UNIVERSITA' DEGLI STUDI DI PADOVA

Sede Amministrativa: Università degli Studi di Padova

Dipartimento di Scienze Animali

SCUOLA DI DOTTORATO DI RICERCA IN SCIENZE ANIMALI

INDIRIZZO: GENETICA, BIODIVERSITÀ, BIOSTATISTICA E BIOTECNOLOGIE
CICLO XXI

INSTITUT NATIONAL POLYTECHNIQUE DE TOULOUSE

SCIENCES ÉCOLOGIQUES, VETERINAIRES, AGRONOMIQUES ET BIOINGENIERIES (SEVAB)

GENETIC, PHENOTYPIC AND PROTEOMIC CHARACTERISATION OF LOCAL CHICKEN BREEDS

Direttore della Scuola : Ch.mo Prof. Luigi Gallo

Supervisori : Ch.mo Prof. Martino Cassandro

Ch.mo Prof Hervè Remignon

Dottorando : Enrico Zanetti

CONTENTS

Summary 5

Riassunto 7

Résumé 9

General Introduction 11

1. Animal genetic resources 11

1.1 Risk status classification 13

1.2 Status of livestock genetic resources 15

2. Avian species and chicken breeds 18

2.1.1 European breeds 18

2.1.2 North American breeds 19

2.1.3 Commercial strains 19

2.1.4 Breeds from other areas 19

2.2 Status of avian genetic resources 20

3. The objectives for conservation 24

3.1 Economic potential 24

3.2 Scientific use 26

3.3 Cultural interest 27

4. Methods for breed characterisation 28

4.1 Molecular genetic level characterisation 28

4.2 Phenotypic qualitative characterisation 29

4.3 Proteomic level characterisation 30

5. An Italian conservation example 32

Literature cited 35

Objectives 39

First contribute: GENETIC CHARACTERIZATION AND POPULATION STRUCTURE OF ITALIAN LOCAL CHICKEN BREEDS UNDERGOING IN-SITU CONSERVATION 41

Second Contribute: CARCASS CHARACTERISTICS AND QUALITATIVE MEAT TRAITS OF THREE ITALIAN LOCAL CHICKEN BREEDS 67

Third Contribute: A PROTEOMIC APPROACH TO STUDY LOCAL CHICKEN BREEDS CHARACTERIZATION 83

General discussion and conclusion *97*
List of publications *99*

SUMMARY

In common domestic species for which varieties, strains or breeds are in danger of extinction, the population levels at which action needs to be taken are object of research in many countries. Different approaches have been developed and exploited to understand the different aspects that contribute to breed differentiation and to study the typical products that originate from them.

The thesis is made up of three contributes. The objectives of the first one were to determine genetic variation and to analyze population structure in six Italian local chicken breeds involved in a conservation program. Twenty microsatellite markers were investigated in 337 animals belonging to six breeds: Ermellinata di Rovigo, Robusta Maculata, Robusta Lionata, Pepoi, Padovana and Polverara; a commercial layer cross was used as reference. One-hundred-twelve alleles were detected in the overall population, with a mean number of 5.6 ± 2.1 alleles per locus. For the local breeds, the observed and expected heterozigosity ranged from a minimum of 0.240 to a maximum of 0.413 and from 0.243 to 0.463 for the Pépoi and Polverara breeds, respectively. Deviation from Hardy-Weinberg equilibrium has been observed in five breeds and in the commercial cross. The overall population heterozygote deficiency F_{IT}, resulted 0.427, the average F_{IS} 0.097, while F_{ST} was 0.437, indicating a high heterozygote deficiency mainly due to breed subdivisions. Reynolds distances were used to draw an unrooted Neighbor-Joining tree, which topology gave information on the genetic origin of these breeds and confirmed their known history. The estimated molecular kinship within breed ranged from 0.559 to 0.769, evidencing high coancestry. Structure analysis was performed to detect the presence of population substructures. Inferred clusters corresponded to the different breeds, without presence of admixture. Exception was the Polverara, for which a more complex genetic structure was found. Obtained results confirmed the usefulness of molecular markers, as microsatellites, to characterize local breeds and to monitor genetic diversity in livestock conservation schemes.

The objective of the second contribute was to describe carcass characteristics and qualitative meat traits of three local chicken breeds showing, at maturity, light, medium-light, and medium live weights. By the fact, those breeds could permit to extend and diversify consumer's offer to fit all the local demands in typical diversified poultry products. The experiment involved 60 male chickens reared in an organic production system where housing was an indoor pen with access to a grass paddock was carried out in order to investigate carcass characteristics and qualitative meat traits of three slow-growing Italian local breeds of chicken

(Ermellinata, Padovana, and Pépoi). Chicks were randomly selected at hatch, raised together under the same conditions, slaughtered at 190 days of age, dissected for carcass traits and meat was stored for subsequent analysis of breast and thigh meat quality. Ermellinata chickens were consistently heavier than Padovana and Pépoi chickens for live, carcass, thigh weight and there were differences among breeds for protein percentage (Ermellinata > Pépoi and Padovana), shear force (Padovana < Ermellinata and Pépoi), and cooking loss (Pépoi > Padovana and Ermellinata). The CIE system values of lightness (L*), redness (a*), and yellowness (b*) evidenced a distinctive darker and lighter colour of Padovana for meat and skin, respectively. Fatty acid composition of breast was similar among the studied breeds, while saturated and monounsaturated fatty acids contents of Ermellinata were higher and lower, respectively than the other breeds.

Aim of the third study was to apply a proteomic approach for characterization of local chicken breeds. The experiment involved a total of 29 males of Pépoi, Padovana, and Ermellinata local chicken breeds. Samples were taken from breast muscle (*Pectoralis superficialis*). Sarcoplasmic protein fractions of breast muscle were analysed by bidimensional electrophoresis. Image analysis followed by statistical analysis enabled to differentiate groups of individuals on the similarities of protein expression. Individuals were distinguished into clusters and groups, corresponding to the breed of origin. SAM analysis enabled identification of the most relevant spots; 10 of these were identified by Mass Spectrometry revealing preliminary evidences on the mechanics of the breed differentiation process. Results evidenced a possible utilisation of proteomic approach in the field of breed characterization studies as an alternative to genomic analyses performed using molecular markers, both for breed and product traceability purposes.

RIASSUNTO

Nelle comuni specie domestiche, alcune razze, varietà o popolazioni risultano a rischio di estinzione. Molte di queste, per le quali si devono prendere provvedimenti, sono oggetto di studio e ricerca in molti paesi. Numerosi approcci sono stati sviluppati ed utilizzati per comprendere i diversi aspetti che contribuiscono alla differenziazione delle razze e per lo studio dei prodotti che da esse derivano.

Questa tesi risulta costituita di tre contributi. Gli obbiettivi del primo riguardavano lo studio della variabilità genetica e l'analisi della struttura di popolazione in sei razze locali italiane di pollo coinvolte in un progetto di conservazione. Sono stati analizzati venti marcatori microsatellite in 337 animali appartenenti a sei razze diverse: Ermellinata di Rovigo, Robusta Maculata, Robusta Lionata, Pépoi, Padovana e Polverara; una linea commerciale ovaiola è stata utilizzata come riferimento. Sono stati rilevati centoventi alleli nel campione complessivo, con un valore medio di 5.6 ± 2.1 alleli per locus. Per quanto riguarda le razze locali, l'eterozigosi osservata variava da un minimo di 0.240 ad un massimo di 0.413 e l'attesa da 0.243 a 0.463, rispettivamente per le razze Pépoi e Polverara. Sono state osservare deviazioni dall'equilibrio di Hardy-Weinberg per cinque razze oltre che per l'incrocio commerciale. Nell'insieme, la deficienza complessiva di eterozigoti nella popolazione (FIT) risultava 0.427, il valore medio di FIS 0.097, mentre l'FST era 0.437, indicando un alta deficienza di eterozigoti dovuta soprattutto alla suddivisione in razze. Sono state utilizzate le distanze di Reynolds per tracciare un albero Neighbour-Joining unrooted, la cui topologia ha fornito informazioni sull'origine genetica di queste razze e ha confermato la loro storia conosciuta. La kinship molecolare stimata entro razza variava da 0.559 a 0.769, evidenziando un alto valore di coancestry. L'analisi della struttura è stata effettuata per evidenziare la presenza di sottostrutture nella popolazione. I cluster ottenuti dividevano chiaramente gli animali in gruppi corrispondenti alle diverse razze, senza mescolanza. Eccezione a questa situazione erano gli animali appartenenti alla razza Polverara, per la quale è stata riscontrata una struttura genetica più complessa. I risultati ottenuti hanno confermato l'utilità di marcatori molecolari come i microsatelliti per la caratterizzazione delle razze locali e per il monitoraggio della diversità genetica negli schemi di conservazione degli animali domestici.

L'obiettivo del secondo contributo è stato di descrivere le caratteristiche della carcassa e i caratteri qualitativi della carne di tre razze locali di pollo che mostravano, alla maturità, pesi vivi medi, medio leggeri e leggeri. In particolare, lo sfruttamento commerciale delle

razze analizzate potrebbe permettere di estendere e diversificare l'offerta ai consumatori locali che richiedono prodotti avicoli diversificati. L'esperimento ha coinvolto 60 polli di sesso maschile allevati in un sistema di produzione di tipo biologico, con accesso ad un areale esterno a prato, con l'obiettivo di studiare le caratteristiche della carcassa e i caratteri qualitativi della carne di tre razze locali italiane a lento accrescimento (Ermellinata, Padovana, and Pépoi).

Gli esemplari sono stati scelti a caso alla schiusa, allevati assieme nelle stesse condizioni e macellati a 190 giorni di età. Dopo aver misurato i parametri qualitativi della carcassa, sono stati analizzati campioni di petto e di coscia. La razza Ermellinata è risultata consistentemente più pesante che la Padovana e la Pépoi in termini di peso vivo, il peso della carcassa e della coscia; inoltre si riscontravano differenze nella la percentuale di proteina (Ermellinata > Pépoi and Padovana), per quanto riguarda lo sforzo di taglio (Padovana < Ermellinata and Pépoi) e la perdita di cottura (Pépoi > Padovana and Ermellinata). I valori di luminosità (L*), indice del rosso (a*) e indice del giallo (b*), che fanno parte del sistema CIE, hanno evidenziato un colore più chiaro della carne e più scuro della pelle della pelle della Padovana. La composizione degli acidi grassi del petto è risultata similare tra le razze studiate, mentre nella razza Ermellinata è stato riscontrato un contenuto di acidi grassi saturi maggiore e un contenuto di monoinsaturi minore che nelle altre razze.

Infine, l'obiettivo del terzo contributo è stata l’applicazione di un approccio proteomico allo studio e alla caratterizzazione delle razze locali di pollo. L'esperimento ha coinvolto un totale di 29 esemplari maschi appartenenti alle razze locali Pépoi, Padovana ed Ermellinata di Rovigo. Sono stati quindi analizzati campioni del muscolo pettorale (*Pectoralis superficialis*). Le frazioni contenenti la classe delle proteine sarcoplasmiche sono state analizzate tramite elettroforesi bidimensionale. L'analisi di immagine, coadiuvata dall'analisi statistica, ha permesso di differenziare gli individui in gruppi, sulla base delle similarità nell'espressione proteica. Gli individui sono stati suddivisi in cluster e gruppi corrispondenti alla razza di appartenenza. L'analisi SAM ha permesso l'individuazione degli spot più rilevanti, 10 dei quali sono state identificati tramite Spettrometria di Massa evidenziando, seppur preliminarmente, i meccanismi dei processi che regolano la differenziazione fra razze. I risultati hanno dimostrato un possibile utilizzo dell'approccio proteomico nel campo degli studi riguardanti la caratterizzazione di razza, e nel campo della tracciabilità di razza o dei prodotti derivati, come alternativa alle analisi genetiche effettuate tramite i marcatori molecolari.

RÉSUMÉ

Pour des espèces domestiques communes parmi lesquelles certaines variétés, populations ou races sont menacées d'extinction, le niveau de la population selon laquelle nous devons prendre des mesures est l'objet de recherches dans de nombreux pays. Plusieurs approches ont été développées et utilisées pour comprendre les différents aspects qui contribuent à la différenciation des races et pour l'étude des produits dérivés.

Cette thèse se compose de trois contributions. Les objectifs de la première concerne l'étude de la variabilité génétique et l'analyse de la structure de la population dans six races locales italiennes de poulet au sein d'un projet de conservation. On a analysé vingt marqueurs microsatellites dans 337 animaux appartenant à six différentes races: Ermellinata di Rovigo, Robusta Maculata, Robusta Lionata, Pépoi, Padovana et Polverara, une ligne commerciale de poulet a été utilisé comme référence. On a détectés 120 allèles dans l'ensemble de l'échantillon, avec une valeur moyenne de 5.6 ± 2.1 allèles par locus. Quant aux races locales, l'hétérozygotie observés variaient de 0.240 à 0.413 et celle attendus variaient de 0.243 à 0.463 pour les races Pépoi et Polverara, respectivement. On a observé des écarts de l'équilibre de Hardy-Weinberg pour cinq races ainsi que pour les croisés commerciaux. Dans l'ensemble, la déficience des hétérozygotes dans la population (FIT) résultait 0.427, la valeur moyenne de FIS était de 0.097, tandis que FST était de 0.437, indiquant une forte carence des hétérozygotes due surtout à la division en races. On a utilisé les distances de Reynolds pour dessiner un arbre Neighbor-Joining unrooted, duquel la topologie a fournie des informations sur l'origine génétique de ces races et a confirmé leur histoire connue. La kinship moléculaire estimée entre race variait de 0.559 à 0.769 en mettant en évidence un haut valeur de coancestry. L'analyse de la structure a été réalisée pour mettre en évidence la présence de substructures de la population. Les clusters obtenues séparaient d'une manière nette les animaux en groupes correspondants aux différentes races, sans aucun mélange. L'exception à cette situation étaient les animaux appartenant à la race Polverara, pour laquelle on a rencontré une structure génétique plus complexe. Les résultats ont confirmé l'utilité des marqueurs moléculaires comme les microsatellites, pour la caractérisation des races locales et de monitorage de la diversité génétique dans les programmes de conservation des animaux domestiques. L'objectif de la deuxième contribution a été de décrire les caractéristiques de la carcasse et les caractères qualitatifs de la viande de trois races locales de poulet qui avait, à la maturité, un poids vif moyens, moyen léger et léger.

En particulier, l'exploitation commerciale des races étudiées pourraient permettre de développer et de diversifier l'offre aux consommateurs locaux qui ont besoin de différents produits de volaille. L'expérience a impliqué 60 poulets mâles élevés dans un système de production biologique, avec un accès à un espace extérieur avec l'herbe, dans le but d'étudier les caractéristiques de la carcasse et les caractère qualitatifs de la viande de trois races Italiennes avec lente croissance (Ermellinata, Padovana et Pépoi).

Les animaux ont été choisis au hasard à éclore, élevés ainsi dans les mêmes conditions et abattus à 190 jours d'âge. Les animaux ont été sectionnés pour mesurer les caractères qualitatifs de la carcasse, après on a analysé des échantillons de poitrine et de cuisse.

La race Ermellinata résultait toujours plus lourde que la race Padovana et Pépoi, en ce qui concerne le poids vif, le poids de la carcasse et de la cuisse; en outre, il y avait des différences en ce qui concerne le pourcentage de protéines (Ermellinata> Pépoi et Padovana), la shear force (Padovana <Ermellinata et Pépoi) et cooking loss (Pépoi> Padovana and Ermellinata). Les valeurs de luminosité (L *), l'indice de rouge (a *) et indice de jaune (b *), qui font partie du système de la CIE, montraient une couleur plus claire de viande et plus sombre de peau pour la Padovana par rapport à d'autres races. La composition des acides gras de la poitrine était similaire entre les espèces étudiées, alors que le contenu des acides gras saturés et mono-insaturés dans la race ermellinata a été respectivement supérieur et inférieur à celui des autres races. Enfin, l'objectif de la troisième contribution a été l'application d'une approche protéomique à l'étude et à la caractérisation des races locales de poulet. L'expriment a impliqué un total de 29 animaux masculins appartenant à des races locales Pépoi, Padovana et Ermellinata di Rovigo. On a analysé des échantillons du muscle pectoral (*Pectoralis superficialis*). Les fractions contenant la classe de protéines sarcoplasmiques ont été analysés en utilisant l'électrophorèse bidimensionnelle. L'analyse d'image, soutenue par l'analyse statistique, a permis de différencier les individus en groupes selon les similitudes dans l'expression des protéines. Les individus ont été répartis en clusters et en groupes correspondants à la race d'appartenance. L'analyse SAM a permis l'identification du spot plus importante, dont 10 ont été identifiés par spectrométrie de masse en mettant en preuve, bien que préliminaires, les mécanismes des processus qui régissent le processus de différenciation entre les races. Les résultats ont montré une possible utilisation de la protéomique dans le domaine des études concernant la caractérisation de race, et ainsi que dans le domaine de la traçabilité de race ou de produits dérivés, comme une alternative aux analyses génétiques effectuées à travers des marqueurs moléculaires.

