Carnitine prevents clastogenic effects induced by hydrogen peroxide in mammalian cells

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ABSTRACT

Carnitine is a small essential molecule that regulates the substrate flux and energy balance across cell membranes by modulating both the transport of long-chain fatty acids into mitochondria and their subsequent β-oxidation. Although humans are capable to synthesize it endogenously, approximately 75% of body carnitine sources come from diet and particularly from food of animal origin such as meat, poultry, fish and dairy products. Due to its intrinsic interaction with the bioenergetics processes, carnitine plays an important role in diseases associated with metabolic compromise, especially mitochondria-related disorders. It has been reported that administration of carnitine by diet or at pharmacological doses can have significant benefit in several physiopathological situations such as ischemia, myocardial injury and neurodegenerative diseases, but there is no data on the possible protective role of carnitine against other oxidative stress-induced pathologies associated with an altered chromosome stability such as cancer. Therefore, we analysed the potential capability of carnitine to protect mammalian cells from genetic instability induced by H₂O₂, using Chinese Hamster Ovary (CHO) cells as a mammalian cell model having a stable karyotype and the chromosome aberration test as genetic end point. Our results showed that in the absence of carnitine H₂O₂ induced a high and dose-dependent induction of structural chromosome aberrations in the concentration range 0.1-0.4 mM whereas at the same H₂O₂ doses, a pre-treatment with 4 mM carnitine produced a strong decrease either of the percent of cells with aberrations or of the aberration frequency. The observed carnitine-mediated prevention of H₂O₂-induced chromosome aberrations reaches almost the control value in the cultures treated with 0.1 mM of H₂O₂ thus evidencing a reduction of about 70%. These data, together with preliminary results showing that carnitine is not able to protect cells from the inhibition of cell growth caused by H₂O₂, suggest that carnitine protects mammalian cells from H₂O₂-induced clastogenic damage and this effect is reproducible and highly specific.
The Comet assay on bovine leukocytes: a sensitive test for genetic studies

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ABSTRACT

The alkaline Comet assay is a sensitive and rapid method for DNA double-and single-strand breaks (DSB, SSB), alkali-labile sites (ALS) and delayed repair sites detection in eukaryotic individual cells. The assay is extensively used in several different areas, such as: genotoxicology, clinical and DNA repair studies, environmental bio monitoring and human monitoring but, as far as we know, data on farm livestock are scanty. In order to evaluate the spontaneous DNA damage in cattle we used the comet assay on unstimulated bovine leukocytes from healthy subjects. The standard alkaline comet assay was performed according to the method developed by Singh and co-workers (1988) with minor modifications. Images of 100 randomly selected cells (50 from each of two replicate slides) were observed from each subject using a fluorescence microscope. Measurements were made by computerized image analysis system using% DNA in tail, Tail length, Comet moment and Tail moment for quantitative evaluation of damage. To validate the test on bovine cells for its application in genetic toxicology studies, we also treated blood samples with increasing concentrations (20-80 μM) of Methyl Methane Sulfonate (MMS), a well known chemical mutagen frequently used as positive control in the comet assay, in order to find the optimal concentration to obtain an adequate number of damaged cells. Furthermore, we evaluated the potential genotoxic activity of trans-asarone, a plant-extract compound involved in several pharmacologic and pest-managing activities. For this purpose we tested concentrations of trans-asarone, selected on the basis of preliminary study on citotoxicity, ranging from 90 to 150 μg/ml. The results obtained showed low basal levels of DNA damage for all parameters investigated. On the contrary a dose-dependent increase of DNA migration was detected in samples treated with MMS, in particular the dose of 40 μM can be recommended as positive control for the use of comet assay when bovine cells are employed. A slight, but not statistically significant, increase of DNA damage was found when increasing concentrations of trans-asarone were tested.
Survey of milk protein polymorphism in the “Bovina Rossa Siciliana”

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ABSTRACT

The “Rossa Siciliana” is an autochthonous small Sicilian dairy cattle population, accounting for 5-7,000 individuals. This breed is characterized by high ability to live on poor pasture lands, moderate milk production, and traditionally linked to cheese production. Aim of this work was to investigate milk protein polymorphisms in this population. A total of 62 individual milk samples were collected from 19 extensive farms spread in the “Parco dei Nebrodi” area (Messina). All samples were analyzed by isoelectrofocusing (IEF) with pH range 2.5-6. High variability was found at the CSN1S1, CSN2, CSN3, LGB and LALBA loci, while the CSN1S2 locus was monomorphic for the A allele. The allele frequencies and the Hardy-Weinberg equilibrium were estimated using the GENEPOP software, further the casein haplotype frequencies and the occurrence of the linkage disequilibrium were computed with the EH software, taking into account the association among loci. Joint analysis at all loci showed that the population is not in Hardy-Weinberg equilibrium (Chi-square=19.0, p-value=0.0407), probably due to genetic drift. Of the three alleles detected at CSN1S1 locus, CSN1S1*B and CSN1S1*C had high frequencies (0.734 and 0.258 respectively), as reported for most breeds. The CSN1S1*D allele was detected with a low frequency (0.008). High frequencies were found for CSN2*A (0.573) and CSN3*B (0.637). High frequencies were observed for LALBA*B (0.903) and LGB*B (0.815). Strong linkage disequilibrium was detected for the polymorphic casein loci CSN1S1-CSN2-CSN3. For the casein haplotype only the hypothesis of association among loci was considered. Out of the 27 expected, only four haplotypes had a frequency higher than 0.10. The most frequent haplotype was BA2B (0.236), followed by CA2B (0.214), BA1A (0.168), BA'B (0.131), and BA'A (0.094). The high frequency of BA'B haplotype and the possible relation with production traits are under investigation.
AFLPs reveal the genetic structure of Italian cattle breeds

