional or intentional errors along the chain from farm to market. As the breed of origin highly contributes in determining the price of the product, reliable methods are needed to detect incorrect declarations. It is well known that the microsatellite markers are the best tool for individual identification, provided that DNA from the live animal is available for comparison (Cunningham, 2000; Arana *et al.*, 2002; Vásquez *et al.*, 2004). However, the creation of DNA archives that include all the animals is an expensive solution, so at present the DNA banks are still of limited use. For this reason there is a need for other genetic markerswhich are suitable to be used even in absence of reference samples.

As most breeds are standardised for coat colour, the Melacortin 1 Receptor gene (MC1R), involved in the regulation of eu-/pheomelanins synthesis (Klungland *et al.*, 1995), has been suggested as a marker for breed traceability of products, such as milk, cheese or meat (Chung *et al.*, 2000; Maudet and Taberlet, 2001; Crepaldi *et al.*, 2003). The aim of this investigation is to characterise the main cattle breeds reared in the Piedmont Region by the MC1R alleles with visible effect and to apply the analysis of the locus to breed traceability of beef cuts purchased in different outlets of the Region.

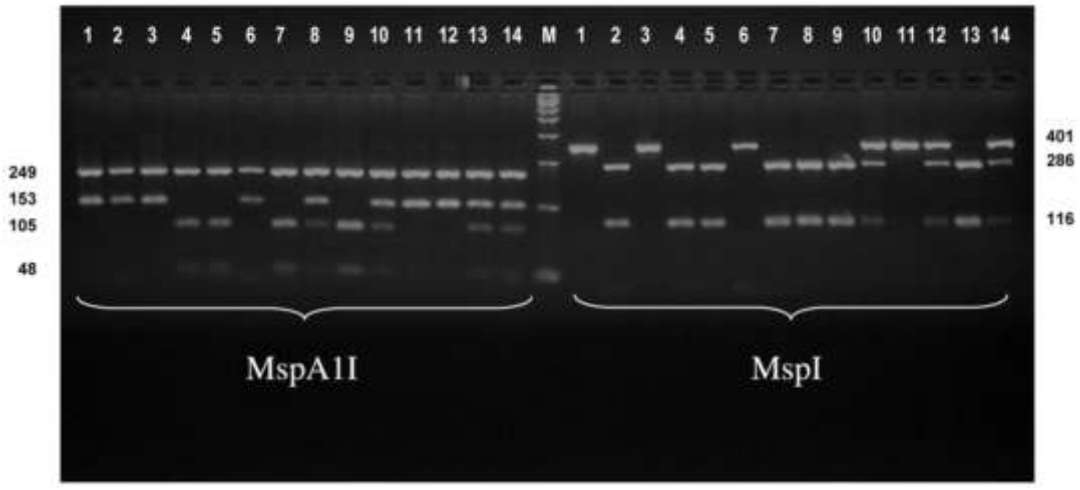
Material and methods

A total of 168 DNA samples from the following breeds were analysed: Piemontese (41), Blonde d'Aquitaine (40), Italian Friesian (44), Aosta Red Pied (43). In addition, 28 DNA samples from beef with breed indication were tested.

A single PCR was employed to amplify a fragment of 402 bp, extending from nt 193 to nt 594 of the MC1R gene (Genbank accession n. U39469) and including the point mutations for E^d allele (296 T/C), responsible for production of eumelanins, and e allele (310 G del), responsible for pheomelanins. The primers M1 and M2 described by Crepaldi *et al.* (2003) were used, with a slight modification in the reverse primer (one A added at the 5' end), introduced in order to have primers with the same annealing temperature. The PCR reaction contained 50-100 ng of genomic DNA, 1X PCR buffer II, 1.5 mM $MgCl_2$, 200 μM each dNTP, 0.16 μM per primer, 1U Red*Taq* DNA polymerase (Sigma, St Louis, MO, USA), in a total volume of 25 μl. The amplification was performed under the following conditions: 5 min at 94°C, 35 cycles of 94°C for 30 sec, 61°C for 30 sec, 72°C for 1 min, and a final extension at 72°C for 5 min.

The PCR products were double digested in two separate reactions, with *Mspa*I1 and *Msp*I enzymes, according to manufacturer's instructions. The digested fragments were electrophoresed on 2.5% agarose gels, stained with ethidium bromide and visualised under UV light.

Figure 1. Polymorphism of MC1R locus.



Left: samples digested with MspA1*I enzyme.*
Right: the same samples digested with Msp*I enzyme.*
M: molecular weight marker.
The genotypes are: 1) ee, 2) E^+E^+, 3) ee, 4) E^dE^d, 5) E^dE^d, 6) ee, 7) E^dE^d, 8) E^dE^+, 9) E^dE^d, 10) E^de, 11) ee, 12) E^+e, 13) E^dE^+, 14) E^de

Results and discussion

The genotype at MC1R locus was identified by integrating the results obtained with the two endonucleases, as shown in Figure 1: *Msp*AI1 discriminated E^d (249, 105 and 48 bp) from E^+ and e (249 and 153 bp), while *Msp*I recognized *e* (401 bp) from E^+ and E^d (286 and 116 bp).