GENERAL INTRODUCTION

1. ANIMAL GENETIC RESOURCES

Agricultural biodiversity is the product of thousands of years of activity during which humans have sought to meet their needs in a wide range of climatic and ecological conditions. Well-adapted livestock have been an essential element of agricultural production systems.

The capacity of agro-ecosystems to maintain and increase their productivity, and to adapt to changing circumstances, remains vital to the food security of the world's population. For livestock keepers, animal genetic diversity is a resource to be drawn upon to select stocks and develop new breeds. More broadly, genetically diverse livestock populations provide society with a greater range of options to meet future demands, so the wise management of the world's agricultural biodiversity is becoming an ever greater challenge for the international community. The livestock sector in particular is undergoing dramatic changes as large-scale production expands in response to surging demand for meat, milk and eggs (FAO, 2007).

Animal genetic resources (AnGR) include all animal species, breeds and strains that are of economic, scientific and cultural interest to humankind in terms of food and agricultural production for the present or the future. Domesticated animals are considered to be those species that are bred in captivity, and modified from their wild ancestors to make them more useful to humans, who control their reproduction (breeding), care (shelter, protection against predators) and food supply (Diamond, 2002; Mignon-Grasteau, 2005)

Only about 40 animal species have been domesticated since the Pleistocene. The small number of animal species successfully domesticated is largely explained by the characteristics required for domestication, which are rarely found together in a single species. All major livestock species were domesticated several thousand years ago. Common species include cattle, sheep, goat, chicken, duck, pig, horse, buffalo, rabbit, camel, donkey, elephants, various poultry species, reindeer, etc.. All these AnGR are vital to the economic development of the majority of countries in the world playing an important role in the subsistence of many communities. Although only a subset of the diversity present in the ancestral species survived in the domestic counterparts, domestic livestock diversity has been continuously evolving. Reshuffling of genes at each generation, mutation, and cross-breeding or admixture of different gene pools has offered new opportunities for natural and human selection. This has been the basis of the enormous gains in output achieved in commercial breeds, and of the

adaptation of indigenous livestock to highly diverse and challenging environments (FAO, 2007).

AnGR represent an important component of global biodiversity in terms of food security and sustainability of agricultural systems, since many of the 6379 recorded livestock breeds are at risk of loss (Hammond, 1996; Ruane, 1999).

Globally, domestic AnGR supply some 30% of total human requirements for food and agricultural production (FAO, 2007). They are particularly vital to subsistence and economic development in developing countries. In rural areas, livestock are an important source of food and cash, hence are crucial for the purchase of consumer goods and procurement of farm inputs. Other functions of livestock include production of such non-food items as leather, skins, wool, transportation and fuel (from dung) in some communities. They also facilitate the use of marginal lands of little or no value for crop agriculture (Anderson, 2003). In some of these production systems the asset and security functions of livestock are particularly important as well. These refer to their role as capital investment yielding interest, for example, in the form of milk or eggs. In view of the environmental and disease stresses, only locally adapted livestock can serve these purposes, especially in low-input smallholder systems.

Livestock genetic resources underlie the productivity of local agricultural systems. They also provide a resource of genetic variation that can be exploited to provide continued improvements in adaptation and productivity. The process of domestication of animals involved only some 40 out of the estimated 40,000 species of vertebrates. The selected species accompanied human populations across the earth, evolving through a combination of natural and human selection to adapt to, and be productive in, all but the most inhospitable environments inhabited by humans. The current enormous genetic diversity of AnGR represented in today breeds and strains, is the result of this 12,000 years process. Once lost, such diversity will be all but impossible to recreate. Existing AnGR thus represent a massive past investment which, if managed appropriately, can provide insurance against an unknowable global future. Although no compelling quantitative data is available, it has been estimated that about 50% of the total livestock genetic variation is between species and the remaining 50% is accounted by variation among breeds within species (Hammond & Leitch, 1996).

However, different species tend to perform particular functions, often in specific environments that have limited overlap with other species and livestock species are unlikely to become extinct. Thus the focus on conservation of AnGR has to be within species.

Moreover, the variation between breeds is likely to be much higher when a global perspective is taken, and when more extreme traits, such as adaptation to harsh environments and disease resistance, are considered. But a more important consideration is the rapidity with which AnGR can be exploited to deliver new levels of production and adaptation, including disease resistance. Within breeds, the amount of genetic change that can be made per unit time is a function of genetic and environmental variance, whereas the rate of change between breeds is a function of range rather than variance. Allowing for this, it is clear that for the majority of traits and production systems the most valuable resource, in terms of providing rapid adaptation to the huge diversity of existing production systems, and for providing flexibility to respond to changing systems and environments, is the variation between breeds (Rege & Gibson, 2003).

Thus genetic erosion within livestock species, including their wild ancestors, is of particular concern because of its implications for the sustainability of locally adapted agricultural practices and the consequent impact on food supply and security.

One of the most difficult issues to deal with in the context of the management of AnGR is the one related to priority setting, both for conservation of endangered or potentially endangered populations or breeds and for breed improvement programmes. Within species, an understanding of the evolutionary history of different breeds in a country or region and quantitative data on the genetic relationships among the breeds, can provide critically important inputs into the decision-making process. Current inferences of evolutionary history of breeds are based on archaeological, anthropological and ethnographic data, but increasingly this is being supplemented or replaced by results of molecular genetic studies. A combination of phenotypic (including classical morphometric) studies, biochemical (e.g. protein polymorphism, blood group) analyses and DNA-level molecular genetic studies, are the main sources of data on genetic relationships among breeds. Results from systematic analyses of molecular genetic data at sub-regional and continental levels have became available since the ‘90s.

1.1 Risk status classification

Very important is the extent to which the particular breed is endangered, relative to other breeds. Risk status classification of breed endangerment is available from many sources. At present, the most widely reported indicators pertinent to livestock biodiversity are found in the list provided by FAO through the “Domestic Animals Diversity Information System” (DAD-IS).

FAO (1992) has developed a framework for classifying breeds on the basis of level of ‘threat’ into various categories based on considerations of population size, fertility, length of reproductive cycle and the exposure of the population to `risk-causing factors'.

DAD-IS monitors breeds worldwide and classifies them into the following risk categories:

- **extinct**: a breed is categorized as extinct when there are no breeding males or breeding females remaining. Nevertheless, genetic material might have been cryoconserved which would allow recreation of the breed. In reality, extinction may be realized well before the loss of the last animal or genetic material.
- **critical**: a breed is categorized as critical if the total number of breeding females is less than or equal to 100 or the total number of breeding males is less than or equal to five; or the overall population size is less than or equal to 120 and decreasing and the percentage of females being bred to males of the same breed is below 80 percent, and it is not classified as extinct.
- **critical-maintained**: are those critical populations for which active conservation programmes are in place or populations are maintained by commercial companies or research institutions.
- **endangered**: a breed is categorized as endangered if the total number of breeding females is greater than 100 and less than or equal to 1000 or the total number of breeding males is less than or equal to 20 and greater than five; or the overall population size is greater than 80 and less than 100 and increasing and the percentage of females being bred to males of the same breed is above 80 percent; or the overall population size is greater than 1000 and less than or equal to 1200 and decreasing and the percentage of females being bred to males of the same breed is below 80 percent, and it is not assigned to any of above categories.
- **endangered-maintained**: are those endangered populations for which active conservation programmes are in place or populations are maintained by commercial companies or research institutions.
- **breed at risk**: a breed that has been classified as either critical, critical-maintained, endangered, or endangered-maintained.
- **not at risk** indicates breeds for which the total number of breeding females and males is greater than 1000 and 20 respectively; or the population size approaches 1000 and the percentage of pure-bred females is close to 100 %, and the overall population size is increasing.

- **unknown** covers breeds for which no data are available.

1.2 Status of livestock genetic resources

While these biological criteria are useful, it is important to remember that conservation of AnGR is not justified in the interest of the biological resources, but rather to contribute to human livelihoods.

Genetic distinctiveness and degree of endangerment are not the only criterions on the basis of which conservation decisions should be made. Conservation priority has to be goal and context-dependent. Therefore, important considerations are the present and future economic and socio-cultural contexts in which the breed exists. If conservation priorities based on biological factors are to have any impact on human livelihoods, they must be determined with a view to implementation within a human socio-political context, and be related to human self-interest (FAO, 2007).

Thus, priority-setting for conservation programmes needs to consider extinction probabilities as well as those factors which are considered important to the livelihoods of the society in question. It is clear that, for each of the major livestock species (cattle, sheep, goats, chickens, pigs, etc.) the main breeds are unlikely to become extinct unless current marketing and production environment change dramatically. In addition, minor breeds that currently make significant contribution to human livelihoods in a given society, and which remain competitive under the present production circumstances, are unlikely to become extinct. Immediate concern, therefore, should be with less-known breeds or species, those with highly restricted geographic distribution. Among them there are some breeds reared only in developing countries (FAO, 2007) but also farm animals breeds reared in restricted rural areas of developed countries (such as some poultry, sheep and pig breeds of the Italian, French or Spanish territory).

However, given the number of breeds that fall into these categories and the fact that resources for conservation will always be limited, there is need for a priority-setting framework.

Over the past decade, the FAO has helped collecting data from some 170 countries on almost 6,500 breeds of domesticated mammals and birds. The FAO Global Databank for Farm Animal Genetic Resources (DAD-IS) contains information on 6,379 breeds of 30 mammalian and bird species. Population size data is available for 4,183 breeds (FAOSTAT, DAD-IS, Barker, 2001).

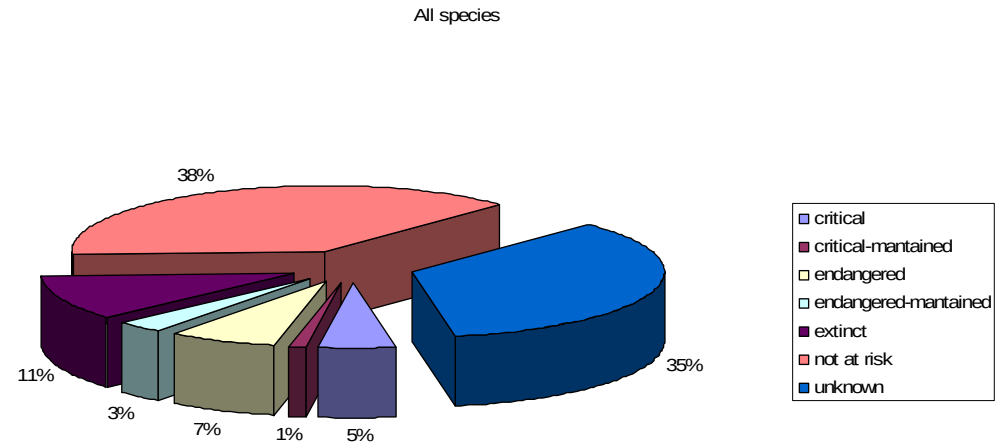
Europe attains a good proportions of all the major livestock species, but the situation of farm animal biodiversity remains particularly critical: 18% of the breeds existing in the early 20th

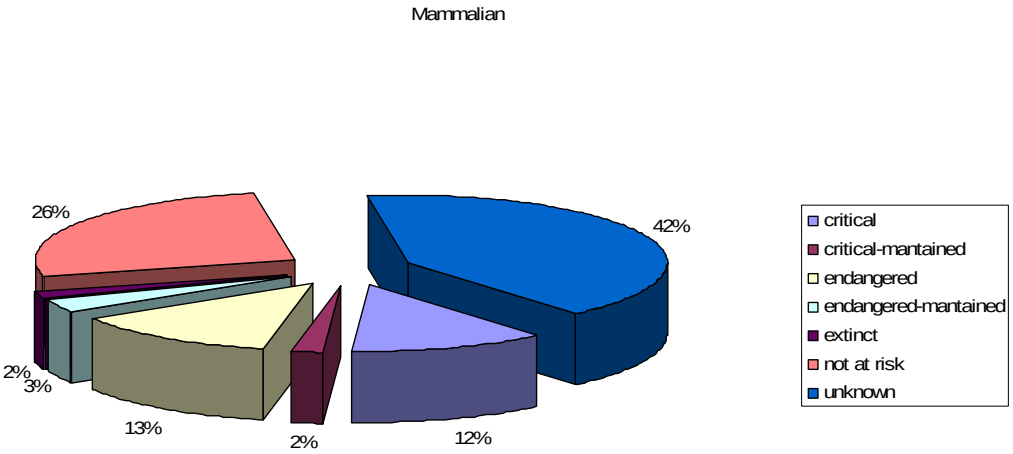
century have already been lost. Unless significant changes take place in the driving forces behind biodiversity depletion, 40% of recorded breeds risks to become extinct over the next 20 years (FAO, 2000).

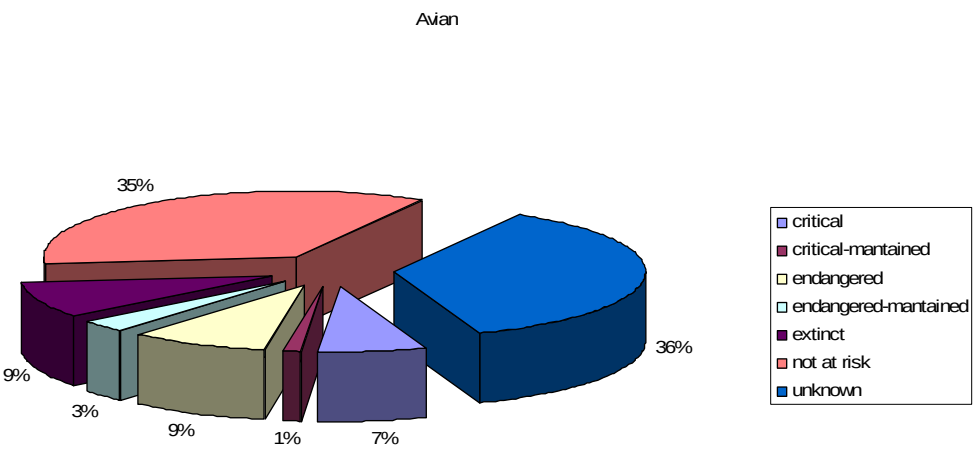
Genetic erosion in farm AnGR is much more serious than in crops because the gene pool is much smaller and very few wild relatives remain. An estimated 82% of the total contribution of AnGR to global food and agricultural production comes from only 14 species (FAO, 2000). The impression that risk of loss could be lower in developing countries compared with the developed world, is presumably is an artefact due to lack of data available in the developing countries, where complete breed surveys have not yet been undertaken. As more data become available, clearer distinctions between populations identify a larger number of breeds/strains in developing countries and indicate that a substantial proportion of them is endangered.

A total of 1 491 breeds (20%) are classified as being “at risk”. Figure 1 shows that for mammalian species, the proportion of breeds classified as at risk is lower overall (16 percent) than for avian species (30 percent). However, in absolute terms, the number of breeds at risk is higher for mammalian species (881 breeds) than for avian species (610 breeds).(FAO, 2007).

Figure 1. Proportion of the world's breeds by risk status category



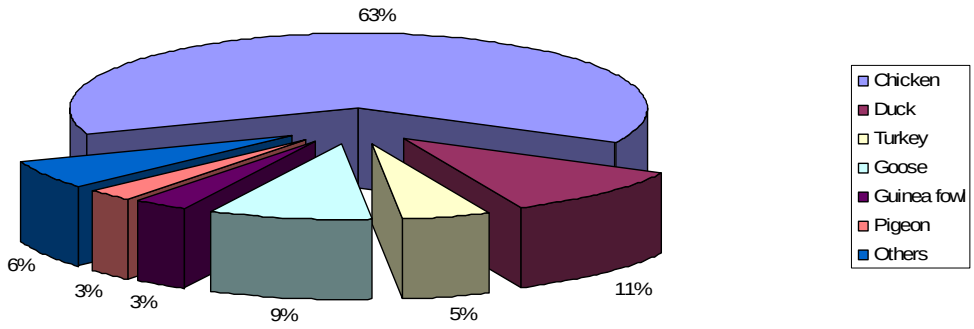




2. Avian species and chicken breeds

In the poultry sector chicken retains the dominant role, representing 63% of all avian breeds (Figure 2).

Figure 2. Distribution of the world's avian breeds by species (avian species with more than 50 recorded breeds are displayed separately; the remaining avian species are aggregated as others)



Source FAOSTAT

However, the most important breeds developed only in the second half of the nineteenth century, including the White Leghorn, New Hampshire and Plymouth Rock.

Chicken breeds are divided between layers (used mainly for egg production), broilers (for meat), dual-purpose breeds (meat and eggs), fighting breeds and ornamental breeds. In the developed countries, commercial strains dominate the production of meat and eggs, while local breeds are restricted to the hobby sector. In the developing countries, however, local breeds continue to play an important role; in making up 70–80 percent of the chicken population in some cases (Guèye, 2005; FAO, 2006). Chickens in the hobby sector look very different from each other, but that does not necessarily mean they are genetically very diverse (Hoffmann *et al.*, 2004). The same may be true for indigenous breeds in developing countries (FAO, 2006).