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ABSTRACT

Assessing the structure, diversity and distinctiveness of livestock populations by means of molecular tools may help to address specific conservation issues. Here we presents the results obtained analysing 411 animals, belonging to 4 cosmopolitan and 14 autochthonous Italian cattle breeds, with three highly polymorphic AFLP primer combinations, which generated 135 polymorphic bands. In spite of different breeding management and effective population size, no significant difference was found among expected heterozygosity (Het) values, ranging from 0.19 in Calvana to 0.23 in Podolica. A relatively high level of diversity is still retained in Calvana, in spite of its small total and effective population size. Probably in this breed genetic drift, bottleneck and founder effects are counteracted by gene flow from neighbouring populations. The Factorial Correspondence Analysis (FCA) of individuals shows: - a tendency to cluster of animals original from Northern Italy and Continental Europe (Piedmontese, Cabannina, Valdostana Red Pied, Rendena, Grey Alpine, Italian Brown, Italian Red Pied, Italian Friesian, Italian Limousin) on one side, and Podolian stock (Romagnola, Marchigiana, Maremmana, Podolica, Cinisara, Modicana and Mucca Pisana) on the other side, with the significant exception of Chianina and Calvana; - within these main clusters, sub-clusters of individuals belonging to the same breed of origin. About 17% of the total molecular variance is due to the divergence between populations, while 83% is retained between individuals within populations. The Analysis of Molecular Variance recovers deep division when the 18 populations are clustered into the three groups evidenced by the FCA analysis (4.80% of the total variance; P<0.001). The Principal Component Analysis based on Reynolds distance between breeds agrees with the analysis of single individuals. In fact it clearly separates the Northern group of breeds from the Podolian stock and clearly separates Chianina and Calvana from the former two groups, underlying the originality of these two breeds present in the Italian Peninsula since historical time.
A QTL for protein percentage on Italian Friesian BTA 28: microsatellites confirm AFLP data

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ABSTRACT

Quantitative Trait Loci (QTL) mapping is the first step towards the understanding of genetic basis of economically important production and functional traits in livestock. In dairy cattle, QTL mapping is mainly carried out using a genome wide approach in existing half sib families according to Daughter (DD) or Grand-Daughter (GDD) designs. The high costs of screening such large families with hundreds markers can be effectively reduced by the combined use of selective genotyping and DNA pooling. This approach was proven to maintain a high statistical power in identifying significant markers-QTL associations. In a previous research carried out in our laboratory we used selective genotyping and DNA pooling in combination with high throughput AFLP technology to identify chromosomal regions candidate to contain QTLs affecting milk protein percentage in 2 GDD half sibs families of Italian Friesian cattle. This approach allowed the detection of 7 significant QTL-AFLP marker associations. Following isolation, sequencing and Radiation Hybrids mapping, these markers resulted located in 6 chromosomes. To confirm these results and validate the strategy, we have typed the family with 8 microsatellites mapped in the region of chromosome BTA28 identified by association analysis with AFLP data. Association analysis carried out by one way ANOVA revealed a significant association (P<0.05) with the trait considered for 5 microsatellites spanning an interval of about 5 cM. Interval mapping analysis by QTL express software confirms the presence of a QTL in the region identified. Further investigations will aim at refining position and effect of this QTL and at confirming the other chromosomal regions detected.
The BovMAS Consortium: a whole genome scan for quantitative trait loci affecting milk yield and protein percentage in the Italian Holstein Friesian cattle breed by selective milk DNA pooling

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ABSTRACT

Many experiments have been set up to identify Quantitative Trait Loci (QTL) associated with economically important traits in different dairy cattle breeds. The Italian Holstein-Friesian breed is the most important dairy cattle breed reared in Italy. So far no complete genome scan for any production trait has been performed in this population. Here we report on the first complete genome scans in the Italian Holstein-Friesian breed for milk yield (MY) and protein percentage (PP), applying a selective DNA pooling strategy in a daughter design. Eight sires each with more than 3500 lactating daughters were chosen. For each sire, milk pools from about 200 daughters with high and 200 with low DYD values for MY and milk pools from about 200 daughters with high and 200 with low estimated breeding values for PP were constructed. The sires were genotyped for 151 dinucleotide microsatellites distributed over all the bovine autosomes. The milk pools were analysed for the heterozygous markers. Shadow corrected estimates of sire allele frequencies were calculated and differences in sire allele frequencies between high and low pools were computed. A total of 798 and 790 individual sire by marker tests were obtained for the MY and PP genome scan, respectively. An adjusted false discovery rate (aFDR) was applied to calculate the experiment wise significant levels. For MY, 41 sire by marker combinations were significant at aFDR of 0.10. At the marker level, 25 microsatellites were significant at aFDR of 0.05. These 25 significant markers (SM) were distributed over 17 chromosomes: 4 SM were on BTA06 and 4 on BTA07. For PP, 30 sire by marker combinations were significant at aFDR of 0.10. For this trait, at the marker level, 38 microsatellites were significant at 0.05 aFDR. The 38 SM were localized on 22 different chromosomes (3 SM were on BTA02, 6 SM were on BTA06, 3 SM were on BTA14). Allele substitution effect was calculated for all significant sire by marker combinations (aFDR P>0.10) and ranged in absolute value from 398 to 1194 kg for MY DYD and from 0.007 to 0.035 for PP.
The BovMAS Consortium: a complete genome scan for identification of QTL for milk yield and protein percent in the Brown Swiss breed

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ABSTRACT

QTL mapping projects have been implemented for numerous traits mainly in Holstein dairy cattle. The aim of this study is to map QTL for milk yield (MY) and milk protein percent (PP) in the Brown Swiss cattle populations of Austria, Germany and Italy, considered in this study as a single population. A selective DNA pooling approach using milk samples was utilised to map QTL in 10 half sib daughter families of Brown Swiss sires with 1000 to 3600 individuals each. Three families were sampled in Germany, three in Italy, one in Austria and three jointly in Austria and Italy. The pools comprised for each sire-trait combination the 200 highest and 200 lowest daughters, ranked by dam-corrected EBV. For each tail two independent pools, each of 100 daughters chosen at random, were constructed. Sire marker allele frequencies were obtained by densitometry and shadow correction analysis at 139 evenly spaced genome-wide autosomal markers. Significance threshold at 5% FDR level was with nominal value of about 0.03 at the marker level and 0.01 at the sire by marker level. 19 markers were significant for PP, 29 for MY, 80 for both traits, and 11 markers were not significant for either. Two families were excluded from the analysis. Out of the 704 (PP) and 697 (MY) sire by marker combinations, 120 and 110 were significant for PP and MY, respectively, and 27 for both traits. From the combinations significant for at least one of the traits, MY and PP showed the same direction of the effect in 113 instances, and opposite effect in 138 instances. The results of this study were compared with results in the other four resource populations (RPs) which are also part of the BovMAS project: Israeli Holstein (8 families), Italian Holstein-Friesian (10), German-Austrian Simmental (10) and Simmental x Red Holstein Backcross (8). Using an approximate interval mapping procedure a QTL-map was constructed for each of the five RPs. All chromosomes had at least one chromosome-wide significant effect for at least one RP. The chromosome fragments showing a significant effect in four or five RPs were chosen as candidate regions for fine mapping.
The BovMAS Consortium: a combined deterministic stochastic simulation for application of MAS in the Brown Swiss breed