Concerning the investigated breeds (Table 1), Piemontese and Blonde d'Aquitaine were monomorphic for E^+ and *e* allele, respectively, as already reported (Rouzaud *et al.*, 2000; Crepaldi *et al.*, 2003). The results on Italian Friesian confirmed the presence of *e* allele, found in heterozygote condition, together with the widespread E^d allele. However, a different scenario can be foreseen for the future for this breed. In fact, in the past the red animals were excluded from the Herd Book, which has led to the present situation, with the E^d allele almost fixed. Since the red colour is no longer selected against (even some Friesian IA bulls are red), it is likely that in the future the frequency of *e* allele will increase. If this happens, the breed will lose its genetic uniformity for MC1R locus, making its use in product traceability less effective.

Table 1. Genotypic frequencies of MC1R locus in the investigated breeds.

Breed	n.	Genotype					
		E^dE^d	E^dE^+	E^de	E^+E^+	E^+e	ee
Piemontese	41	-	-	-	1.00	-	-
Blonde d'Aquitaine	40	-	-	-	-	-	1.00
Italian Friesian	44	0.98	-	0.02	-	-	-
Aosta Red Pied	43	0.05	0.07	-	0.16	0.14	0.58

As far as we know, these data represent the first report on MC1R polymorphism in Aosta Red Pied, which showed a remarkable polymorphism with the presence of the three alleles and five genotypes out of six. As expected, the most frequent was *ee* (0.58), but also E^+E^+ and E^+e had quite high frequencies (0.16 and 0.14 respectively). In addition, E^dE^+ and E^dE^d genotypes were observed with a cumulative frequency of 0.12. The high genetic variability observed is not so surprising if some historical and geographical aspects are considered. In fact, Aosta Red Pied is an autochthonous population of the Aosta Valley, which has not been so intensively selected as the main standardised breeds. For this reason it may have maintained a higher genetic variability, as also observed for other local breeds like the Aubrac and Gasconne in France (Rouzaud *et al.*, 2000). This variability could in part be traced back to the documented, though sporadic, use of Simmental, Jersey and Montbéliard bulls introduced in the past century to improve the breed (Dupont, 1992). Furthermore, the breeding history of Aosta Red Pied is closely linked to that of two other local populations reared in the same area and characterised by different colours: the Aosta Black Pied and Aosta Chestnut. Their common origin (Del Bo *et al.*, 2001) and uncontrolled genetic admixtures could contribute in maintaining some variability for coat colour genes as well.

The comparison of the genotypic distribution in the four breeds clearly indicates that it is possible to distinguish among Piemontese, Blonde d'Aquitaine and Friesian breeds, but the same does is not true for the Aosta Red Pied, which has genotypes in common with the other breeds.

The results on beef samples (Table 2) revealed a high percentage of mislabelling (about 18%), which concerned Friesian breed (three samples) and crossbreds (two samples). The two beef cuts labelled as 'Piemontese x Friesian' derived most likely from pure Frisian breed, as their genotype is E^dE^d. As beef from the Piemontese x Friesian crossbred has a higher quality than beef from Friesian (Destefanis *et al.*, 2000), intentional mislabelling is a possibility. The MC1R genotype also resulted incompatible for three samples labelled as 'Friesian'; they were all heterozygous and different from each other (E^+E^+, E^+e, E^dE^+). Some hypotheses could be put forth as respects the real origin of these beef samples, but none of them seemed to be more probable than the others. In any case, it is most likely that the inconsistencies found were due to casual errors.

In evaluating the effectiveness of the MC1R analysis in breed traceability, a basic aspect is to be taken into account: if genetic incompatibilities are detected, the error in the declarations is proved, while in case of compatibility the assignment of a breed is never proved, because beef could derive from another breed with the same genotypes as the declared one. When incompatibilities are observed, hypotheses on the real breed of origin can be put forth, while investigating each case individually and taking into consideration not only the MC1R genotype, but also any other avail-

Table 2. Breed traceability of beef samples.

Declared breed	Consistent with MC1R genotype	
	Yes	No
Piemontese	3	-
Blonde d'Aquitaine	2	-
Italian Friesian	12	3
Aosta Red Pied	1	0
Crossbred	5	2
Total	23	5

able information. However, when the inconsistencies are due to unintentional errors, the assignment of a putative breed is usually very difficult, because such errors occur at random, without any logical basis.

Conclusions

The MC1R locus has been shown to be an effective marker in breed traceability of beef when the involved breeds are characterised by different genotypes. Moreover, compared to other genetic markers, it has the great advantage of not requiring DNA reference samples. This survey, though limited, has revealed a high percentage of incompatibilities. Therefore, the analysis of MC1R locus is recommended in the framework of product certification, at least for random controls within a system aimed at preventing fraud.

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