2.1.1 European breeds

Breeds that definitively originated in Europe account for 26 of the 67 chicken breeds reported in five or more countries. The Leghorn is the most widespread; it is found in 51 countries, and ranks second overall. It is also an important contributor to commercial strains. The second most common European breed is the Sussex from the United Kingdom, which is found in 17 countries (tenth overall).

2.1.2 North American breeds

Chickens were introduced to North America by the Spanish and then by other Europeans in the 1500s. These birds gradually developed into distinct breeds. North American breeds now account for three of the top five most widely distributed breeds worldwide, and seven of the 67 breeds reported in five or more countries. The top three are Rhode Island Red, Plymouth Rock and New Hampshire. All three are dual-purpose layers/broilers developed in the northeastern United States of America.

2.1.3 Commercial strains

Commercial strains dominate the worldwide distribution of chickens, accounting for 19 of the top 67 breeds. Because the companies involved keep their breeding information secret, there is no information on the provenance of these strains. However, most appear to be derived from White Leghorn, Plymouth Rock, New Hampshire and White Cornish (Campbell & Lasley, 1985). Commercial strains are controlled by a small number of transnational companies based in northwestern Europe and the United States of America. There has been further consolidation in the industry in recent years. Today, only two primary breeding companies (Erich Wesjohann based in Germany and Hendrix Genetics from the Netherlands) dominate the international layer market, and three primary breeders (Erich Wesjohann, Hendrix Genetics and Tyson, a company from the United States of America) dominate the market for broilers. The companies maintain many separate breeding lines, and different units within a company may even compete with one another for market share (Flock & Preisinger, 2002).

2.1.4 Breeds from other areas

The most widespread breed not included in the categories above is the Aseel, which hails from India, and is reported from 11 countries, ranking only 17th in the world. It is followed by several Chinese breeds: the Brahma and Cochin (which were developed further in the United States of America) and the Silkie (a breed with fur-like feathers). Other Asian breeds are considered as “ornamental” in the West: Sumatra (from Indonesia, eight countries), Malay Game and Onagadori (a long-tailed breed from Japan). Also worth mentioning is the Jungle Fowl (five countries) from Southeast Asia, which is the ancestor of modern chickens. The

only Australian breed in the top 67 breeds is the Australorp, derived from the Black Orpington, a British breed. Reported from 16 countries, this breed ranks 12th overall in terms of distribution.

2.2 Status of avian genetic resources

Europe has the highest number of avian local breeds (851), followed by Asia (408), Africa (146), Latin America regions (138). Near Middle East, North America and Southwest Pacific regions have the lowest number of reported local breeds (Table 1).

Table 1. Avian species: number of reported local breeds

Species	Africa	Asia	Europe & Caucasus	Latin America & Caribbean	Near & Middle East	North America	Southwest Pacific	World
Chicken	89	243	608	84	24	12	17	1077
Duck	14	76	62	22	4	1	7	186
Turkey	11	11	29	11	3	11	2	78
Goose	10	39	100	5	2	0	2	158
Muscovy duck	7	10	10	3	1	0	3	34
Partridge	2	8	3	0	0	0	0	13
Pheasant	0	7	5	6	0	0	0	18
Pigeon	7	12	30	7	8	1	2	67
Ostrich	6	2	4	0	0	0	1	13
Total	146	408	851	138	42	25	34	1644

extinct brees are excluded

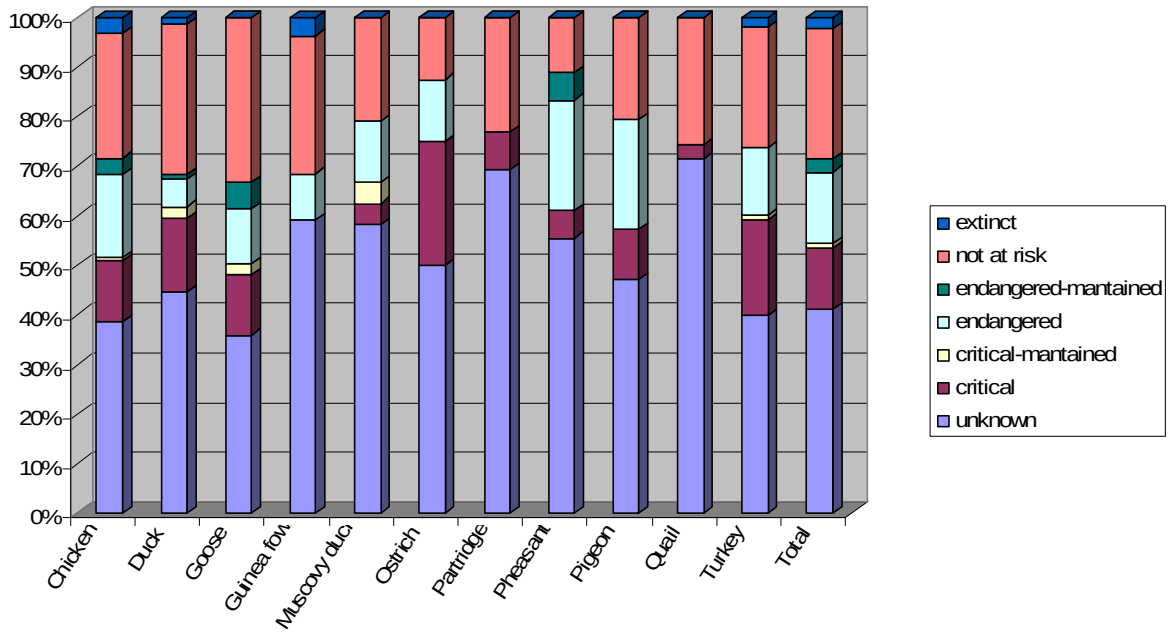
Compared to the other regions, Europe has also the highest number of transboundary chicken breeds, defined as breeds that occur in more than one country. The existence of significant numbers of regional transboundary breeds clearly has implications for management and conservation of AnGR, and highlights the need for cooperation at regional or subregional levels.

In a worldwide context, 9% of all avian breeds are classified as ‘extinct’. 9%, 7% critical, 1% critical maintained, 9% endangered, 3% endangered maintained, 35% not at risk and for the remaining 36% the situation is unknown because no information is available (FAO-STAT).

Among avian species, chickens have by far the highest number of breeds at risk on a world scale (Figure 3). This is partly related to the large number of chicken breeds in the world, but

the proportion of breeds at risk is also high in chickens (33%). Forty breeds are already declared extinct (Table 1), 34 of witch in Europe. However, relatively high proportions and numbers of breeds at risk are also found among turkeys and geese (FAO, 2007)

Figure 3. Risk status of the world's avian breeds. Percentage (chart) and absolute (table) figures by species (January 2006).



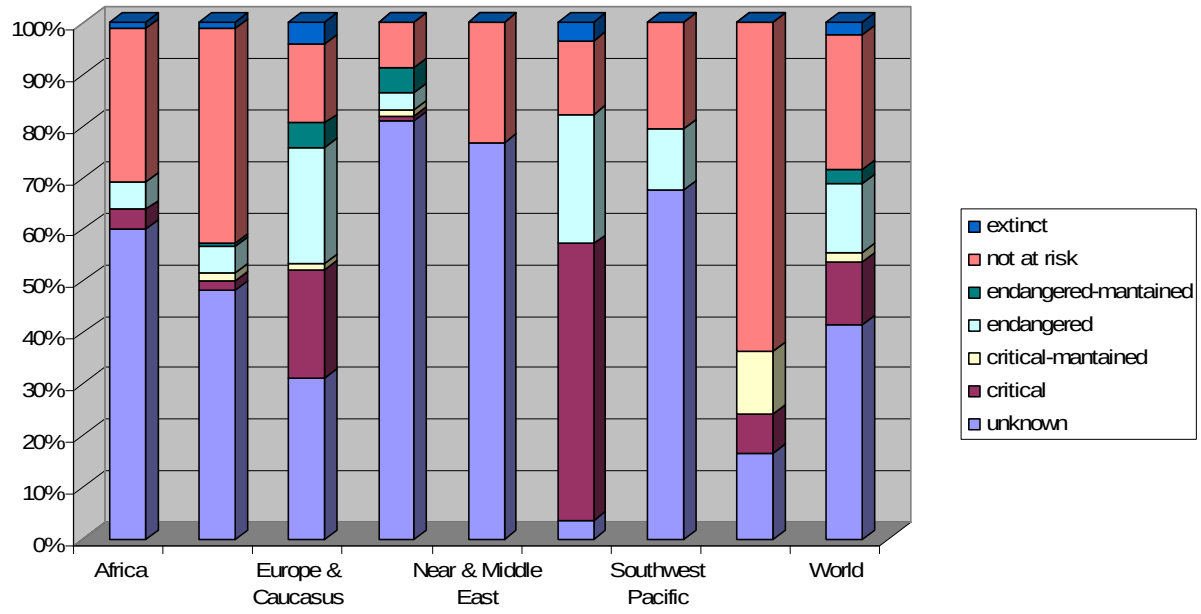
Status	Chicken	Duck	Goose	Guinea fowl	Muscovy duck	Ostrich	Partridge	Pheasant	Pigeon	Quail	Turkey	Total
unknown	493	96	65	32	14	8	9	10	32	25	41	825
critical	156	32	22	0	1	4	1	1	7	1	20	245
critical-mantained	9	5	4	0	1	0	0	0	0	0	1	20
endangered	212	12	20	5	3	2	0	4	15	0	14	287
endangered mantained	42	2	10	0	0	0	0	1	0	0	0	55
not at risk	321	65	60	15	5	2	3	2	14	9	25	521
extinct	40	3	0	2	0	0	0	0	0	0	2	47
Total	1273	215	181	54	24	6	13	18	68	35	103	2000

Table 2. Number of extinct avian breeds.

Species	Africa	Asia	Europe & the Caucasus	North America	World
Chicken	0	5	34	1	40
Duck	0	0	3	0	3
Turkey	0	0	2	0	2
Guinea fowl	2	0	0	0	2
Total	2	5	39	1	47

Figure 4 shows the distribution of avian breeds at risk by region. The regions with the highest proportion of their breeds classified as at risk are Europe and the Caucasus (49%), and North America (79%). Europe and the Caucasus, and North America are the regions that have the most highly specialized livestock industries, in which production is dominated by a small number of breeds. In absolute terms, Europe and the Caucasus has by far the highest number of “at risk” breeds. Despite the apparent dominance of these two regions, problems in other regions may be obscured by the large number of breeds with unknown risk status. In Latin America and the Caribbean, for example, 81 percent of avian breeds are classified as being of unknown risk status, while the estimate for Africa is 60% (FAO, 2007)

Figure 4. Risk status of the world's avian breeds. Percentage (chart) and absolute (table) figures by region. (January 2006)



Status	Africa	Asia	Europe & Caucasus	Latin America & Caribbean	Near & Middle East	North America	Southwest Pacific	Int. Transboundary b.	World
unknown	113	214	305	120	33	1	23	26	835
critical	7	8	204	1	0	15	0	12	247
critical-mantained	0	6	12	2	0	0	0	19	39
endangered	10	23	220	5	0	7	4	0	269
endangered-mantained	0	3	45	7	0	0	0	0	55
not at risk	56	184	151	13	10	4	7	100	525
extinct	2	5	39	0	0	1	0	0	47
Total	188	443	976	148	43	28	34	157	2017

(Source Fao, 2007)

3. THE OBJECTIVES FOR CONSERVATION

The idea of conserving animal genetic resources focuses on two separate but interlinked concepts. The first is the conservation of ‘genes’ and the second, the conservation of ‘breeds’ or populations. The conservation of ‘genes’ refers to action to ensure the survival of individual genetically controlled characteristics inherent within a population or group of populations. Such programmes require that the characteristic to be conserved is clearly recognized and identified. It does not, however, require that the genetic function at the chromosome or DNA level should be understood. Such a characteristic may in fact be a complicated biochemical function controlled by several sections of DNA on more than one chromosome, but it can be identified in the appearance or function of the animals that exhibit it.

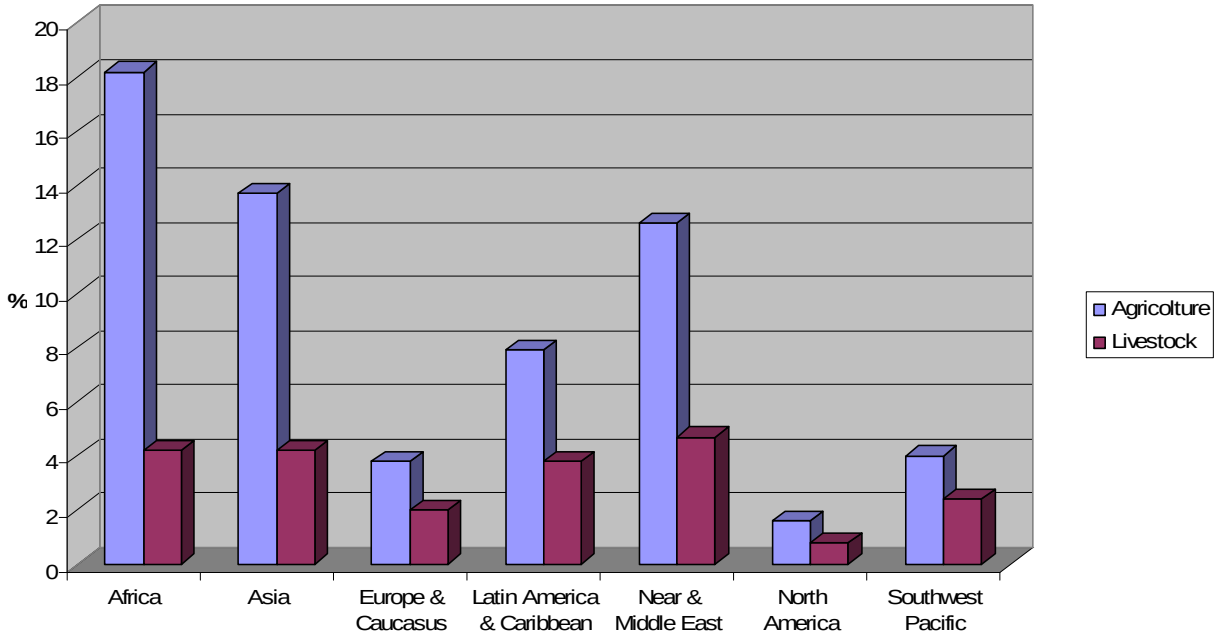
Instead, the conservation of populations or breeds refers to actions aiming to ensure the survival of a population of animals as defined by the range of genetically controlled characteristics that it exhibits. This form of conservation is applied to endangered species as well as to breeds, and is developed to ensure the conservation of all the characteristics inherent with a given population, including many which may not have been recognized, defined, identified or monitored. The differences between breeds may often be due to differences in the frequency of quantitative genes rather than the presence or absence of unique genes. Such a difference in gene frequency may result in dramatically different populations with respect to appearance and production in a given environment (FAO, 2007).

The FAO definition of animal genetic resources eligible for conservation includes animal populations with economic potential, scientific use and cultural interest.

3.1 Economic Potential

Agriculture and livestock contribute greatly to the world gross domestic product (GDP), especially in the developing countries were they retain a fundamental role for the economic sustenance of millions people (Figure 5). Although in Europe the contribution given by livestock activities attains to about 2% to the total GDP, in Africa, Asia and Middle East it reaches the levels of 18%, 13.5%, and 12.5% respectively, underlying the fundamental importance for the populations inhabiting these regions.

Figure 5. Contribution of agriculture and livestock to total GDP by region



Source FAO 2007

Endangered populations should be therefore conserved for their potential economic use in the future. Their economic potential may be the production of meat, milk, fibre, skin or draught power. This potential production may be in diverse climatic and environmental conditions. Endangered populations with economic potential may have regional adaptations developed for the country of origin, or adaptations which may be beneficial in other areas of the world where similar or complementary conditions exist. Economic potential cannot be measured by looking simply at performances. Rare or endangered breeds are often highly adapted and their performances should be measured comparatively, within their own environmental conditions. They should not be compared with other breeds in improved or modified conditions or under intensive management. Furthermore, they should be examined with respect to the products for which they were selected and valued in the conditions under which they evolved. There are many examples where growth rate, prolificacy, or milk production have been measured and used to illustrate the inferiority of purebred indigenous stock over that of exotic imported breeds or their crosses. However, when survivability of the offspring, fertility and longevity are taken into account, the indigenous stock are often found to be very productive overall. When considering economic potential it is important to remember that bioefficiency is not the same as bioeconomic efficiency. The economic success of a breed or agricultural system is

dependent on many manmade variables. These variables include the value of land, the cost of oil and other fuels, the international currency markets and exchange rates, the production efficiency of other breeds and populations in this and other regions of the world, the product shelf-life, travel and storage characteristics, health controls, current marketing strategies, consumer preferences and international political objectives. Changes in any one of these features may shift the balance and enhance the economic value of one breed type over another. Finally, crosses between unrelated breeds are not completely predictable in their production characteristics. There are many instances where two pure breeds produce crosses which far exceed the production

characteristics of either parent breed due to heterosis (Dickerson, 1969). This may be particularly important between breeds which are historically distant or which are each inbred and this may be due to the two breeds carrying genes of different allelic pairs which complement each other. This 'matching' of breeds is not predictable. The total number of possible crosses is potentially infinite, and many un-tried crosses could produce valuable production stocks.