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ABSTRACT

The aim of this study is to assess the possible increase in genetic gain in the Brown Swiss population applying marker assisted selection (MAS) for the pre-selection of young bulls by means of a simulation. For each animal in the base population 29 autosomes, each 100 cM long, harbouring 49 evenly distributed loci (12 QTL affecting four traits and 37 markers) are simulated. At mating each individual randomly inherits one allele of each locus from each parent. Phenotypes and estimated breeding values (EBV) are computed. To result in a realistic polygenic background of the population, the simulation runs through an evolutionary process, a phase of domestication (phenotypic selection on milk yield) and finally, before the introduction of MAS, selection on EBV is performed to mimic intensive selection as conducted in the last few decades. The 5 QTL with the largest allelic substitution effects identified in the genome scan on the Brown population performed within the BovMAS consortium are used as an input to simulate 5 specific QTL in the population. Segregation analysis is performed for each sire of bull to identify the linkage between the QTL and the closest heterozygous marker. Selection of individuals is on EBV for the bull dams and the sire of bull’s pathways. Two different scenarios for selection of young bulls are compared: conventional selection on EBV where young bulls are selected randomly from 2 or 4 full sibs and preselection of young bulls based on marker index (MAS). Within MAS the differences in genetic gain are evaluated for 2 different daughter group sizes for segregation analysis with a 100 or 200 informative daughters per sire, respectively. Genetic gain is investigated for 5 generations in active sires, young bulls, elite dams and first lactation cows. The results of this simulation aim to provide information for the decision-making process of whether or not to implement MAS in the actual breeding strategies for the Brown Swiss population. The simulation can also be a working tool for such kind of decisions for other breeding organisation who would like to explore the extra benefit of MAS with available information for that particular population.
Estimate of variance components for productive, type and functional traits in the Italian Brown Swiss

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ABSTRACT

In recent years the importance of economic impact of functional traits like milk somatic cell score (LS), milking speed (MS), functional longevity (FL) has been identified as a significant source of herd profitability. The National Brown Breeders Association aims to introduce MS and FL as new selection criteria in the economic index. The aim of this study is to estimate the genetic correlation among productive traits, conformation traits, LS, MS, and FL. First lactation records of cows calving from 1985 to 2003 are considered. Editing was performed in order to maximise comparisons across traits originating different data set. Estimates were obtained adopting a sire model with equal design matrix. Canonical transformation was used to calculate correlation estimates among a large number of traits. Herd-year-season, age at first calving, classifier were fixed effects in the model, while days in milk was utilised as linear covariate. LS was defined as the log\textsubscript{2} transformation of milk somatic cell count. MS was defined as the amount of liquid (kg) released per time unit (min.). Cow FL was obtained from total herd life after correction for production level within herd. Estimation were obtained with a multivariate linear sire model using the REML algorithm. Heritabilities for LS, MS, and FL (standard errors in parenthesis) are .14 (.02), .33 (.07) and .05 (.01). The LS shows correlation values of .18 (.09) with milk yield, -.19 (.08) with milk fat content, and -.22 (.08) with milk protein content. The largest correlation estimates between FL and udder traits are with udder depth, -.46 (.07), and with teats length, .22 (.08). The MS is positively correlated with milk production .30 (.18) and with fat and protein yield, .24 (.17) and .16 (.20) respectively while is negatively correlated with protein content -.26 (.16). MS with udder conformation traits shows the largest correlation values with udder width, .55 (.14), udder depth, -.25 (.19) and fore udder attachment, .19 (.17). Finally the correlation between LS and MS has a value of .46 (.26) indicating that faster cows are more susceptible to mastitis, in this study indirectly measured as LS.
Identification of QTL for productive traits and milk somatic cell count in the Italian Simmental cattle

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ABSTRACT

A number of genomic regions have already been reported to affect milk production and functional traits and, in few cases, a specific gene has been identified. The discovery of markers linked to genes affecting the expression of traits of interest to the dairy industry makes possible the inclusion of MAS in ongoing selection schemes and in breeding decisions. In this study a genome scan on selected autosomes was carried out in two families (650 and 480 individuals) of the Italian Simmental population to identify quantitative trait loci (QTL) affecting milk yield (MY), milk protein percent (PP), fat protein content (FP) and milk somatic cell count (MSCC). Samples from all lactating daughters were sampled across Italy and stored at -20°C. To detect suggestive QTL found in other populations, a selective DNA pooling approach, using milk somatic cells as source of DNA, was adopted in a daughter design (DD). Daughters were distributed according to the EBV of each trait corrected for the maternal effect and the best and the worst 20% of the individuals from the distribution, each randomly divided into 2 sub pools, were used to construct the pools. Duplicates of sub-pools were also constituted ending in a total of 64 pools (8 for each sire-trait combination). Milk samples were included in the pools according to their somatic cell count in order to have an equivalent amount of DNA for each individual. Milk PCR was carried out to amplify fragments for subsequent genotyping. Densitometric estimates of sire marker allele frequencies in the pools were obtained after shadow bands corrections. Same frequency of inherited sire marker alleles in both tails was the expected null hypothesis. Significance at 5% adjusted false discovery rate was set as threshold to identify association at marker and sire by marker level. Results of this study indicate association of markers investigated with MK in distal region of BTA1, with FP in proximal region of BTA 3 and BTA 6 and in central region of BTA1, with MSCC in central/distal part of BTA 7 and with PP in central region of BTA1 and 3.
Sexing of beef by PCR analysis of Amelogenin gene