While at a global level, food of animal origin will to a large extent be produced in high-input high-output systems with highly specialized breeds or cross-breeds, small-scale farming continues to be important, and the significance of organic farming is increasing. These systems require well-adapted dual-purpose or multipurpose breeds. These breeds are better fitted to the production goals of less-intensive farming systems than are highly specialized breeds or cross-breeds. Breeding companies rarely invest in these breeds because of the limited size of the markets. More emphasis should be given to the development of these breeds and to the conservation of their genetic diversity. The development of special products for niche markets offers the possibility to use local breeds and to make them profitable again.

3.2 Scientific Use

Endangered populations should be conserved for their possible scientific use. This may include the use of conservation stocks as control populations, in order to monitor and identify advances and changes in the genetic makeup and production characteristics of selected stocks. They may include basic biological research into physiology, diet, reproduction or climatic tolerance at the physiological and genetic level. Genetically distinct breeds are needed for research into disease resistance and susceptibility which could help in the development of better medication or management of disease. It could also help with the identification of

specific genes involved in natural disease or parasite control. Some populations may also be used as research models in other species, including man.

3.3 Cultural Interest

Many populations have played an important role in specific periods of national or regional history or have been associated with social and cultural development. Some examples are the Texas Longhorn cattle in the colonization of the USA, Spanish Merino sheep in the creation of Spain's seventeenth century wealth, or llamas, important as pack animals and fibre producers for the Inca nation of Peru.

4. METHODS FOR BREED CHARACTERIZATION

To understand the potential of the livestock genetic resources is a complex task, that implies the study of various aspects in order to obtain a good characterisation. Different approaches have been developed to analyse breed multiple features, including registration of their performances and molecular genetic or proteomic characterisations.

4.1 Molecular genetic level characterisation

Molecular characterization can play a role in uncovering the history, and estimating the diversity, distinctiveness and population structure of AnGR. It can also serve as an aid in the genetic management of small populations, to avoid excessive inbreeding. A number of investigations have described within and between-population diversity, some at quite a large scale. However, these studies are fragmented and difficult to compare and integrate. Moreover, a comprehensive worldwide survey of relevant species has not been carried out. As such, it is of strategic importance to develop methods for combining existing, partially overlapping datasets, and to ensure the provision of standard samples and markers for future use as worldwide references. Marker technologies are evolving: microsatellites analysis played and is still playing a fundamental role in molecular studies aiming to genetic characterisation of breeds and populations, but it is likely that microsatellites will increasingly be complemented by SNPs. These markers hold great promise because of their large numbers in the genome, and their suitability for automation in production and scoring. However, the efficiency of SNPs for the investigation of diversity in animal species remains to be thoroughly explored.

Methods of data analysis are also evolving. New methods allow the study of diversity without a priori assumptions regarding the structure of the populations under investigation; the exploration of diversity to identify adaptive genes (e.g. using population genomics); and the integration of information from different sources, including socio-economic and environmental parameters, for setting conservation priorities. The adoption of a correct sampling strategy and the systematic collection of phenotypic and environmental data, remain key requirements for exploiting the full potential of new technologies and approaches.

Characterization at the molecular genetic level based on molecular markers is undertaken mainly to explore genetic diversity within and between animal populations, and to determine genetic relationships among such populations. More specifically, the results from the

laboratory work are used to determine within and between-breed diversity parameters (FAO, 2005):

- identify the geographical locations of particular populations, and/or of admixture among populations of different genetic origins.
- provide information on evolutionary relationships and clarify centres of origin and migration routes.
- implement gene mapping activities, including identification of carriers of known genes.
- identify parentage and genetic relationships within populations.
- support marker assisted genetic improvement of animal populations.
- develop DNA repositories for research and development.
- In populations with limited or no information on pedigrees and population structure, molecular markers can also be used to estimate the effective population size (Ne).

In the absence of comprehensive breed characterization data and documentation of the origin of breeding populations, molecular marker information may provide the most easily obtainable estimates of genetic diversity within and between a given set of populations. Furthermore, marker assisted selection offer new opportunities in AnGR management (FAO, 2004), as DNA markers are useful in both basic (e.g. phylogenetic analysis and search for useful genes) and applied research (e.g. marker assisted selection, paternity testing and food traceability).

4.2 Phenotypic qualitative characterisation

Production and successful marketing of goods and services that are highly valued by consumers can promote maintenance of minor breeds. For example, in Italy, the population of the Reggiana cattle increased from 500 in the early 1980s to approximately 1200 by 1998 because of the development of Parmigiano Reggiano cheese that is made exclusively from milk obtained from Reggiana cows (De Roest & Menghi, 2000), providing an economic incentive for farmers to conserve and use a breed that may otherwise be lost.

Market identification is a type of incentive approach that has also been successful in Mediterranean countries where local or regional products are highly valued by consumers. Market-based linkages have also been established for meat products that are derived from locally adapted breeds.

The study of production factors affecting poultry meat quality, including organoleptic properties and nutrient contents, has a fundamental role for breeds exploitation and valorisation. Growth rate is central to many eating quality characteristics (Dransfield & Sosnicki, 1999), but it is not the only valuable feature. Factors affecting growth rate and live weight at slaughter (e.g. genotype, duration of the growing period, diet specification, and ambient temperature), influence meat flavour and texture, carcass conformation and nutrient content. Raging may also affect qualitative characteristics, and in breast muscles the fibre length may be increased, influencing meat texture. Pasture intake and the contribution of pasture to the birds total nutrient intake is likely to be variable, but low.

4.3 Proteomic level characterisation

Aim of proteomics is the description of identity, quantity and state of all proteins in a cell under a specific set of conditions. Proteomics complements and extends the study of genomes and transcript data, reflecting the true biochemical outcome of genetic information. While genomic and transcriptomic data provide the "blueprint" for the possibility of cell function, they do not always inform on the actual protein content and thus the structural and biochemical effectors of a cell (Doherty et al., 2007).

Avian proteome studies have been limited, and include muscle development, egg production, craniofacial disorders and the chicken lens using proteomic technologies.

Proteomic approaches aiming to characterize breeds and to study differentiation have not been yet exploited. Advances made in avian genomics, particularly the publication of the chicken genome sequence, should improve confidence in the protein identifications provided by a typical proteomics experiment and provide the basis for further exploration of the protein component of avian species (Doherty et al., 2007).

The systematic study of protein structures, posttranslational modifications, protein profiles, protein–protein, protein–nucleic acid, and protein–small molecule interactions, and the spatial and temporal expression of proteins, are crucial to understanding complex biological phenomena. The number of different protein variants arising from protein synthesis (alternative splicing and/or post-translational modifications) is significantly greater than the number of genes in a genome.

Mass spectrometry in combination with chromatographic or electrophoretic separation techniques, is currently the method of choice for identifying endogenous proteins in cells, characterizing post-translational modifications and determining protein abundance (Zhu et al., 2003). Two-dimensional gel electrophoresis is unique with respect to the large number of

proteins (>10 000) that can be separated and visualized in a single experiment. Protein spots are cut from the gel, followed by proteolytic digestion, and proteins are then identified using mass spectrometry (Aebersold and Mann, 2003). However, developing high-throughput technologies would be useless without the capacity to analyse the exponentially growing amount of biological data. These need to be stored in electronic databases associated with specific software designed to permit data update, interrogation and retrieval.

5. AN ITALIAN CONSERVATION EXAMPLE

In the Veneto region of Italy, the increasing interest in the conservation and development of the indigenous chicken breeds is due to both historical, social and economical reasons. The local poultry breeds provide an interesting alternative to commercial strains, providing typical products with particular meat qualities that are of great interest in the regional local markets (De Marchi et al. 2005a,b); the demand for meat products from these indigenous breeds has increased because of their perceived image as nutritious, healthy and natural products obtained from birds reared in accordance to the organic European standards.

In 2000, an important project to safeguard domestic animal biodiversity of the Veneto region of Italy has been implemented. The "Conservazione e Valorizzazione di Razze Avicole Locali Venete (Co.Va.)" was developed by the Veneto Agricultural Agency, along with the scientific support of the Department of Animal Science of the University of Padova, to provide economic support for organic production systems using local breeds (De Marchi et al. 2005a,b) and for the development of a marker-assisted conservation scheme.

This *in-situ* conservation programme involves four different species (chicken, duck, helmeted guinea fowl and turkey breeds) and four conservation nuclei flocks located in different areas of the region. Five chicken breeds (Robusta Maculata, Robusta Lionata, Ermellinata di Rovigo, Pépoi, and Padovana), two duck breeds (Germanata Veneta and Mignon), two turkey breeds (Ermellinato di Rovigo and Comune Bronzato) and one guinea fowl breed (Camosciata) are included in the project (Cassandro et al. 2004).

Each nucleus flock is divided in three different zones per breed: the hatching zone, working from February to May, the adult box, composed of an indoor pen with access to a grass paddock, and the chicks box, used between April and October, similar to the box of adults but split in two zones, one for each family.

The breeding activities and the conservation scheme are developed at the same time and in the same manner in all flocks. Each breed within each conservation flock consists of 34 females and 20 males; males are divided in two distinct groups (families) based on genetic relations estimated at the beginning of the project using molecular markers information. Females of each breed are grouped all together. The two families that originate from the two male groups are maintained through the years.

The reproduction season starts in February and birds are hatched from April to June. The first group of males is used to fecundate females for a maximum period of 3 weeks; eggs are collected and hatched. Females need two weeks to empty the spermatic sac in order to be

ready to be fecundated by the second male group for other 3 weeks. Each reproduction period ends when 90 weaning chicks per breed per flock are attained (180 chicks per breed). At hatch, chicks are individually tagged with wing tags. Usually at the end of April the reproduction programme finishes and chicks are placed in a unique box. In October, new males and females are selected to be used in the next season.

The conservation programme is based on a biannual change of all animals to extend the generation interval, to increase effective population size and reduce genetic drift (Meuwissen, 1999). Every year, for each breed within flock, 50% of males (10 males per year) and females (17 females) are replaced. In December birds are weighted and blood sample of all males are collected for DNA analysis. In January, all males of each breed are rotated among the three flocks.

When all chicken breeds attained adult weight, usually in later fall, new males and females are selected to be used in the next season. Four threshold indexes are used in the chicken selection: group of origin, breed phenotypic standards, productive and reproductive performances.

The first and very important threshold index is the group origin (two groups were defined at the beginning of the Co.Va. project for each breeds within flock using molecular markers) that influenced the selection action. In fact it is important that 50% of males and females derives from group 1 and the other 50% from group 2.

At the same time the molecular markers indications are used to monitor genetic variability within breed and within and among nuclei flocks. Secondarily the young selected chickens must observe the phenotypic standards breed requirements as colour of plumage, morphological appearance, and size. Finally the reproductive and productive performances of the fathers of young chicken are considered. These threshold indexes guaranteed the identification of the most suitable chickens that can be used to replace the middle old males and females.

Until 2005 the genotyping of the individual animals for marker assisted conservation scheme was carried out using the AFLP technique (De Marchi et al., 2006). Afterward, microsatellites have been applied because these molecular markers are well dispersed in the genome and highly polymorphic (Cheng et al., 1995); their application to characterise chicken breeds has been used in many countries to study the genetic relationships among native breeds (Takahashi et al., 1998; Hillel et al., 2003).

LITERATURE CITED

Aebersold, R., Mann, M. 2003. Mass spectrometry based proteomics. Nature, 422 (6928): 198–207. Review.

Anderson, S. Animal genetic resources and sustainable livelihoods Ecological Economics Volume 45, Issue 3, July 2003, pp. 331-339 Valuing Animal Genetic Resources

Barker, J.S.F. 2001. Conservation and management of genetic diversity: a domestic animal perspective Can. J. For. Res.31(4): 588–595.

Campbell, J.R., Lasley, J.F. 1985. The science of animals that serve humanity. New York, USA. McGraw-Hill

Cassandro, M., De Marchi, M., Targhetta, C., Dalvit, C., Ramanzin, M., Baruchello, M. 2004. An in situ marker-assisted conservation scheme of 11 Italian avian breeds. 55th Anual meeting of the European Association for Animal Production (EAAP), Bled, 5-9 September. Wageningen Academic Publishers. Book of abstracts 10:30.

DAD-IS. 2006. Domestic Animal Diversity Information System (DAD-IS). FAO (available at www.fao.org/dad-is/).

De Marchi, M., Cassandro, M., Targhetta, C., Baruchello, M., Notter, D.R. 2005a. Conservation of poultry genetic resource in the Veneto region of Italy. Anim. Genet. Res. Inf. 37: 63-74.

De Marchi, M., Dalvit, C., Targhetta, C., Cassandro, M. 2006. Assessing genetic diversity in indigenous Veneto chicken breeds using AFLP markers. Anim. Genet., 37: 101–105.

De Marchi, M., Dalvit, C., Targhetta, T., Cassandro, M. 2005b. Assessing genetic variability in two ancient chicken breeds of Padova area. Ital. J. Anim. Sci. 4(3), 151-153.

De Roest, K., Menghi, A. 2000. Reconsidering'traditional'food: the case of Parmigiano Reggiano cheese. Sociologia Ruralis, 40(4): 439-451

Diamond, J. 2002. Evolution, consequences and future of plant and animal domestication. Nature, 418: 700–707.

Dickerson, G. E. 1969. Experimental approaches in utilising breed resources. Anim. Breed. Abstr. 37:191-202.

Doherty, M.K., McLean, L., Beynon, R.J. 2007. Avian proteomics: advances, challenges and new technologies. Cytogenet. Genome Res. 117:358-369.

Dransfield, E., Sosnicki, A.A. 1999 Relationship between muscle growth and poultry meat quality. Poultry Science 78:743–746

FAO (Food and Agriculture Organisation of the United Nations). 1992. The New World Screw Worm Eradication Programme. Rome

FAO (Food and Agriculture Organisation of the United Nations). 2000. World Watch List for Domestic Animal Diversity, third ed. FAO, Rome, Italy.

FAO (Food and Agriculture Organisation of the United Nations). 2004. Conservation strategies for animal genetic resources, by D.R. Notter. Background Study Paper No. 22. Commission on Genetic Resources for Food and Agriculture. Rome.

FAO (Food and Agriculture Organisation of the United Nations). 2005. Genetic characterization of livestock populations and its use in conservation decision making, by O. Hannotte & H. Jianlin. In J. Ruane & A. Sonnino, eds. The role of biotechnology in exploring and protecting agricultural genetic resources, pp. 89–96. Rome.

FAO (Food and Agriculture Organisation of the United Nations). 2006. Poultry gene flow study: the relative contribution of indigenous chicken breeds to poultry meat and egg production and consumption in the developing countries of Africa and Asia, by R.A.E. Pym. Draft report for FAO. Rome.

FAO (Food and Agriculture Organisation of the United Nations). 2007. The State of the World's Animal Genetic Resources for Food and Agriculture, edited by Barbara Rischkowsky & Dafydd Pilling. Rome.

FAOSTAT. (available at http://faostat.fao.org/).

Flock, D.K., Preisinger, R. 2002. Breeding plans for poultry with emphasis on sustainability. In Proceedings of the 7th World Gongress on Genetics Applied to Livestock Production, held 19–23 August 2002, Montpellier, France.

Guèye, E.F. 2005. Editorial: Family poultry must no longer be a 'hidden harvest'. INFPD Newsletter, 15(1):1.

Hammond, K. 1996. The status of global farm animal genetic resources. In: Proceedings of the Symposium on the Economics of Valuation and Conservation of Genetic Resources for Agriculture, Centre for International Studies on Economic Growth, Tor Vergata University, Rome, 13–15 May.

Hillel, J., Groenen, M.A., Tixier-Boichard, M., Korol, A.B., David, L., Kirzhner, V.M., Burke, T., Barre- Dirie, A., Crooijmans, R.P., Elo, K., Feldman, M.W., Freidlin, P.J., Maki-Tanila, A., Oortwijn, M., Thomson, P., Vignal, A., Wimmers, K., Weigend, S. 2003. Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Gen. Sel. Evol., 35: 533–557.

K Hammond, HW Leitch, 1996. The FAO global program for the management of farm animal genetic resources

Mignon-Grasteau, S., Boissy, A., Bouix, J., Faure, J.-M., Fisher, A.D., Hinch, G.N., Jensen, P., Le Neindre, P., Mormède, P., Prunet, P., Vandeputte, M., Beaumont, C. 2005. Genetics of adaptation and domestication in livestock. Livestock Production Science, 93(1): 3–14.

Rege, J.E.O., Gibson, J.P. 2003. Animal genetic resources and economic development: issue in relation to economic valuation. Ecological Economics 45, 319-330.