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ABSTRACT

It is well known that male beef is of higher quality than female beef and therefore it yields higher prices; moreover, the intervention buying of EC brought into force to stabilize the market concerns only male beef. This represents a temptation for fraud, that only reliable methods for sexing can hopefully discourage. The PCR-based techniques for sexing have proved to give the most reliable results, being simple, fast and cheap. Among them, the analysis of the Amelogenin gene, developed for embryo sexing (Ennis and Gallagher, 1994), is of particular interest. In cattle there are two different Amelogenin genes, located on sex chromosomes: the Amelogenin gene on chr Y differs from that on chr X for a deletion of 63 bp. Therefore amplification of the region including deletion gives rise to PCR products of different sizes for the two different chromosomes. We applied this method for beef sexing. DNA was extracted from fresh or lyophilized meat using the NucleoSpin® Tissue kit (Macherey-Nagel) and the PCR was performed according to Ennis and Gallagher (Anim. Genet, 1994, 25: 425-427). The reliability of the method was tested on 42 samples from animals of known sex (20 males and 22 females). In addition, 66 meat samples randomly collected in different shops in Piemonte were analysed to verify the declared sex (24 females and 42 males). In the test group, the sex of the animals was correctly determined in 100% of cases, confirming the efficacy of the method. The analysis of the random samples of beef revealed 3 misclassifications (4.5%). Rather unexpectedly, in all cases male beef was labelled as female beef, which could be explained as casual errors in data recording. The results confirm the reliability of the method. Moreover, compared to other PCR-based techniques, it has the great advantage of not requiring any enzymatic restriction nor the use of an internal control to exclude PCR failure in the absence of sex-specific bands. The high percentage of errors in such a limited sample indicates the need for careful controls within the framework of product certification.
MC1R polymorphisms in some Italian local cattle breeds

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ABSTRACT

Coat colour and colour pattern distribution is, in general, a distinctive trait in most cattle breeds. The relative amounts of black/brown (eumelanin) and red/yellow (phaeomelanin) pigments produced in melanocytes are controlled primarily by two loci, Extension and Agouti. The Extension (E) locus produces the melanocortin-1 receptor (MC1R). In cattle, four main alleles have been identified in the MC1R gene: E+, the “so called” wild type allele that produces a variety of colours; E0, the dominant allele that gives black coat colour; e, the recessive allele that yields red/yellow coat colour; E1 that is determined by a 12 bp duplication and whose effect on coat colour is not well understood. As a first step to identify breed specific DNA markers that could be used for the traceability of dairy cattle products obtained with “mono-breed” milk, here we investigated the presence and distribution of the mentioned alleles at the MC1R locus in some local breeds that have different coat colour: Reggiana (red or fromentino), Bianca Val Padana (white) and Rendena (dark brown). A total of 442 animals (Reggiana, 284; Bianca Val Padana, 74; Rendena, 84) were genotyped at this locus using a PCR-restriction fragment length polymorphism (RFLP) protocol and a PCR-amplified product length polymorphism (APLP) method. Sequencing of a 740 bp fragment of the MC1R gene was carried out to confirm the results of the PCR-RFLP and PCR-APLP genotyping techniques. All Reggiana animals were homozygous “ee”. This result confirm the effect of the e allele on coat colour in this breed. This allele was also identified, but with much lower frequency, in Bianca Val Padana (0.04) and in Rendena (0.01). In Rendena allele E+ was 0.73 and allele E1 was 0.26. Allele E0 was not observed in any of the three considered breeds. Comparing these data with results previously obtained for other major cattle breeds, it could be possible to consider, at least in some cases, the MC1R locus to distinguish or to exclude the dairy products obtained from milk of some breeds.
Horse coat colour genes: relationship between genotype and phenotype

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ABSTRACT

The horse coat colour description is based on detailed terminology traditionally used to identify single animals. In 1991, Lauvergne et al. proposed a new phenotypic description, based on the scientific knowledge then available, with 4 dimensions (pigmentary pattern; type of eumelanin; pigment alterations: dilution or intermixing; white patches). This study, based on PCR analyses of 80 horses chosen according to coat colour, aims at comparing the known polymorphisms of the MC1R, ASIP, TYRP1 and MATP genes with this description of equine coat colour. Our results confirm the correspondence between the first dimension of Lauvergne’s description and the observed genotypes for MC1R and ASIP genes. 15 black eumelanic/black-nearly black animals are homozygous for 11 bp deletion in exon 2 of the ASIP gene (a allele), while 31 pheomelanic/chestnut-sorrel horses are homozygous for C901T mutation at MC1R gene (e allele). 32 animals with different pigmentation patterns (pheomelanic with black eumelanic extremities/bay-dark bay, eumelanic and tan/dark bay and horses with spinal stripe - wild pattern pigmentation) are characterized by the absence of these two mutations at the homozygous state. We have also observed the presence of the epistatic effect of MC1R/e allele on ASIP/a allele in the genotype eeaa carried by one chestnut horses. Furthermore we observed a particular genotype at MATP gene and a specific shade of coat colour dilution characterizing some equine phenotypes. In fact, the 2 white/perlino horses of our panel are homozygous for G72A mutation at MATP gene. The 4 dilute/palomino-buckskin animals are heterozygous for this substitution. Whilst, all the 74 solid coat colour horses are characterized by the absence of this mutation. No relation was found between the C189T substitution in exon 2 of the TYRP1 gene and horses with brown or chestnut phenotypes. Further studies are needed to explain molecular background of brown coat colour in horses and in other mammalian species such as bovine. The other dimensions of Lauvergne’s description could be better explained when more causative polymorphisms of pigment alterations and white patches and more information about the epigenetic control of pigmentation have been studied.
Analysis of the myostatin gene in different horse breeds

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ABSTRACT

Myostatin (MSTN), previously called growth and differentiation factor-8 (GDF8), is a member of the transforming growth factor beta (TGF-b) superfamily that acts as a negative regulator of muscle growth in mammals. The aim of the work is to study the sequence, the genomic organization and polymorphisms of the horse MSTN gene. At this purpose we used PCR, sequencing of PCR products and PCR-RFLPs analysis. The PCR primers were designed based on available horse cDNA sequence, our sequence data, and conserved sequences within the promoter of the cattle, pig and human MSTN gene. Ten primer pairs were used to amplify regions of MSTN in nine animals of the following nine breeds: Italian Heavy Draught (IHD), Breton (BR), Bardigiana (BA), Haflinger (HA), Ventasso Horse (VE), Italian Trotter (IT), Italian Saddle Horse (IS), Thoroughbred (TH) and Arabian (AR). The PCR products of the ten primer pairs were sequenced. On the basis of our sequencing results and of the available MSTN gene sequences in other species the genomic organization of horse MSTN was determined. The nucleotide sequences of the three exons and of the two introns were determined. The introns 1 and 2 have 1828 nt and 2018 nt, respectively. Parts of the promoter region (663 bp upstream to ATG translation start codon) and 85 bp of the 3’UTR were also sequenced. The promoter region of the horse MSTN gene contains several potential transcription factors binding sites. Of particular interest was the identification of four E boxes which are binding sites for the myogenic regulatory factors and of a region homologous to MEF2 binding site. The sequence data obtained for the PCR-products were aligned and compared. A polymorphism at –646 and a polymorphism at –516 (relative to ATG) were found. Five polymorphism within the intron 1 and one within the intron 2 were also detected. For two SNPs, the T→C at –646 and the A→C in intron 1, position 1282 from the exon/intron boundary, PCR-RFLP protocols were set up and further one hundred animals of eight breeds (IHD, BR, BA, HA, VE, IT, IS and TH) were genotyped. Differences in allelic frequencies between the breeds were observed for both polymorphisms.
Evaluation of the genetic diversity in some Sicilian horse populations by microsatellites

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ABSTRACT

Scarce data are currently available on the genetic diversity of autochthonous horse breeds of Southern Italy. In Sicily three horse populations are reared, that play an important role in the local habitats. The genetic diversity of these populations (Sanfratellano - SAN, Sicilian Oriental - ORI and Sicilian Indigenous - IND) has been investigated by microsatellite markers, in order to improve their genetic characterization and to define their within-breed variability. The study was carried out on 150 horses (70 SAN, 50 ORI and 30 IND). Blood samples were collected from unrelated animals belonging to Sanfratellano and Sicilian Indigenous Horse populations, whereas in the case of Sicilian Oriental breed the sample consisted of nearly the whole population. Twelve microsatellite markers (HTG6, HTG10, VHL20, HTG7, HTG4, AHT5, AHT4, HMS3, HMS6, HMS7, HMS2 and ASB2) were amplified in a multiplex reaction. The PCR products were mixed with GeneScan 350 ROX internal size standard. Gel electrophoresis and genotype determination were performed on an ABI Prism 377 DNA Sequencer equipped with Genescan and Genotyper softwares. The GENEPOP and DISPAN packages were used to calculate allele frequencies, expected and observed heterozygosity, gene diversity parameters and genetic distances. All microsatellites were polymorphic in each breed. A total of 113 alleles (from 4 to 13 alleles per locus) were detected. The number of observed alleles was higher in IND (87) than in SAN (85) and ORI (73). The three populations showed good levels of heterozygosity, higher in IND and SAN (0.803 and 0.751, respectively) than in ORI (0.702). The genetic differentiation coefficient was low (Gst=0.052). The dendrogram, constructed from the genetic distance matrix using Neighbor-Joining algorithm, reflects the different phylogenetic origin of the populations: IND and SAN are the less distant (0.0803), while their distances from ORI are 0.1526 and 0.1882, respectively.
Genetic diversity in three Italian donkey populations assessed by microsatellite markers

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ABSTRACT

We analysed the genetic variability of three Italian donkey populations, Romagnolo (ROM), Ragusano (RAG) and Martina Franca (MF), using 18 horse microsatellites approved for identification and parentage test by International Society for Animal Genetics (ISAG). We tested 100 unrelated animals: 46 Romagnolo, 19 Ragusano and 35 Martina Franca. The 18 microsatellites were amplified in two multiplex-PCR, one with 12 and the other one with 6 microsatellites. We calculated: - the allele number and the allelic frequency; - the Polymorphism Information Content (PIC); - the heterozygosity; - the deviation from Hardy-Weinberg equilibrium (HWE); - the Inbreeding coefficients (F IS); - the Wright diversification (F ST) according to Weir and Cockerham, using the computer packages GENEPOP and Fstat. We calculated genetic distance using the packages Microsat and PHYLIP. As regards the amplification in the asinine species, we had positive results for all horse microsatellites, except for the locus ASB2, that had null amplification in all the samples. The loci HMS1 and HMS8 resulted monomorphic. The total number of the alleles was 117. Considering the three tested donkey populations, allele number varied from 4 for AHT4 locus to 15 for HTG7 and AHT5 loci; PIC varied from 0.874 for AHT5 to 0.161 for HTG6. The mean heterozygosity for each population was: 0.530 (Romagnolo), 0.402 (Ragusano) and 0.459 (Martina Franca). The inbreeding coefficient (F IS) was 0.0571±0.02, while the Wright diversification (F ST) was 0.0856±0.02. Most loci did not show a significant deviation from HWE. According to Neighbour-joining diagram of the individual distance, we observed a significant assembling (90.3%) only for Martina Franca population. We observed a considerable genetic variability even if the three populations are similar and show reciprocal influences, considering all tested parameters.
Study on PrP locus in four Veneto sheep breeds