Ruane, J. 1999. In: Genebanks and the conservation of farm animal genetic resouces. J.K. Oldenbroek ed. DLO institute for Animal Science and Health. The Netherlands, pp.59-73.

Takahashi, H., Nirasawa, K., Nagamine, Y., Tsudzuki, M., Yamamoto, Y. 1998. Genetic relationships among Japanese native breeds of chicken based on microsatellite DNA polymorphisms. J. Hered. 89(6), 543-546.

Zhu, H., Bilgin, M., Snyder, M. 2003. Proteomics. Annual Review of Biochemistry, 72: 783–812.

OBJECTIVES

The thesis is made up of three contributes, dealing with different approaches developed and exploited aiming to understand the different aspects that contribute to breed differentiation and to study the typical products that originate from them. Objectives of the different contributes were:

- to determine genetic variation and to analyze population structure in six Italian local chicken breeds undergoing *in-situ* conservation, using twenty microsatellite markers, using such information to monitor the conservation scheme.

- to describe carcass characteristics and qualitative meat traits of three local chicken breeds showing, at maturity, light, medium-light, and medium live weights, so to evaluate their performances in an organic extensive rearing system.

- to perform breed characterization of three local chicken genotypes, aiming to group animals on the basis of protein expression differences as an alternative to molecular genetic analysis and to identify the most relevant spots playing a role on the mechanics of the breed differentiation process.

GENETIC CHARACTERIZATION AND POPULATION STRUCTURE OF ITALIAN LOCAL CHICKEN BREEDS UNDERGOING *IN-SITU* CONSERVATION

E. Zanetti, M. De Marchi, C. Dalvit, M. Cassandro.

Department of Animal Science. University of Padova. Viale dell'Università 16, 35020 Legnaro (PD), Italy. Ph. (+39) 049 827 2616. Fax (+39) 049 827 2669

Corresponding author: massimo.demarchi@unipd.it

ABSTRACT

The objectives of this study were to determine genetic variation and to analyze population structure in six Italian local chicken breeds involved in a conservation program. Twenty microsatellite markers were investigated in 337 animals belonging to six breeds: Ermellinata di Rovigo, Robusta Maculata, Robusta Lionata, Pepoi, Padovana and Polverara; a commercial layer cross was used as reference. One-hundred-twelve alleles were detected in the overall population, with a mean number of 5.6 ± 2.1 alleles per locus. For the local breeds, the observed and expected heterozigosity ranged from a minimum of 0.240 to a maximum of 0.413 and from 0.243 to 0.463 for the Pépoi and Polverara breeds, respectively. Deviation from Hardy-Weinberg equilibrium has been observed in five breeds and in the commercial cross. The overall population heterozygote deficiency F_{IT}, resulted 0.427, the average F_{IS} 0.097, while F_{ST} was 0.437, indicating a high heterozygote deficiency mainly due to breed subdivisions. Reynolds distances were used to draw an unrooted Neighbor-Joining tree, which topology gave information on the genetic origin of these breeds and confirmed their known history. The estimated molecular kinship within breed ranged from 0.559 to 0.769, evidencing high coancestry. Structure analysis was performed to detect the presence of population substructures. Inferred clusters corresponded to the different breeds, without presence of admixture. Exception was the Polverara, for which a more complex genetic structure was found. Obtained results confirmed the usefulness of molecular markers, as microsatellites, to characterize local breeds and to monitor genetic diversity in livestock conservation schemes.

Key words: chicken breeds, genetic diversity, microsatellite, population structure.

INTRODUCTION

In the recent years, animal biodiversity management has became an important issue for the international scientific community, because of big changes in large-scale production systems (FAO, 2007). In North America, Europe, and China about 50 percent of documented breeds are classified as extinct, critical or endangered (Hammond, 1996) and local breeds have often been diluted by indiscriminate cross-breeding with imported stocks (FAO, 2007). The dramatic size contraction of local poultry breeds due to replacement with cosmopolitan improved ones evidences the need for local genetic resources conservation.

In the absence of comprehensive breed characterization data and documentation of the origin of breeding populations, molecular marker information may provide the most reliable estimates of genetic diversity within and between a given set of populations. It is useful mainly to explore genetic diversity within and between breeds or populations, to analyze genetic relationships and admixture and to provide information on evolutionary relationships and parentage within populations. Anyway, for breeds undergoing conservation, molecular data should be integrated with other information (i.e. adaptative, productive and reproductive performances, extinction probabilities) to guide decision makers.

In Italy, the interest in conservation of local poultry breeds has been concretized in 2000 by the regional government with the “Conservazione e Valorizzazione delle Razze Avicole Venete” (Co.Va.) conservation program (De Marchi et al., 2005a). Co.Va. is an *in situ* program involving 12 breeds belonging to four poultry species (chicken, duck, helmeted guinea fowl, and turkey) reared in distinct flocks distributed in the Veneto region of Italy. Molecular markers information has been used to monitor genetic diversity of populations (Targhetta et al., 2005, De Marchi et al., 2006) and to valorize genetic resources using genetic traceability systems (Dalvit et al., 2007). Among molecular markers, microsatellite have been preferred because are well dispersed in the genome and highly polymorphic (Cheng et al., 1995). They have been used in many countries to study the genetic relationships among local

breeds (Takahashi et al. 1998; Hillel et al., 2003; Baumung et al., 2004; Muchadeyi et al., 2007), and their use allows meta-analysis and comparisons between different independent research units.

The aim of this study was to analyze genetic diversity, genetic relationships, population structure, and molecular coancestry in the Italian local chicken breeds undergoing conservation using microsatellite markers.

MATERIALS AND METHODS

Conservation Program

The Co.Va. is an *in situ* marker assisted conservation program, started in 2000, that involves 3 organic flocks located in different environments: plain, hill, and mountain, as reported by De Marchi et al (2005a). Initially the program involved 5 local chicken breeds: Ermellinata di Rovigo (ER), Pèpoi (PP), Robusta Lionata (RL), Robusta Maculata (RM), and Padovana (PD) with 2 different strains: Dorata (PDd) and Camosciata (PDc). In 2006 the Polverara breed (PV) with 2 different strains Nera (PVn) and Bianca (PVb) was also included. The origin of these local breeds is documented in literature (De Marchi et al., 2005a; De Marchi et al., 2006) with the exception of PV that, until 1899, was confused with PD. As reported by De Marchi et al. (2005b), just in the 1900 the PV and PD breeds were described separately, nevertheless in the last 30 years the PV has been crossed with other breeds and so its features are not fully fixed.

Sample Collection and DNA Extraction

A total of 337 animals were analyzed: ER (n = 45), PP (n = 45), RL (n = 43), RM (n = 45), PV (n = 88, of which PVn = 52 and PVb = 36), PD (n = 50, of which PDd = 24 and PDc = 26) and a commercial brown layer cross (Hubbard Golden Comet) (BL, n=21) was used as

reference breed. Each of the six local breeds is reared in the 3 conservation flocks mentioned above and samples were randomly taken from all of them. The population sizes of the 6 local breeds have been estimated about 1500 animals for ER, PP, RL, RM, PV, and about 2000 for and PD.

Whole blood samples were taken from the wing vein onto a sterile collecting vacuum tube (Vacutainer) containing Sodium Citrate and Citric Acid and stored at 4°C. Genomic DNA was isolated from blood using a modified DNA purification kit (Gentra System PUREGENE DNA) and stored at -20°C until subsequent use as a template for PCR reaction.

DNA Polymorphisms

A set of 20 microsatellite markers, included in the list of recommended microsatellites for chicken analysis by the ISAG/FAO Standing Committee (MoDAD project, FAO, 2004), were used to amplify SSR regions in the genome (Table 1). The PCR primer pairs were synthesized and 5' ends of the forwards primers were fluorescently labeled with cy5 or cy5.5 dyes. The 20 microsatellites were individually amplified by a PX2 Thermohybaid thermal cycler at the following conditions, the X temperature being the annealing t° of each primer (NCBI): initial denaturation step of 10 min at 94°C, 35 cycles of 45 s at 94°C, 1 min at X°C and 1.5 min at 72°C and a final extension of 10 min at 72°C. A reaction volume of 15 μl contained 25 ng of genomic DNA, 1.5 mM $MgCl_2$, 1.5 μl of Taq Buffer 1X, 0.04 U Taq Gold (Sigma), 3mM dNTPs and 10 μM of each primer. Amplified fragments were pooled in four multiplex and analysis was performed using an automated DNA sequencer (CEQ 8000 Genetic Analysis System, Beckman Coulter). Electropherogram processing was carried out using the CEQ 8000 software (Beckman Coulter). Alleles were scored according to PCR product size.

Marker Polymorphisms and Diversity Within and Among Breeds

Total number of alleles, average number of alleles per locus across breeds, allelic frequencies, expected (H_E) and non biased observed heterozygosity (H_O) (i.e. observed heterozygosity corrected for bias due to sampling according to Nei, 1978) were estimated using the Genetix software (Belkhir, 1996-2002). Exact tests for deviation from Hardy–Weinberg equilibrium (HWE) (Guo and Thompson, 1992) were applied using the Markov Chain Monte Carlo simulation (100 batches, 5,000 iterations per batch, and a dememorization number of 10,000) as implemented in GENEPOP version 3.4 (Raymond and Rousset, 1995). The polymorphism information content (PIC) (Botstein et al., 1980), that is a general measure of how informative a marker is, was calculated using the MOLKIN software (Gutièrrez and Goyache, 2004). Wright's fixation indices (F_{IS}, F_{ST} and F_{IT}) estimated according to Weir and Cockerham (1984), were calculated for the whole population using the FSTAT 2.9.3 software (Goudet, 2001) in order to quantify the within and between breed partitioning variance. F_{ST} distances among breeds were computed using MOLKIN (v3.0). Reynolds distances (D_R) (Reynolds et al., 1983) were estimated using the PHYLIP 3.66 software package (Felsenstein, 2005). A consensus tree was reconstructed and tree robustness was evaluated by bootstrapping over loci (1,000 replicates). Neighbor-Joining trees were plotted from D_R distances using TREEVIEW (v.1.6.6) (Page, 2001).

Molecular Coancestry and Kinship Distances

Average molecular coancestry within breed (*fij*) and kinship distances among breeds (*Dk*) were estimated using the software MOLKIN 3.0 following the formula suggested by Caballero and Toro (2002), and previously presented by Eding and Meuwissen (2001). To avoid bias, because of unequal sample sizes, 100 samples with exactly 50 individuals per breed were generated with a bootstrap-procedure. To help setting conservation priorities,

MOLKIN 3.0 (Gutiérrez et al., 2005) was used to quantify the contribution of each analyzed population to the diversity of the whole dataset using the method proposed by Caballero and Toro (2002). Because BL is not a local breed involved in the conservation scheme, but a commercial cross used as reference population, its data were not included in the approach for setting conservation priorities. *Dk* between breeds was simply computed averaging the corresponding values for all the within or between-breeds pairs of individuals.

Structure Clustering Analysis

STRUCTURE software 2.1 (Pritchard et al., 2000) implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers. Applications of the method include detection of the presence of population structure, identification of distinct genetic populations (K), assignment of individuals to populations, and identification of migrants and admixed individuals. The analysis was performed setting an admixture model with correlated allele frequencies. For the burn-in phase 50,000 iterations were used followed by 300,000 repetitions for K values ranging from 2 to 14, with 50 runs for each K. K is the value corresponding to the assumed number of cluster to be examined during the analysis. The best number of clusters fitting the data was established by plotting the mean Ln Pr(X|K) over the 50 independent runs for each K, as suggested by Pritchard et al. (2000). SIMCOEF procedure (Rebbeck et al., 2002) of the statistical package R (v. 2.6.0) was used to make a comparison of the 50 solutions, defining identical the solutions with 95% of similarity or more, and considering the most frequent solutions as the most probable. DISTRUCT software (Rosenberg, 2004) was used to graphically visualize the clustering pattern of the animals.

RESULTS AND DISCUSSION

Marker Polymorphisms

All loci studied were polymorphic and 112 alleles were detected, showing a mean number of 5.6 ± 2.1 allele per locus (Table 1). Polymorphism information content (PIC) per each marker ranged from 0.233 to 0.702, with an average (±SD) of 0.546 ± 0.124. According to Botstein et al. (1980) PIC at all loci analyzed resulted reasonably informative, with the exception of MCW0098. Within breeds, several loci were monomorphic: 4 for PP (MCW0295, MCW0123, MCW0222, MCW0098), 3 for RL (MCW0078, MCW0014, ADL0278), 4 for RM (MCW0104, MCW0037, MCW0098, ADL0268), and 1 for PD (MCW0081). This situation could be due to a rather high inbreeding or to the choice of the markers. The SSR investigated in the present study are included in the list of recommended microsatellites for chicken analysis by the ISAG/FAO Standing Committee (MoDAD project, FAO, 2004) and should display at least four alleles per locus; however this could be difficult to assure for previously unanalyzed breeds. On the whole, 34 private alleles, corresponding to 30.3%, were found (Table 2); 8 showed a frequency greater than 10%: 3 for BL, 2 for PV and 1 for ER, PP and RL, respectively. Taking into account that these local Italian breeds came from a relatively close geographic area, the presence of private alleles was rather high; Tadano et al (2007) detected just 15% of private alleles in their study on twelve chicken lines bred based on five well distinct breeds. Such differentiation can be explained considering the different origin and management practices that did not allow crossbreeding in local Venetian breeds. The average number of alleles per locus within breed ranged from 2.17 to 3.80 (Table 3). This finding is comparable with what found by Tadano et al. (2007) in the above mentioned study and by Bodzsar et al. (2009) in their paper on Hungarian chicken breeds, but it is much lower than what found by Muchadeyi et al. (2007) on different chicken ecotypes from Zimbabwe.

Genetic Diversity and Genetic Distances Among Breeds

Expected and observed heterozigosity estimates and molecular coancestry values within breed (*fij*) are shown in Table 3. Values of H_O and H_E ranged from 0.240 to 0.243, and from 0.413 to 0.463 for the PP and PVn breeds, respectively. The values of H_O and H_E for BL were 0.622 and 0.559, respectively. The low frequency of heterozygotes may be explained by the high number of monomorphic loci detected in the studied breeds. Heterozigosity estimates are comparable with those reported in literature for highly specialized breeds selected in Europe in the last centuries (Hillel et al., 2003; Granevitze et al., 2007). On the other hand, free range ecotypes, usually reared in developing countries where no selection for morphological, productive and reproductive traits is accomplished, showed usually higher heterozigosity values (Berthouly et al., 2008; Muchadeyi et al. 2007). It is worth of mention that the PD breed was included in the studies of Granevitze et al. (2007) and Hillel et al. (2003); H_O values reported in their papers (0.36 and 0.17, respectively) were similar to those reported in our study (0.287 and 0.329 for PDc and PDd, respectively). The low genetic diversity owned by the analyzed breeds and, more in general, by European chicken breeds, could be due to the loss of variability observed in all animal species outside their centre of domestication (Mignon-Grasteau et al., 2005). Moreover, for the local breeds studied here, a founder effect when the breeds were involved in the conservation scheme, could also be responsible of a loss of genetic variation. Deviation from HWE has been observed for ER, PP, RL , PVb, and PVn (Table 3). This deviation was due to a heterozygotes deficiency suggesting either a rather high inbreeding or a Wahlund effect; the last hypothesis seemed the most reliable for PV as the presence of population substructures was proved by the STUCTURE software and it will be better examined in the next paragraphs. On the contrary, BL showed a significant heterozygotes excess, as one may expect from a commercial line that is produced by

crossbreeding. F_{IT} value, that is the overall population heterozygote deficiency, was 0.427 (99% confidence interval 0.427–0.533). Average F_{IS} value for the whole population resulted 0.097 (99% confidence interval 0.045–0.165), while F_{ST} was 0.437 (99% confidence interval 0.371–0.498) indicating a high heterozygotes deficiency mainly due to breed subdivisions. This result underlined a high degree of breed differentiation, which is comparable to the values reported in literature for native Japanese poultry breeds (Tadano et al., 2008). The cause of such a high differentiation could be the selective breeding carried out in these breeds and the absence of gene flow among them proved also by the analysis of population structure. Apart from the study by Tadano et al. (2008), usually chicken breeds evidenced a lower genetic differentiation; in a study on eight Finnish breeds Vanhala et al. (1998) estimated a F_{ST} of 0.303 while Tadano et al. (2007) found a F_{ST} of 0.298 in twelve commercial lines. F_{ST} distances among the analyzed breeds (Table 4) ranged from 0.035 (BL-PV) to 0.142 (RM-PP). The low distance among PV and the commercial cross is difficult to explain and there are no evidences supporting this data. A close relationship among the tufted breeds (PD and PV) was highlighted; this result is expected since their common origin and the presence of gene flow between them has already been documented. Kinship distances among breeds (Table 4) ranged from a minimum of 0.262 (RL–RM) to a maximum of 0.359 (PV–ER). The low distance evidenced between RL and RM is justified by the genetic origin of these breeds, which were both selected in the ‘50s and ’60s from Orpington and White America breeds. Reynolds distances are recommended by Eding and Laval (1999) for populations with short divergence time. The D_R estimates among breeds were used to draw an unrooted Neighbour-Joining tree (Figure 1). The common origin of RL and RM, already highlighted by kinship distances, seemed to be confirmed by tree topology and by the rather high bootstrap values (74.8%). ER and BL probably share common ancestors, while the PP seems to have a mixed but not well defined origin.