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ABSTRACT

A conservation scheme is currently running for four indigenous Veneto sheep breeds. Aim of this project is to characterize for PrP locus the Alpagota, Brogna, Foza and Lamon sheep breeds. To safeguard an endangered breed is important to know all the genetic characteristics, in particular the susceptibility and incidence of severe diseases. Scrapie is one of a group of slow neurodegenerative diseases known as the transmissible spongiform encephalopathy (TSE) in the small ruminant species. This disease is characterized by a long incubation period and by slowly progressing neurological signs and occurs naturally in sheep, goats, and mouflon. The occurrence of natural scrapie seems to be influenced by polymorphisms at codons 136, 154 and 171 of the host gene that encodes the prion protein (PrP). The indigenous sheep breeds analyzed were Alpagota (50 animals), Brogna (30 animals), Foza (23 animals) and Lamon (16 animals). The analysis of PrP polymorphisms at codons 136, 154 and 171 was carried out by the allelic discrimination technique and Real Time PCR. DNA was extracted from rams and ewes by means of High Pure PCR Template Preparation Kit (Roche). The allelic discrimination technique is based on the 5’ nuclease assay and allows distinguishing between two alleles of a single nucleotide polymorphism (SNP). PCR amplification and allelic discrimination analysis were carried out using the ABI PRISM 7700 apparatus (Applied Biosystems) following standard thermal profiles. The Hardy-Weinberg equilibrium within breed was studied with a chi-squared test. The average allele frequencies at locus PrP were: 5% for VRQ, 7% for AHQ, 68% for ARQ, 1% for ARH and 19% for ARR. Chi-square test did not show any statistical difference. The ARR/ARR and VRQ/VRQ genotypes were respectively the 7% and 1% of the observed genotypes. The low frequency of VRQ allele and high frequency of ARQ allele found in this study suggest a large monitoring for scrapie genotyping in the whole Veneto indigenous sheep populations.
Preliminary allele frequencies for the PrP gene of Valle del Belice pilot farms

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ABSTRACT

To avoid human health risks the EU is implementing policies for selection against certain alleles of the PrP gene. Generally, PrP alleles are described for polymorphisms linked to scrapie susceptibility at three codons (136, 154 and 171). At these codons, the following alleles are distinguished: ARR, AHQ, ARH, ARQ and VRQ in order of increasing susceptibility. An investigation of the PrP allele frequencies of 482 Sicilian Valle del Belice dairy sheep in three flocks is presented. Verification of Hardy-Weinberg equilibrium resulted in a probability of 0.41. Therefore the population was not in disequilibrium and selection did not strongly affect the PrP locus. A simulation of drift with 1 million repeats resulted in approximate confidence intervals of allele frequencies. Absence of selection and mutation was assumed. The following PrP allele frequencies and 95% confidence intervals were estimated ARR (36.7%; 27-46%), AHQ (3.6%; 0-7%), ARH (1.1%; 0-3%), ARQ (57.3%; 47-67%) and VRQ (1.2%; 0-3%). The most susceptible allele VRQ appears to have a very low frequency and therefore it can be eradicated from the population relatively fast. If necessary all carriers could be culled. Also ARH is at such a low frequency that it could be eradicated easily. The second most susceptible allele ARQ is however the most frequent allele found. This allele can only be removed in several generations. The eradication of ARQ can be achieved by using only sires which are not carriers of ARQ and understandably not of ARH and VRQ either. The 95% confidence intervals indicate that the VRQ allele is highly likely to remain at a low frequency because its frequency could only be substantially increased if a disproportionate part of the sires used are carriers. The chance of that occurring is low. The 2.5% of the genotypes which include a VRQ allele are considered as highly susceptible genotypes. The resistant animals are ARR/ARR, ARR/AHQ, ARR/ARH and ARR/ARQ, which together comprises 59.8%. The remaining 37.8% is considered less resistant to scrapie. In total 84.6% of the animals carries at least one ARQ allele and 61% carries an ARR allele.
Polymorphism of the \textit{CSN1S1}, \textit{CSN1S2} and \textit{CSN2} genes in Sarda bucks

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ABSTRACT

This research was carried out on 50 Sarda bucks, belonging to 15 farms in the Province of Nuoro, assigned to natural reproduction. The aim was to assess allele frequencies of calcium-sensitive caseins. At the αs1-casein locus the presence of the F allele, was investigated by restriction fragment length polymorphism (PCR-RFLP; Ramunno \textit{et al.}, 2000). This method allows distinction between F and strong alleles A, B and C (indicated as A*). The E and O1 alleles were investigated according to Jansa-Perez \textit{et al.} (1994) and to Cosenza \textit{et al.} (2001) respectively. At the αs2-casein locus the A, B and C alleles were investigated by allele specific PCR (AS-PCR; Ramunno \textit{et al.}, 2000). The E allele (Veltri \textit{et al.}, 2000) and the F, D and O alleles (Ramunno \textit{et al.}, 2001) were detected by PCR-RFLP. At the β-casein locus AS-PCR allowed distinction between A and O alleles (Ramunno \textit{et al.}, 1995). The results at the \textit{CSN1S1} locus showed the following genotype frequencies: A*A* 0.60, A*F 0.32, FF 0.08; no subject was found to carry the intermediate (E) and null (O1) alleles. At the \textit{CSN1S2} locus the frequencies were: AA 0.42, AC 0.06, AF 0.10, BB 0.02, CC 0.22, CF 0.10, EE 0.02, FF 0.06; the D and O variants were not detected. It is clear from the excess of homozygotes, a significant deviation from Hardy-Weinberg expectations. This result, which may be due to the small sample size, has been observed at the \textit{CSN1S2} locus also in Girgentana breed goats. Genotype frequency at the β-casein locus was: AA 0.82, A0 0.18. On the whole, 21 genotypes were found; the most frequent genotype combination (26%) was homozygote AA at all loci. These results confirm that the Sarda goat mostly produces a kind of milk suitable for cheese making. Nevertheless, genetic variability is such that allows the setting up of genetic lines for production of drink milk. Considering the goals reached in the field of molecular biology, and the great applicability of genomic investigations, it is desirable that it will be considered, at least for those males that have to be chosen for reproduction, the possibility of genetic characterisation for the major milk protein genes.
Characterization of eight single nucleotide polymorphisms (SNPs) in sheep

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ABSTRACT

Single nucleotide polymorphisms (SNPs) showing different frequencies among breeds may be used in biodiversity studies and in commercial tasks like traceability, paternity testing and selection for suitable genotypes. For SNP discovery and characterization in sheep, we designed pairs of PCR primers on genomic sequences published in Genebank belonging either to Ovis aries or to the most genetically related species available. PCR amplifications and sequencing of genomic fragment were performed in a panel of 16 unrelated sheep belonging to 8 different European breeds. The sequences obtained were BLASTed against those published in Genebank to validate their identity. Eight SNPs (in genes GHRHR, TYRP1, IGF1, MSTN, GHR, CTSB, ITGB1, MYH1) were characterised and genotyped on 1900 individuals belonging to 57 breeds to assess the basic population parameters.