Mean molecular coancestry estimates within breed are listed in Table 3. They are very similar for all the breeds studied, ranging from 0.559 to 0.769 for PVn and PP, respectively. Data about molecular coancestry in chickens are not yet available for comparison, while, if compared to results obtained in other species, the values appear quite high. In particular Fabuel et al. (2004), analyzing the genetic diversity of Iberian pig breeds involved in conservation measures in Spain, reported values lower than those obtained in our study (0.322-0.556). Marletta et al. (2006) showed even lower estimates in their study on some local endangered Spanish and Italian horse breeds (0.210-0.302). Anyway our high molecular coancestry values could be explained by the low number of animals of these endangered breeds available at the beginning of the conservation program, and by a high inbreeding. Moreover, this results are in accordance with the observed low level of genetic diversity and with the high genetic differentiation among breeds.

Results obtained with the Caballero and Toro approach to set up conservation priorities are illustrated in Table 5. The removal of one breed from the dataset resulted in both loss or gain of the total genetic diversity in the population which ranged from -4.23% to +1.34% when ER and PD were removed, respectively. The highest gain of between breed diversity was found removing the PV breed (+3.48%); on the other hand, its removal resulted in a loss of the within breed diversity (-6.78%). On the contrary, removal of PP gave a high contribution to the internal diversity (+3.41%) and a loss in the between breed diversity (-2.85%), resulting in a global modest gain of total genetic diversity (+0.56%). The high contribution to internal diversity due to PP extinction depend on its high inbreeding, evidenced by the very high *fij*. In fact, ignoring the within breed variability will, favor inbred populations and populations with extreme allele frequencies (Glowatzki-Mullis et al., 2008). As already mentioned, PV and PD are closely related breeds. This is confirmed by genetic distances, morphology and known historic origin. The exclusion of one of this two breeds, seemed to compromise poorly the

total genetic diversity, but if both breeds are removed a high loss of genetic diversity in the whole population (-6.90%) was detected. This loss was mostly due to the among breeds diversity (-25.19%), in fact, the extinction of PV and PD will result in a loss of the only two tufted breeds involved in the conservation program.

Genetic Structure Analysis

The analyzed animals showed a particular underlying genetic structure. The Ln Pr(X|K) increased sharply from K = 2 to K = 8 and it reached a plateau without showing any significant decrease from K = 9 to K = 14. However, the highest Ln Pr(X|K) was found at K = 10 suggesting that this was the most probable number of clusters in the population. In Figure 2, the results of the analysis with K ranging from 2 to 10 are displayed. Only the most probable solutions are shown for each K. Results obtained using the program STRUCTURE, reflected the fact that probably high inbreeding levels and no gene flow has occurred in the last decades among these breeds, leading to a strict breed differentiation. A similar population structure, characterized by very low level of admixture has been observed also in some Hungarian chicken breeds (Bodzsar et al., 2009); this was not the case for Vietnamese chickens studied by Ngo Thi Kim Cuc et al. (2006) and Zimbabwean ones analyzed by Muchadeyi et al. (2007) which evidenced a low differentiation and high level of gene flow and admixture among studied populations. These contrasting findings suggested a different management of chicken breeding; in Europe pure breeding is preferred while in Asiatic and African countries exchange of genetic material among villages is preferred allowing gene flow among breeds. With the only exception of the PV breed, no structures within flock were visible using the genetic structure analysis method. The ten inferred clusters were basically formed by the different breeds. The two PD strains were assigned to the same cluster, while, most remarkably, the PV breed was divided in 2 populations: the white strain being more

homogeneous and the black one evidencing a more complex pattern that cannot even distinguish the animals of the different flocks; however a high level of admixture could be observed in both strains. This complex situation could be due to the fact that PV was involved in the conservation program only in 2006, that is the same year in which samples were collected. Though, for PV animals, no sire rotation was applied and probably there was not exchange of genetic material between breeders before involvement in the conservation scheme. Detected deviations from HWE could be explained by this sub-structure in genetically distinct populations that have been reproductively isolated each other for years. In fact, sub-structure of the populations violates the basic assumptions of HWE based on random mating. It must also be considered that PV, almost completely lost during the mid 80's, has been submitted to indiscriminate crosses with other breeds before the conservation phase, to re-establish the breed and to enhance performance traits; this could also be the reason of its low *fij* estimates if compared with the other local breeds. At present efforts to obtain purebred animals are in act. However, further exchange of males among flocks will promote gene flow and homogenization between the animals that presented a complex structure, increasing the whole gene diversity of the breed. To obtain unstructured populations was one of the most important objectives that the conservation scheme has accomplished. In the case of the other Italian local breeds, the highly significant deficit of heterozygotes seemed to be caused mainly to inbreeding as STRUCTURE did not detect any substructure. This consideration is also supported by the high molecular coancestry estimates within breed. Further exchange of animals, although conveniently chosen among the most different, could not lead to a decrease of inbreeding coefficients. An increase of genetic variability could be obtained introducing new unrelated animals in the conservation program, which could be found looking for fancy breeders.

CONCLUSIONS

Results highlighted the high level of genetic diversity among the local chicken breeds. Whatever the method used to analyze genetic differentiation (i.e. genetic distances, structure clustering), breeds resulted well distinct, with no admixture, and homogeneous within breed, with the single exception of PV which presented complicate population substructures. The high level of genetic differentiation, the clear distinction among breeds, and the low level of admixture, are important factors that support the idea to conserve these breeds with unique genetic features. According to Ruane (1999), adaptative features, traits of scientific and economic interest, cultural\historical value, strong link to regional traditions and ability to generate incomes from tourism justify conservation efforts and this is the case for Italian chicken genetic resources. For this reason sampling for molecular analysis should be combined with surveying and/or monitoring of productive and phenotypic traits, as molecular information alone cannot be used for conservation decisions. Finally, once decision about conservation have already been taken, molecular markers can be a useful tool to perform chicken characterization, to monitor the conservation program and to arrange matings.

ACKNOWLEDGEMENTS

This research was financed by Veneto Agricoltura, the regional agriculture agency of Veneto region. The authors wish to thank all the staff working on the CoVa project and Prof. Steffen Weigend for his useful comments on the first draft of the manuscript.

REFERENCES

Aldrovandi U. 1600. In: Ornithologiae treatise. Vol. II, Book XIV.

Baumung R., H. Simianer, and I. Hoffman. 2004 Genetic diversity studies in farm animals – a survey. J. Anim. Breed. Genet., 121:361-373

Baumung R., V. Cubric-Curik, K. Schwend, R. Achmann, and J. Sölkner. 2006. Genetic characterisation and breed assignment in Austrian sheep breeds using microsatellite markers information. J. Anim. Breed. Genet. 123:265-271.

Belkhir K., P. Borsa, L. Chikhi, N. Raufaste, and F. Bonhomme. 1996-2002. GENETIX v. 4.04. Logiciel sous Windows TM pour la génétique des populations. Laboratoire Genome, Populations, interactions CNRS UMR 5000, Université de Montpellier. France.

Berthouly C., B. Bed'Hom, M. Tixier-Boichard, C. F. Chen, Y. P. Lee, D. Laloe, H. Legros, E. Verrier, and X. Rognon. 2008. Using molecular markers and multivariate methods to study the genetic diversity of local European and Asian chicken breeds. Anim. Genet. 39:121-129.

Botstein D., R. L. White, M. Skolnick, and R. W. Davis. 1980. Construction of a genetic linkage map in man using Restriction Fragment Length Polymorphisms. Am. J. Hum. Genet. 32:314- 331.

Caballero A., and M. A. Toro. 2002. Analysis of genetic diversity for the management of conserved subdivided populations. Conserv. Genet. 3:289-299.

Cheng H. H., I. Levin, R. Vallejo, H. Khatib, J.B. Dodgson, L. B. Crittenden, and J. Hillel. 1995. Development of a genetic map of the chicken with markers of high utility. Poult. Sci. 74:1855-1874.

Dalvit C., M. De Marchi, and M. Cassandro. 2007. Genetic traceability of livestock products: A review. Meat Sci. 77:437-449.

De Marchi M., M. Cassandro, C. Targhetta, M. Baruchello, and D. R. Notter. 2005a. Conservation of poultry genetic resource in the Veneto region of Italy. Anim. Genet. Res. Inf. 37:63-74.

De Marchi M., C. Dalvit, C. Targhetta, and M. Cassandro. 2006. Assessing genetic diversity in indigenous Veneto chicken breeds using AFLP markers. Anim. Genet. 37:101-105.

De Marchi M., C. Dalvit, C. Targhetta, and M. Cassandro. 2005b. Assessing genetic variability in two ancient chicken breeds of Padova area. Ital. J. Anim. Sci. 4(3):151-153.

Eding H., and T. H. E. Meuwissen. 2001. Marker based estimates of between and within population kinships for the conservation of genetic diversity. Anim. Breed. Genet. 118:141-159.

Eding J. H., and G. Laval. 1999. Measuring genetic uniqueness in livestock. Pages 33–58 in Genebanks and the Conservation of Farm Animal Genetic Resources. J.K. Oldenbroeck (ed.). DLO Institute for Animal Science and Health, Lelystad, The Netherlands.

Fabuel E., C. Barragán, L. Silió, M. C. Rodriguez, and M. A. Toro, M.A. 2004. Analysis of genetic diversity and conservation priorities in Iberian pigs based on microsatellite markers. Heredity 93:104-113.

FAO. 2007. The state of the world's animal genetic resources for food and agriculture. Commission on genetic resources for food and agriculture. Food and agriculture organization of the united nations. Rome.

FAO. 2004. Secondary Guidelines: Measurement of Domestic Animal Diversity (MoDAD): New Recommended Microsatellite Markers. Home page address: http://dad.fao.org/.

Felsenstein J. 2005. PHYLIP v.3.6. Department of Genome Sciences, Washington University, Seattle http://evolution.genetics.washington.edu/phylip/getme.html.

Glowatzki-Mullis M. L., J Muntwyler, E. Bäumle, and C. Gaillard. 2008. Genetic diversity measures of Swiss goat breeds as decision-making support for conservation policy. Small Rumin. Res. 74:202-211.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Granevitze Z., J. Hillel, G. H. Chen, N. T. K. Cuc, M. Feldman, H. Eding, and S. Weigend. 2007 Genetic diversity within chicken populations from different continents and management histories. Anim. Genet. 38:576–583

Guo S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48:361–372.

Gutièrrez P., and F. Goyache. 2004. MolKin (version 2.0) A Computer Program for Genetic Analysis of Populations Using Molecular Coancestry Information. Universidad Complutense de Madrid, Spain.

Hammond K. 1996. The status of global farm animal genetic resources. In: Proceedings of the Symposium on the Economics of Valuation and Conservation of Genetic Resources for Agriculture, Centre for International Studies on Economic Growth, Tor Vergata University, Rome, 13–15 May.

Hillel J., M. A. M. Groenen, M. Tixier Boichard, A. B. Korol, L. David, V. M. Kirzhner, T. Burke, A. Barre Dirie, R. P. M. A. Crooijmans, K. Elo, M. W. Feldman, P. J. Freidlin, A. Mäki-Tanila, M. Oortwijn, P. Thomson, A. Vignal, K. Wimmers, and S. Weigend. 2003. Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Genet. Sel. Evol. 35:533-557.

Mignon-Grasteau S, A. Boissy, J. Bouix, J. Faure, A. D. Fisher, G. N. Hinch, P. Jensen, P. Le Neindre, P. Mormède, P. Prunet, M. Vandeputte, and C. Beaumont. 2005. Genetics of adaptation and domestication in livestock. Liv. Prod. Sci. 93:3-14.

Muchadeyi F. C., H. Eding, C. B. A. Wollny, E. Groeneveld, S. M. Makuza, R. Shamseldin, H. Simianer, and S. Weigend. 2007. Absence of population substructuring in Zimbabwe chicken ecotypes inferred using microsatellite analysis. Anim. Genet. 38:32-339.

NCBI, Home page address http://www.ncbi.nlm.nih.gov Accessed Dec.08

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Page R. D. M. 2001. TREEVIEW: An application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12:357-358.

Pritchard J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Raymond M., F. Rousset. 1995 GENEPOP v. 3.1d. J. Hered. 86:248-249.

Rebbeck C. A., O. R. Jones, and I. J. Tsai. SimCo: A program to automate the comparison of multiple structure runs (available from howen.jones@imperial.ac.uki).

Reynolds J., B. S. Weir, and C. C. Cockerham. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics 105:767-769.

Rosenberg N. A., J. K. Pritchard, J. L. Weber, H. M. Cann, K. K. Kidd, L. A. Zhivotovsky, and M. W. Feldman. 2002. Genetic Structure of Human Populations. Science 298(5602):2381 - 2385

Rosenberg N. A. 2004. DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. Notes 4(1):137-138

Ruane J. 1999. Pages 59-73 in Genebanks and the conservation of farm animal genetic resources. J. K. Oldenbroek ed. DLO institute for Animal Science and Health. The Netherlands.

Tadano R., M. Nishibori, N. Nagasaka, and M. Tsudzuki. 2007. Assessing genetic diversity and population structure for commercial chicken lines based on forty microsatellite analysis. Poultry Sci. 86:2301-2308.

Tadano R., M. Nishibori, Y. Imamura, M. Matsuzaki, K. Kinoshita, M. Mizutani, T. Namikawa, and M. Tsudzuki. 2008. High genetic divergence in miniature breeds of Japanese native chickens compared to Red Junglefowl, as revealed by microsatellite analysis. Anim. Genet. 39:71–78

Takahashi H., K. Nirasawa, Y. Nagamine, M. Tsudzuki, and Y. Yamamoto. 1998. Genetic relationships among Japanese native breeds of chicken based on microsatellite DNA polymorphisms. J. Hered. 89(6):543-546.

Targhetta C., C. Dalvit, M. Baruchello, and M. Cassandro. 2005. Application of AFLP molecular markers to genetic characterisation of duck (Anas platyrhyncos), turkey (Meleagris gallopavo) and helmeted guinea fowl (Numida meleagris) Veneto breeds. Ital. J. Anim. Sci. 4:109-111.

Wahlund S. 1928. Zusammensetzung von Populationen und Korrelation sercheinungen vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas 11:65-106.

Weir B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

TABLES AND FIGURES

Table 1 Chromosomal location (Chr), number of alleles per locus (Na), fragment size, and polymorphism information content (PIC) for the analyzed chicken breeds.

Locus	Chr	Na (±SD)	Fragment size (bp)	PIC (±SD)
ADL0268	1	6	104-119	0.702
ADL0278	8	6	102-121	0.648
LEI0094	4	7	251-283	0.604
LEI0166	3	3	251-261	0.592
MCW0014	6	6	166-189	0.415
MCW0020	1	4	183-189	0.701
MCW0037	3	5	151-159	0.554
MCW0078	5	7	134-150	0.534
MCW0081	5	7	143-155	0.620
MCW0098	4	2	255-257	0.233
MCW0103	3	2	268-272	0.320
MCW0104	13	10	190-228	0.546
MCW0111	1	4	98-106	0.607
MCW0123	14	7	112-134	0.584
MCW016	3	8	136-154	0.589
MCW0165	23	4	112-123	0.587
MCW0216	13	4	141-147	0.615
MCW0222	3	5	217-225	0.531
MCW0248	1	8	213-245	0.350
MCW0295	4	7	86-102	0.597
Total		5.6±2.1		0.546±0.124

Table 2 Private alleles in bp (frequencies in brackets) for the analyzed breeds: Brown layer (BL) Ermellinata di Rovigo (ER), Pepoi (PP), Robusta Lionata (RL), Polverara (PV) and Padovana (PD). Alleles with frequencies higher than 0.10 are reported in bold.

Locus	BL	ER	PP	RL	PV	PD
ADL268						119(0.07)
ADL278				**108(0.0119)**	102(0.0057)	
LEI94	279(0.0476)		259(0.0119)			271(0.08)
MCW104	202(0.05) **210(0.125)** 228(0.05)		204(0.0179) 216(0.0357)		**218(0.1)**	
MCW123		126(0.0114)			**119(0.1824)**	
MCW14	168(0.0526) 170(0.0263)	**176(0.1333)**			189(0.0132)	
MCW16					136(0.0161)	
MCW16	**148(0.4048)** 152(0.0476) 154(0.0476)					
MCW165			123(0.0116)			
MCW222	217(0.0238)					
MCW248	213(0.0556) 227(0.0556)				230(0.0059) 245(0.0059)	
MCW295					102(0.0132)	
MCW37	**151(0.119)**					
MCW78	144(0.0476)		146(0.0233)			150(0.0104)
MCW81			**147(0.2889)**			

Table 3 Average number of alleles per breed (Na), sample size, expected (H_E) and observed (H_O) heterozigosity and molecular coancestry estimates (*fij)* of the analyzed breeds and strains: Brown layer (BL) Ermellinata di Rovigo (ER), Pepoi (PP), Robusta Lionata (RL), Robusta Maculata (RM), Polverara nera (PVn), Polverara bianca (PVb), Padovana camosciata (PDc) and Padovana dorata (PDd).

Breed	Na	Sample size	$H_E \pm SD$	$H_O \pm SD$	P	*fij*
BL	3.80	21	0.559±0.141	0.622±0.233	***	0.439
ER	3.14	45	0.420±0.175	0.384±0.248	***	0.573
PP	2.51	45	0.243±0.239	0.240±0.236	*	0.769
RL	2.43	43	0.367±0.229	0.317±0.264	***	0.657
RM	2.17	45	0.293±0.225	0.292±0.226	n.s.	0.721
PVb	3.01	36	0.436±0.190	0.366±0.201	***	0.577
PVn	3.45	52	0.463±0.177	0.413±0.170	***	0.559
PDc	2.27	26	0.305±0.257	0.287±0.271	n.s.	0.704
PDd	2.66	24	0.340±0.199	0.329±0.230	n.s.	0.689

***=P<0.001; *=P<0.05; n.s.= not significant

Table 4 F_{ST} distances (above diagonal, bold) and Kinship distances (below diagonal) among the analyzed breeds: Brown layer (BL), Ermellinata di Rovigo (ER), Pepoi (PP), Robusta Lionata (RL), Robusta Maculata (RM), Polverara (PV) and Padovana (PD).

	BL	ER	PP	RL	RM	PV	PD
BL		**0.067**	**0.098**	**0.073**	**0.075**	**0.035**	**0.070**
ER	0.325		**0.129**	**0.102**	**0.116**	**0.087**	**0.115**
PP	0.330	0.340		**0.125**	**0.142**	**0.087**	**0.111**
RL	0.319	0.332	0.318		**0.084**	**0.070**	**0.112**
RM	0.298	0.327	0.318	0.262		**0.099**	**0.110**
PV	0.312	0.359	0.313	0.316	0.343		**0.059**
PD	0.315	0.347	0.290	0.328	0.298	0.285	

Table 5 Loss or gain (%) of genetic diversity (GD) in the whole population when one of the analyzed breed is removed according to Caballero and Toro: Brown layer (BL), Ermellinata di Rovigo (ER), Pepoi (PP), Robusta Lionata (RL), Robusta Maculata (RM), Polverara (PV) and Padovana (PD).

Breed	GD	Internal diversity (%)	Between breed diversity (%)	Loss/Gain
All breeds	0.597			
ER	0.575	-2.06	-2.18	-4.24
PP	0.603	+3.41	-2.85	+0.56
RL	0.598	-0.29	-0.11	-0.40
RM	0.598	+1.62	-2.03	-0.41
PV	0.580	-6.78	+3.48	-3.30
PD	0.608	+0.47	+0.87	+1.34
PD + PV	0.499	+8.28	-25.19	-16.91

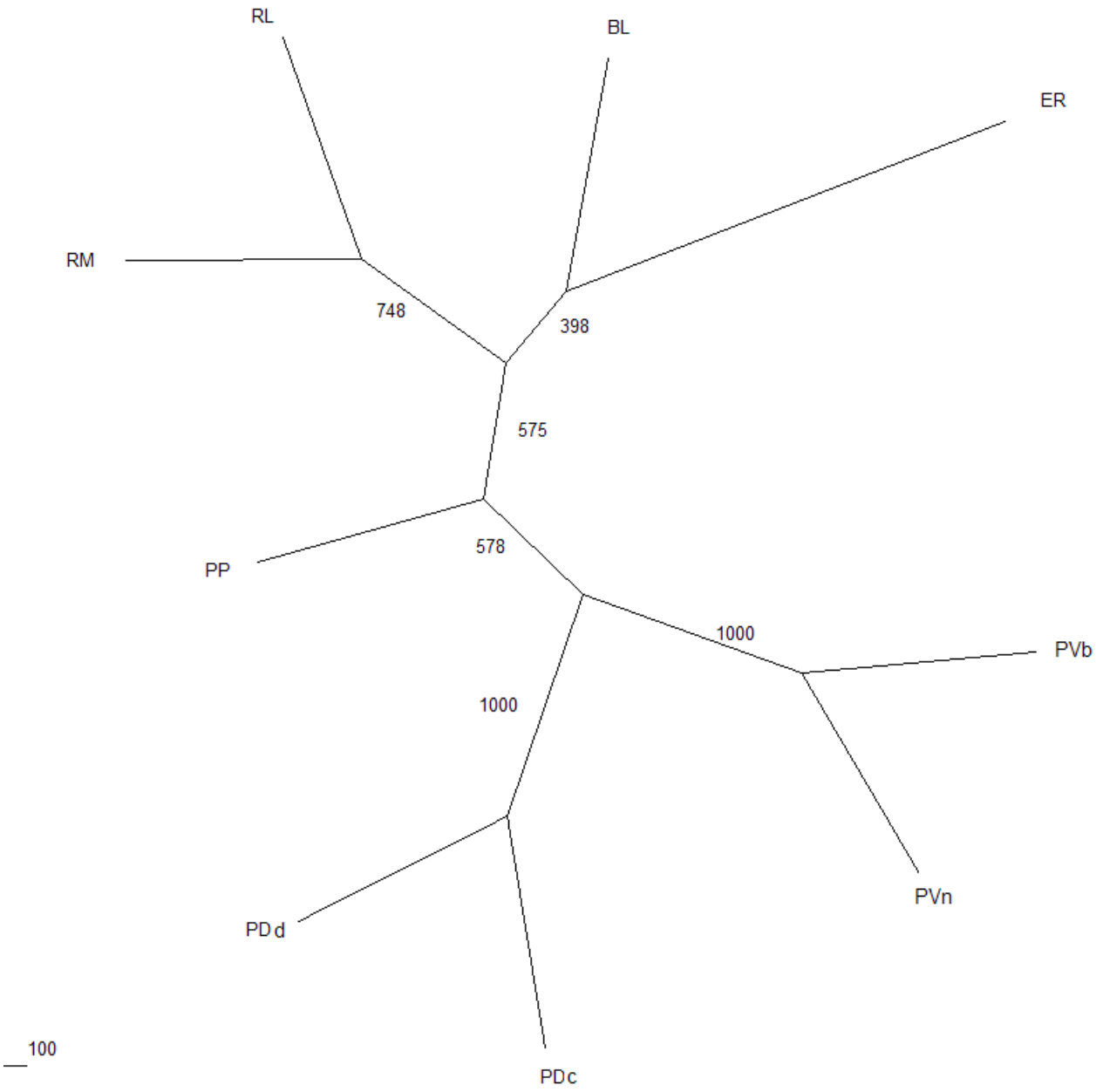


Figure 1 Neighbour-Joining tree drawn from D*R* distances between breeds (1000 bootstrap repetitions). Brown layer cross (BL), Ermellinata di Rovigo (ER), Pépoi (PP), Robusta Lionata (RL), Polverara Bianca (PVb), Polverara Nera (PVn), Padovana Camosciata (PDc), Padovana Dorata (PDd).

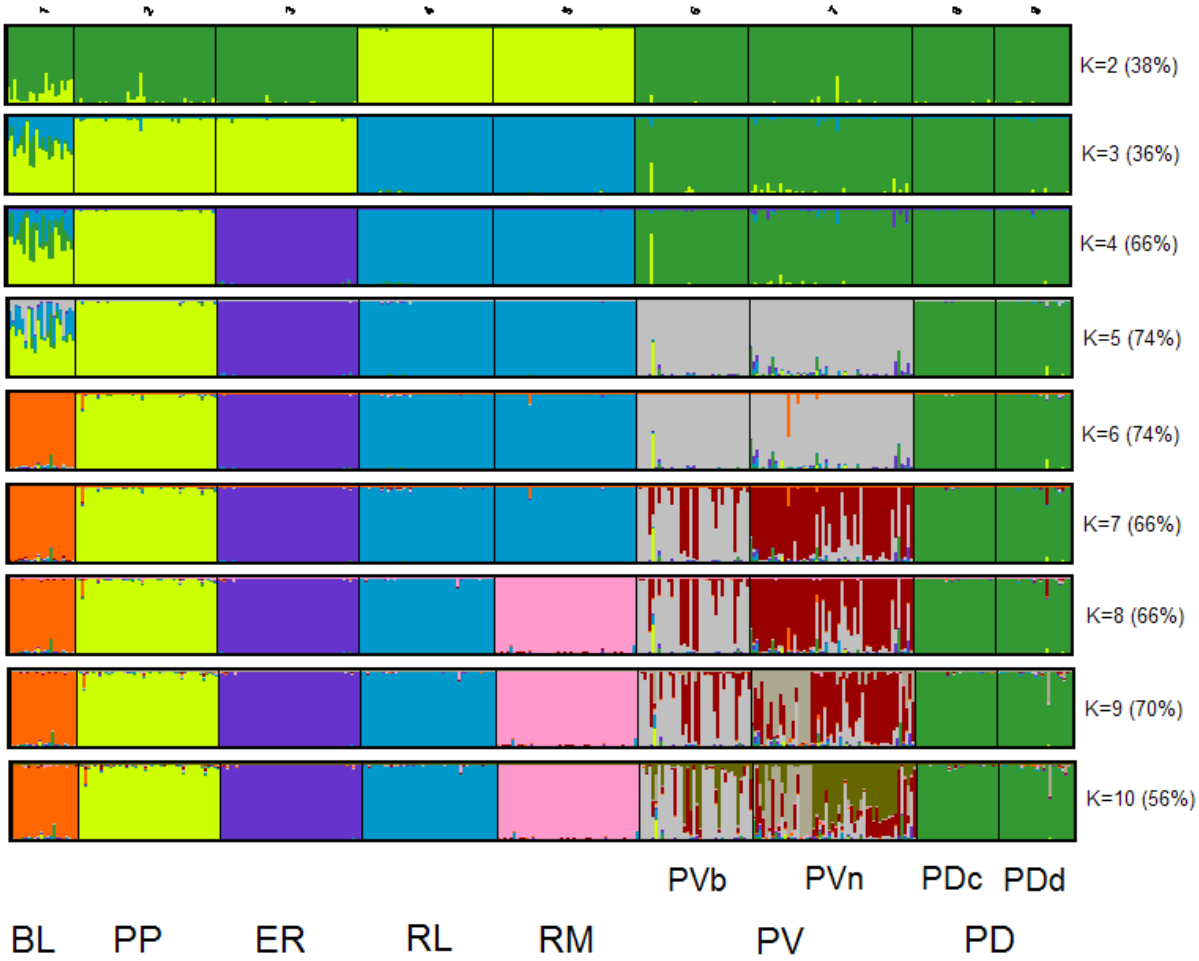


Figure 2 Structure analysis of six Italian local chicken breeds assuming K = 2, 3, 4, 5, 6, 7, 8, 9, 10. In brackets percentage of identical solutions with 95% of similarity, only most probable solutions for each K are shown. Brown layer cross (BL), Ermellinata di Rovigo (ER), Pépoi (PP), Robusta Lionata (RL), Polverara (PV), Polverara Bianca (PVb), Polverara Nera (PVn), Padovana (PD), Padovana Camosciata (PDc), Padovana Dorata (PDd).

CARCASS CHARACTERISTICS AND QUALITATIVE MEAT TRAITS OF THREE ITALIAN LOCAL CHICKEN BREEDS

E. Zanetti[1], M. De Marchi[1], C. Dalvit[1], C. Molette[3],[2,4], H. Remignon[3],[2,4], M. Cassandro[1]

[1] Department of Animal Science, University of Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy

[2] INRA, UMR 1289 Tissus Animaux Nutrition Digestion Ecosystème et Métabolisme, F-31326 Castanet-Tolosan, France

[3] Université de Toulouse, INPT ENSAT, UMR 1289 Tissus Animaux Nutrition Digestion Ecosystème et Métabolisme, F-31326 Castanet-Tolosan, France

[4] ENVT, UMR 1289 Tissus Animaux Nutrition Digestion Ecosystème et Métabolisme, F-31076 Toulouse, France

ABSTRACT

An experiment involving 60 male chickens reared in an organic production system, where housing was an indoor pen with access to a grass paddock, was carried out in order to investigate carcass characteristics and qualitative meat traits of three slow-growing Italian local breeds of chicken (Ermellinata, Padovana, and Pépoi).

2. Chicks were randomly selected at hatch, raised together under the same conditions, slaughtered at 190 days of age, dissected for carcass traits and meat (breast and thigh) was stored for subsequent analysis of quality parameters.

3. Ermellinata chickens were significantly different from Padovana and Pépoi chickens for live, carcass and thigh weights. Breeds were also different for breast muscle protein content (Ermellinata > Pépoi and Padovana, $p<0.05$), shear force (Padovana < Ermellinata and Pépoi, $p<0.05$) and cooking loss (Pépoi > Padovana and Ermellinata, $p<0.05$) values.

4. The CIE system values of lightness (L*), redness (a*), and yellowness (b*) evidenced a distinctive darker meat and lighter skin colour of Padovana breast meat.

5. Total fatty acids composition of breast meat was similar among the analysed breeds, while saturated and monounsaturated fatty acids contents of Ermellinata were higher and lower, respectively, than the other breeds.

INTRODUCTION

Consumer's interest is growing in specialty poultry products, particularly in Europe. Examples exist in France with "Label Rouge" (Westgren, 1999) or Poulet de Bresse as well as in Italy with Padovana chicken (De Marchi *et al.*, 2005). Those chicken production systems require extensive rearing conditions, with an outdoor access, and have eared grated success in national markets despite a higher retail price than conventional poultry products (Westgren, 1999; Fanatico & Born, 2001). Other production parameters such as the use of slow-growing lines (rearing period >2 months) or cereals based feeding programs are also appreciated in gourmet market (Westgren, 1999; De Marchi *et al.*, 2006). Among those slow-growing genotypes there are several local chicken breeds, especially in Italy, that showed very interesting meat quality traits such as peculiar color and flavour (De Marchi *et al.*, 2006). Moreover only the slow-growing strains can fully benefit from organic system (pasture availability, older age), whereas the fast-growing strains are characterized by a very low degree of adaptation and resistance to natural environment (Reiter & Bessei, 1996). Slow-growing and local strains have an intensive foraging behavior (Bokkers & Koene, 2003; Minh

& Ogle, 2005) and spend a lot of time outdoor (65-78% of budget time vs. 35-40 % for fast-growing strains; Gordon *et al*., 2002).

Several researches (Touraille *et al*., 1981; Jaturasitha *et al*., 2008) have evaluated differences in quality of meat from fast and slow-growing birds showing a great deal of variation in relation to the breed and the production systems. In particular, slow-growing birds reared with an outdoor access and slaughtered at an older age, present higher meat quality traits which please consumer's expectations from conventional poultry products. Lonergan *et al*. (2003) compared meat quality parameters among unique chicken populations with varying growth rates including broilers, Leghorns, and their crosses, and showed a high diversity of breast meat characteristics in terms of composition and quality. It has been reported that selection for fast growing rates and high meat yields are likely to have affected the sensory and functional qualities of the meat (Dransfield & Sosnicki, 1999; Le Bihan-Duval *et al*., 2001; Le Bihan-Duval, 2003); therefore, it is likely that differences in meat quality may exist between fast- and slow-growing broilers.

Although research has been conducted to evaluate meat and sensory quality of meat from fast- and slow-growing birds, there is a great deal of variation in the types of birds (e.g., breed, strain, age) and the production systems that have been used in these reports. The meat of slow-growing birds grown with outdoor access and harvested at an older age is expected to be more firm and more flavorful than conventional poultry, and, in a European study, consumers preferred it to conventional poultry meat (Touraille *et al*., 1981).

In the Veneto region of Italy, the increasing interest in the conservation (De Marchi *et al*., 2006) and development of the local chicken breeds is due to historical, social and economical reasons. A few breeds of chicken, Padovana (PA), Ermellinata (EA), and Pépoi (PI) which are typically reared in extensive systems, provide an interesting alternative to commercial broilers. The recent development of organic animal production and consumer requests for food safety, sustainable systems of production and more environmentally rural relations might encourage the use of local chicken breeds at least for a gastronomical niche market. Moreover, the demand for meat products from these indigenous breeds of chicken has also increased because of their perceived image as nutritious, healthy and natural products obtained from birds reared in a clean and natural environment without industrial residues. Previous research has been focused on the carcass characteristics and quality meat traits of Padovana breed of chicken (De Marchi *et al*. 2005) because it is actually the more developed system of production.

The interest on local genotypes increased noticeably in the last decade, mostly because biodiversity conservation and management has become an important issue for the international

scientific community (Fao, 2007). Productive performance analysis and peculiar phenotypical traits, together with genetic diversity, reproductive and adaptative performances and historical interest, are hence of great relevance for including local breeds in conservation programmes (Ruane, 1999).