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Analysis of genetic differentiation of a sheep population by Cluster Analysis on milk serum proteins: preliminary results

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ABSTRACT

The aim of this research was to evidence if the animals with similar phenotypic quantitative expression of milk whey proteins, present also a high genetic similarity and some alleles with a particularly high frequency. In a previous work several microsatellites with alleles significantly linked to milk whey proteins have been pointed out. A cluster analysis, with the link “Sum of Square” method, has been made on milk serum proteins of 68 Massese ewes reared in a farm of Tuscany. Besides the serum protein content, also standard chemical analysis, casein and its fractions, pH and rheological parameters were considered. For this study 17 microsatellites were analyzed using an ABI PRISM 310 automated sequencer. Genetic similarities within and among clusters were estimated using the Individual Multilocus Genotype (IMG) and the differences in milk quality among groups were tested by ANOVA. Three clusters respectively of 33 (group 1), 15 (group 2) and of 20 subjects (group 3) have been highlighted. Significant differences in milk traits were found mainly between the group 3 and the other groups, for serum proteins, with the exception of the β-serumalbumin, and also the majority of the parameters. In particular group 3 has a greater significant value of total serum protein, α-lactoalbumin, immunoglobulin, β-CN, γ-CN, lactose, and fat. The average similarities within the groups resulted respectively of 0.410, 0.410 and 0.442 (total average similarities 0.416). The group 3 presented not only a high genetic homogeneity, but also some alleles with different frequency in comparison with the other groups. In particular such group presents a higher percentage of the OMHC1, allele 4 associated with a low immunoglobulin value, of the OIFNG allele 1 associated with a higher content of α-lactoalbumin value, and of the BL4 allele 10 associated with low immunoglobulin value and high γ-CN value. None of the subjects of group 3 presents the allele 2 of OIFNG, allele linked with low α-lactoalbumin and lactose values, and high immunoglobulin, β-CN and fat values. The study has allowed to point out a subpopulation more genetically and phenotypically differentiated from the other groups.
Multilocus genotype assignment methods among six ovine breeds

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ABSTRACT

One of the most important problems in the rearing of small ruminant breeds, especially if they are at risk of extinction, is the breeding management. Too often in fact we are not able to infer exactly the belonging of two or more similar subjects to a particular breed only on the basis of the morphological traits. On the other hand, if we are in presence of a breed at risk of extinction, in which the inbreeding level is high, we could try to perform a crossbreeding with another breed, but how can we choose the appropriate breed? Microsatellites are particularly suitable for this kind of studies because they could be multiplexed and used in an automated sequencer, so we can analyse a relatively high number of loci in a short time. Aim of this work is to test the reliability of a genetic assignment of 5 ovine Italian breeds, namely Pomarancina, Garfagnina Bianca, Appenninica, Massese, and Zerasca (a Mexican breed considered as an out-group), by the use of a small number of microsatellites. Assignment tests are one of the most suitable tools in evaluate breeds like these, where a lack of genealogical information exists. In order to perform the genetic assignment 42 blood samples for each of the mentioned breeds were obtained with single-use Na-Citrate Vacutainer, for a total of 252 head. DNA was isolated from whole blood samples with a “Nucleo Spin Blood®” commercial kit following the manufacturer’s procedures. A total of 10 microsatellite loci were analyzed (MCM8, MCM11, OARAE119, FCB4, MAF70, OARCP49, JPM29, HH55, VH72, JPM8). We evaluate three main assignment methods: Bayesian, distance based, and frequencies based. The assignment test was carried on using the Geneclass II and Arlequin software packages. Analyzing the different computational methods we can confirm that Bayesian and frequency-based methods are in general more accurate than distance-based ones (under Infinite Allele Model). Results from log-likelihood are also useful to obtain a visual representation of the genotypic assignment, and also to put in evidence which genotype is closer to the others. Finally, we can suggest that in order to obtain a satisfactory genotypical assignment 10 loci could be adequate, and that the best assignment method results the Bayesian one.
Associations between microsatellite markers and milk traits in Massese sheep: preliminary results

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ABSTRACT

Current research aims to establish statistical associations between DNA microsatellites and milk chemical composition to be used for improvement procedure and genetic progress. A trial was carried out on 68 Massese ewes reared in a farm of the Tuscany. On fresh milk the following parameters were evaluated: standard chemical composition, casein and its fractions, whey proteins, pH and rheological parameters. On DNA extracted from peripheral blood, 17 microsatellites were analyzed using an ABI PRISM 310 automated sequencer. Genetic similarities among individuals have been tested using the Individual Multilocus Genotype (IMG) and classical genetic parameters using the software Arlequin. For each locus, the significance of the differences of each quality traits between the subjects carrying or not carrying a given allele, was estimated using the software J.M.P. The average number of alleles per locus resulted of 7.18 and the observed heterozygosity ranged from 0.403 to 0.867 (0.677 mean value). The genetics similarity among individuals was 0.460 (0.018 SD). Five markers pointed out a significant deviation from the Hardy-Weinberg proportions (BM8124, CSN3, BM1258, BMS468 and TGLA387); we found quite a high rate of linkage disequilibrium partly because many loci mapped on the same chromosome. The study revealed several microsatellites with alleles significantly linked to milk composition traits (P<0.01). In particular the highest significance (P<0.001) has been found for the OIFNG marker, for which subjects carrying the allele 2 showed higher values of immunoglobulin and lower values for α-lactoalbumin. On the other hand, subjects carrying the allele 10 of the BL4 marker showed significantly lower value of immunoglobulin (P<0.001). We can also assume that allele 2 of BMC1009 influenced fat, while allele 9 of ILSTS42 influenced a30 (P<0.001). Further analyses are needed to validate these preliminary results, in particular increasing the number of subjects and of typed loci above all on the chromosomes 3 and 20 where the more interesting markers map.
Validation of oligo-array LPL and SCD expression profiles in sheep mammary gland by real-time PCR

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ABSTRACT

Real-Time PCR method and cDNA sequencing were applied to validate differentially expressed genes (Lipoprotein Lipase and Stearoyl-CoA-Desaturase) identified in a previous work by DNA oligo-array in the comparison of two Italian sheep breeds; Sarda and Gentile di Puglia. An investigation of the results obtained with DNA oligo-array approach could bring to identify mutations in the promoter region and/or in the codifying gene and also this study could be important in the perspective of animal breeding. A biopsy of the mammary gland has been effected in three stages of the lactation, beginning, middle and end, to be able to effect a comparison of gene expression pattern both among the two breeds and among groups of the same breed in different physiological stages. Real-Time PCR assay was performed by using ovine Glyceraldehyde 3-phosphate Dehydrogenase as gene reference and the amount of target, normalized to an endogenous reference and relative to a calibrator, was calculated by using the comparative CT method. Gene codifying for ovine Lipoprotein Lipase (LPL) showed an expression profile in mammary gland completely different in the two sheep breeds during the three stage of lactation. It can be concluded that Real-Time PCR approach confirms that the LPL gene is more expressed in Sarda breed rather than in Gentile di Puglia. Gene codifying for ovine Stearoyl-CoA-Desaturase (SCD) was first analysed for cDNA structure in five Sarda sheeps and three Gentile di Puglia sheeps belonging to the group analysed by DNA oligo-array approach. An individual of this group showed high SCD expression at the end of lactation. The alignment of cDNA sequences was conducted by using MultiAlign program and it revealed (by comparing to the EMBL sequence) in all the analysed animals the existence of three polymorphic sites which cause a change in amino-acid sequence and one in the 3’-UTR region without aminoacidic differences. Moreover the animal with a higher expression profile did not show any exclusive mutation.
Evaluation of the expression of $\text{H}^+$/K$^+$ ATPase gene by real time RT-PCR in fundic gastric mucosa of weaned pigs

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ABSTRACT

Gastric acid secretion contributes to the gut barrier against pathogens. However, during suckling, pigs show a reduced hydrochloric acid secretion, in proportion to fermentative activity of milk lactose in the stomach. When the passage of maternal milk in the stomach ceases at the moment of weaning, the acid secretion in the stomach is still limited. Then, the progressive intake of solid feed will gradually stimulate this secretion. H$^+$/K$^+$-ATPase is responsible for acid secretion into the stomach and catalyses electro neutral exchange of cytoplasmic H$^+$ and external K$^+$ coupled with ATP hydrolysis. The aim of the research was to set up in fundic gastric mucosa a technique to quantify the expression levels of gene involved in H$^+$/K$^+$-ATPase production during weaning by Real Time RT-PCR. To quantify the copy number of the H$^+$/K$^+$-ATPase gene, the absolute quantification method, by using an external standard curve (MSE 0.125, r=-1, slope=-3.576; intercept=39.23), was performed on stomach samples. The nucleic acid sequence of pig gastric H$^+$/K$^+$-ATPase was found in GenBank (accession number: M22724) and the external and internal primers were designed by OLIGO Primer Analysis Software version 5.0, respecting the parameters suggested by Roche. Each final value was obtained from at least 2 replicates per amplification in at least 2 different days. Data obtained from 60 weaned pigs showed a great variability with a minimum of 10 copies/µl to a maximum of 471,398 copies/µl, with a standard deviation of 72,726 and an average value of 21,847. The large individual variability could be explained by the fast regulation on parietal cell, which occurs after various stimuli. The evaluation of the expression H$^+$/K$^+$-ATPase gene was useful to evidence the effect of different diets on gastric functionality. We also observed a positive correlation (r=0.385, P<0.01) of this parameter with the number of parietal cells. The Quantitative Real Time RT-PCR technique permitted to cover the lack of literature concerning the gene expression in pig gastric mucosa with a sensitive method, detecting even low amount on mRNA molecules. It so offers important physiological insights on mRNA expression level.
Genetic characterization of Casertana pig breed using microsatellite markers

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ABSTRACT

The Casertana is an autochthonous pig breed of ancient origins, found in southern Italy, for centuries appreciated for the quality of its productions. Currently the breed consists in very few individuals often crosses with cosmopolite breeds, so its status is defined “critical” by the Food and Agricultural Organisation (FAO). The aim of this work was to verify the genetic identity of Casertana (CE) and to compare the genetic structure of the breed to four cosmopolite breeds: Large White (LW), Duroc (DU), Pietrain (PT) and Landrace (LD). The genetic characterization has been realised analysing 5 microsatellite loci in 24 individuals for Casertana, Pietrain and Landrace and 23 individuals for Large White and Duroc breeds. Markers were chosen within the FAO World Watch List of Domestic Animal Diversity (FAO WWL-DAD). DNA has been extracted from the whole blood, microsatellite loci have been amplified by means of PCR, amplified fragments have been separated by capillary electrophoresis. Standardisation has been carried out analysing four control samples (2 French F1 animals and 2 Swedish F1 animals) chosen within the PiGMaP reference families. For each locus, allelic number and frequencies, observed and expected average heterozygosity based on Hardy-Weinberg equilibrium have been computed. The coefficient of inbreeding ($F_{IS}$) for each locus and breed has been calculated and the genetic variability within breeds has been estimated by fixation index, $F_{ST}$. Genetic distances (Chord distance, $D_{c}$) among breeds have been obtained, which were used to construct a Neighbour-Joining tree (unrooted tree). The average number of alleles ranges from 3,8(CE) to 6,2 (LD). Locus heterozygosity varies from 0.417 to 0.792 for the CE breed, with a estimate mean of 0,633, while mean heterozygosity of cosmopolite breeds ranges from 0.532 (DU) to 0.705 (PT). “Important” differentiation achieves among breeds according to the Wright classification by the total $F_{ST}$ value (0.177). In the Neighbour-Joining diagram CE results to be closely related (bootstrap value 95/126) to DU breed. Equally LD results related to LW. PT breed is in a single branch.