The objective of this research was to describe carcass characteristics and qualitative meat traits of three local chicken breeds showing, at maturity, light, medium, and heavy live weights. By the fact, those breeds could permit to extend and diversify consumer's offer to fit all the local demands in typical diversified poultry products.

MATERIALS AND METHODS

Animals, diets and experimental procedures

A trial was conducted at the Agricultural High School "Duca degli Abruzzi" in Padova (northeast of Italy). Three slow-growing genotypes were compared Pépoi (PI), Padovana (PA), and Ermellinata (EA); they were categorized with regard to the different weight they reach in 190 days (market weight): [1] PI live weight of 1400 -1600 g, [2] PA live weight of 2000 - 2200 g [3] EA live weight of 2800 - 3000 g (DE MARCHI et al., 2006). Twenty-five 30d male chicks were obtained from the PA, EA, and PI breeds and housed in an indoor pen with access to a grass paddock.

All birds were provided with the same diets, which included a starter diet (provided for 4 wk) consisting of 23.0% crude protein, 4.0% lipids, 5.0% fiber, 8.5% ash, and 3,300 kcalME/kg to 21 days of age and a grower diet (provided until slaughter) made up of a crumbled vegetable diet consisted of 18.5% crude protein, 4.0% lipids, 4.0% fiber, 6.0% ash, and 2,800 kcalME/kg. Feed and water were supplied *ad libitum*. The diets were devoid of animal products, antibiotics and anticoccidial medication. Access to feed and water was freely available, and the diets were formulated to contain adequate nutrient levels.

Fifty nine animals (20 males for PA and PI, and 19 males for EA) were slaughtered at 190 days of age. Feed was withdrawn 18 h prior to slaughter and weights were obtained from live animals just before slaughter. After the slaughtering process, the carcasses were cooled in a tunnel and refrigerated at 4° C for 24 h and the weight of the carcasses was recorded (ready to cook weight). The breast and thigh meat from all chickens were then harvested and processed for meat quality parameters determination. Other breast meat samples were also collected and stored at -20°C for further analyses.

Analytical determinations

Breast (*Pectoralis superficialis)* and thigh (*Peroneus longus)* muscles pH were measured 24 h *post-mortem* using a Delta Ohm HI-8314 pH-meter (Delta Ohm, Padova Italy). Color, tenderness and cooking loss were also determined at 24 h *post-mortem*. The color of the breast meat, with and without skin and thigh skin was evaluated using a Minolta CM-508c (illuminate: D65, Observer: 10°). The readings were taken on same anatomical positions for all breast and thigh samples. For each sample, 3 measurements were performed and the final value for each chicken is the average of those readings. Skin color of breast and thigh and meat color of breast were expressed in the CIELab color space by reporting L*, a* and b* values (CIE, 1978).

Cooking loss percentage (CL%) was measured on the left part of the breast muscle without the skin.using 2-cm thick samples sealed in a polyethylene bag and heated in a water bath to an internal temperature of 70 °C for 40 min (ASPA, 1996). Cooking loss percentage was then calculated from the ratios between the weights before and after cooking.

For the calculation of Shear force (SF) on breast muscle, measures were obtained on five cylindrical cores of 1.13 cm in diameter taken parallel to muscle fibers. Shear force was measured by a TA-HDi Texture Analyser (Stable Macro System) with a Warner-Bratzler shear attachment (10 N load cell, crosshead speed of 2 mm/s) and interpreted using texture expert software (ASPA, 1996).

All chemical analyses were performed on the right breast, without skin, and were in accordance to Aoac (1990) standards. Moisture was determined after drying at 102°C for 16 h. Ash was determined after mineralization at 525°C. Total lipids were analyzed by extraction with petroleum ether (Soxtec method). Protein content was estimated by difference.

For the determination of total fatty acids composition, lipids were extracted according to the method of Folch *et al*. (1957). Briefly, a 5 g homogenized meat sample was blended twice with extraction solvent chloroform/methanol (1:2, v/v), filtered, placed in separator funnels, and mixed with saline solution (0.88% KCl). After separation in two phases, the methanol aqueous fraction was discarded, and the lipid chloroform fraction washed with distilled water/methanol (1:1, v/v). Following a further filtration and evaporation by means of a rotary evaporator, lipid extracts were prepared for trans-esterification following the protocol of Christie (1982) and transferred to test tubes for subsequent gas chromatographic analysis, performed on a Thermo Quest (Italia, model 8000 Series Top) instrument equipped with a Omegawax 250 capillary column (length 30 m, internal diameter 0.25 mm).

Statistical analysis

Data were subjected to ANOVA by the GLM procedure considering breed as a fixed effect using SAS® software (1996, SAS Institute, Cary, NC). For breed effect a multiple comparison of means was performed using the Bonferroni's test ($P < 0.05$). Breast meat L* values were also calculated using breed as fixed effect and pH as covariate.

Comparisons among breeds were performed two by two by means of principal component analysis was performed using PROC PRINCOMP of SAS®, using data on skin color of breast and thigh and meat color of breast were expressed in the CIELab color space.

RESULTS

Final live weights at … days of age (just before slaughter) clearly differed ($P<0.001$) among breeds as reported in Table 1. Ermellinata had heavier live, carcass, and thigh weights than PA and PI. Pépoi had lighter breast weight than PA and EA ($P<0.001$). Percentage dressing was greater for PA and EA than PI, while PA and PI had greater breast percentage than EA ($P<0.05$). The pH values measured in the breast muscle were significantly ($P< 0.01$) higher in PA than in the other local breeds, while measures in the thigh revealed a higher pH value in PI respect to EA and PA ($P<0.01$) (Table 1).

Dry matter, protein and lipid contents of the breast muscle only slightly differed among breeds ($P<0.05$) (Table 1).

Maximum shear force values measured on cooked breast muscle were significantly different between PA and the other breeds ($P<0.05$) (Table 1). The PA breed showed the highest tenderness (12.51 N) followed by EA (16.76 N) and PI (18.84 N) while cooking loss values were higher for PI breed respect to PA and EA breeds ($P<0.001$).

Breast and thigh color values recorded in the three chicken breeds are shown in the Table 2. The PA breed showed the highest L* value of breast skin followed by PI and EA ($P<0.01$), while the PI breed showed the lower a* and b* values ($P<0.01$) respect to PI and EA. The color values of thigh skin were similar to breast skin with the exception of the b* index that was higher for EA followed by PI and PA. The breast meat color evidenced lower L* value for PA than for EA and PI, while higher b* values were recorded for PI than for PA and EA.

Raw breast meat (without the skin) fatty acid composition of the three chicken breeds is shown in the Table 3. Ermellinata breed evidenced a higher content of saturated (SFA) and lower amounts of monounsaturated (MUFA) fatty acids than PA and PI ($P<0.01$). Percentages of n-3 and n-6 polyunsaturated fatty acids did not evidence differences among breeds.

Ermellinata differs from other breeds because of a higher $C_{16:0}$ content and a lower production of $C_{18:1cis\ n-9}$ and $C_{18:2cis\ n-6}$ fatty acids ($P<0.01$). No differences in DPA nor CLA fatty acids were recorded among breeds while Pépoi breast meat showed a higher content of DHA than Padovana.

Padovana and PI breeds showed significantly higher levels of MUFA (23.45% and 24.92%, respectively) than EA (20.21%) ($P<0.01$). Oleic acid ($C_{18:1cis\ n-9}$) wass the most abundant MUFA present in all the analyzed breeds (20.70% PA, 17.79% EA and 21.53% PI), while, amongst PUFA, linoleic acid ($C_{18:2\ n-6}$) was the most representative (25.25% for PA, 21.79% for EA and 21.84% for PI).

DISCUSSION

The Ermellinata, Padovana and Pépoi chickens exhibited medium, light and very light carcass weights respectively. Padovana dressing and breast percentages were slightly lower than those reported by De Marchi et *al*. (2005) in an experiment done with 60 Padovana chickens. Dressing percentages for the PA, EA, and PI breeds were also slightly lower than those reported for local Thaï chicken genotypes (Jasurasitha *et al*., 2008) and greatly lower than that reported from commercial broilers (Cortinas *et al*., 2004; Havenstein *et al*., 2003). These results showed that the Italian local chicken breeds studied here had moderate carcass weight, dressing and breast percentages.

The pH values measured in the breast muscle were significantly ($P< 0.01$) higher in PA when compared to the other local breeds, while, for the thigh, PI showed the highest values. These values were higher than expected and could reflect unfavourable conditions of transport and slaughter probably because the old unselected breeds have a more aggressive and alert behavior than the modern selected breeds (Jaturasitha *et al*., 2004). Nevertheless the breast pH values of the PA breed were lower than those reported previously by De Marchi *et al*. (2006). High pH values in meat are generally associated with increased stressful conditions before slaughter resulting in poor glycogen content in muscle at the time of death. Here, this could reflect a higher sensibility to environmental conditions prior to slaughter of those local breeds. In consequence, it should be recommended that specific precautions before slaughter might be taken to reduce those impacts and ensure optimal conservation of extensive rearing.

Dry matter, protein and lipid contents of the breast muscles slightly differed among breeds. The chemical composition of the PA breed breast was consistent with values reported by De Marchi *et al*. (2006). Dry matter and protein contents of the studied local Italian breeds were

similar to those reported for other organic chickens (Castellini *et al*., 2002; Castellini *et al*., 1994). On the contrary, protein, lipids, and ash contents were higher for those Italian local breeds than for Thaï indigenous chickens or standard commercial broilers (Wattanachant *et al*., 2004). As expected, local chicken muscle contains high percentage of protein and low fat and ash contents as previously reported by Wattanachant *et al*. (2005).

The PA breed showed a greater value of cooking loss compared to the values reported by De Marchi *et al*. (2006) for the same breed. However, recorded cooking loss values (around 18-22%) were lower to the 33% reported for organic breeds by Castellini *et al*. (2002) and similar to the 23% reported for a Thaï indigenous chickens or to the 20% recorded for standard broilers (Wattanachant *et al*., 2004; Liu *et al*., 2004).

The Padovana breed appeared to have darker breast muscle (low L* values). Nevertheless, the presence of an higher ultimate pH value in the breast muscle, which favours the post-mortem formation of metmyoglobin through myoglobin oxidation, could explain why such difference exists between PA and other studied breeds. For this reason, the analysis of breast meat L* values were also performed by using the fixed effect of breed and the effect of pH as covariate. In the preliminary analysis the interaction between breed and pH was not significant. The multiple comparison of estimated least square means, performed using the Bonferroni's test ($P < 0.05$), confirmed that PA had a significant lower breast meat L*value than the other breeds.

Principal component analysis was used as alternative statistical approach to study individual grouping based on color differences among breeds. Figure 1 outlines the bidimensional plot of the first and second principal component scores for Ermellinata-Padovana, Pépoi- Padovana and Pépoi-Ermellinata comparisons, respectively. First principal component can fully distinguish the analysed animals into two groups, corresponding to the different breeds, and a good amount of variability is explained by this component (29%, 30%, and 31% for Ermellinata-Padovana, Pépoi- Padovana and Pépoi-Ermellinata comparisons, respectively), enabling breed differentiation using color parameters; while the same was not possible taking into account carcass yield ratios, chemical composition or fatty acids compositions of the different breeds.

Regarding fatty acid composition of the breast meat, the results of this study are similar to those reported by De Marchi *et al*. (2005) and by Castellini *et al*. (2006) for Ross 205 and Kabir chickens reared in organic rearing system. The observed differences in SFA and MUFA among the studied breeds can be attributable only to the genetic determinism, since diets and rearing system were completely similar for all breeds during the whole experimental period. It is

however possible that this trend could be attributable to the greater ingestion of grass (not measured) by EA chickens. Highest saturated fatty acids contents recorded in EA breed remains lower than those reported for organic (38%) and Thai local chickens (62%) (Wattanachant et al., 2004). Among SFA, palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids were the most abundant as generally observed in chicken breast meat.

In conclusion the present study evidenced differences in meat quality traits among the studied local chicken breeds. The breeds differed from each other for some aspects such as carcass yield, colour, tenderness, and fatty acids composition. From a consumer point of view, each breed presents unique features. Beyond a more incisive traditional interest and a high historical and cultural value, PA has the highest tenderness, a peculiar darker color able to differentiate its meat, and, with PP, the lower content in saturated fatty acids. However EA presents a good carcass weight to meet the demands of the modern consumer. Performed analysis did not evidence particular factors exalting the meat quality traits of the PP breed. Successful individual grouping corresponding to the breed of origin was achieved based on color differences of skin and meat. Besides that, also adaptability features, traits of scientific and economic interests, the cultural\historical value of these breed, its strong link to the local traditions and its ability to generate incomes from tourism justify the efforts for its conservation and characterization (Ruane, 1999). Then, the commercialization and the capitalization on local markets represent a big opportunity for the future valorisation and exploitation of those local genetic resources. Alternative strategies, such as the use of crosses of these breeds with more productive commercial breeds, are currently under evaluation to enhance their diffusion for niche and regional markets.

REFERENCES

AOAC, 1990: Official Methods of Analysis (15th ed.). Association of Official Analytical Chemists. Washington, DC.

ASPA. 1996. Associazione Scientifica di Produzione Animale, Metodiche per la determinazione delle caratteristiche qualitative della carne, Università degli studi di Perugia.

Bokkers, E.A.M. & Koene, P. 2003. Behaviour of fast- and slow growing broilers to 12 weeks of age and the physical consequences. Applied Animal Behaviour Science, 81(1): 59.

Castellini, C., Mugnai, C. & Dal Bosco, A. 2002. Effect of organic production system on broiler carcass and meat quality. Meat Science 60(3), 219-225.

Castellini, C., Dal Bosco, A., Mugnai, C. & Pedrazzoli, M. 2006. Comparison of two chicken genotypes organically reared: oxidative stability and other qualitative traits of the meat. Italian Journal Animal Science. 5 :355-368.

Castellini, C., Pannella, F. & Lattaioli, P. 1994. Impiego della razza Ancona per l'impiego di un pollastro di qualità. Rivista di Avicoltura 5, 41-45.

CIE. 1978: International Commission on Illumination, recommendations on uniform color spaces, color, difference equations, psychometric color terms. CIE publication No. 15 (Suppl. 2), (E-1.31) 1971/(TC-1.3). Bureau Central de la CIE, Paris, France.

Cortinas, L., Villaverde, C., Galobart, J., Baucells, M.D., Codony, R. & Barroeta, A.C. 2004. Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level. Poultry Science 83:1155-1164.

Christie, W.W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesterol ester. Journal of Lipid Research 23:1072-1075.

De Marchi, M., Cassandro, M., Targhetta, C., Baruchello, M. & Notter, D.R. 2006. Conservation of poultry genetic resource in the Veneto region of Italy. Animal Genetic Resources Information (FAO/UNEP). 37:63-74.

De Marchi, M., Cassandro, M., Lunardi, E., Baldan, G. & Siegel, P.B. 2005. Carcass characteristics and qualitative meat traits of the Padovana breed of chicken. International Journal of Poultry Science 4:233-238.

Dransfield, E. & Sosnicki, A.A. 1999. Relationship Between Muscle Growth and Poultry Meat Quality. Poultry Science 78(5):743-746.

Fanatico, A.C. & Born, H. M. 2001. Label Rouge: Pastureraised poultry in France. ATTRA publication. Natl. Center Appropriate Technol., Fayetteville, AR.

FAO. 2007. The state of the world's animal genetic resources for food and agriculture. Commission on genetic resources for food and agriculture. Food and agriculture organization of the united nations. Rome.

Folch, J., Lees, M. & Sloane-Stanley, H. (1957) A simple method for the isolation and purification of total lipids from animal tissue. Journal of Biological Chemistry 226:497-509.

Gordon, S. H., Charles, D. R. & Green, G. 2001. Metabolic age: a basis for comparison of traditional breeds of meat chickens. British Poultry Science 42:S118–S119.

Havenstein, G.B., Ferket, P.R. & Qureshi, M.A. 2003. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science 82:1509-1518.

Jaturasitha, S., Srikanchai, T., Kreuzer, M. & Wicke, M. 2008. Differences in Carcass and Meat Characteristics Between Chicken Indigenous to Northern Thailand (Black-Boned and Thai Native) and Imported Extensive Breeds (Bresse and Rhode Island Red). Poultry Science 87:160-169.

Jaturasitha, S., Khiaosaard, R., Pongpaew, A., Leawtharakul, A., Saitong, S., Apichatsarangkul, T. & Leaungwunta, V. 2004. Carcass and indirect meat quality of native and Kai Baan Thai chickens with different sex and slaughter weight. In: Proc. 42nd Annu. Conf. Kasetsart Univ., Bangkok, Thailand. pp 116–126.

Le Bihan-Duval, E. 2003. Genetic variability of poultry meat. In Proceedings of the 52nd Annu. Natl. Breeders Roundtable, St. Louis, MO. US Poult. Egg. Assoc., Tucker, GA. pp 11–20

Le Bihan-Duval, E., Berri, C. Baeza, E. Millet, N. & Beaumont, C. 2001. Estimation of the genetic parameters of meat characteristics and their genetic correlations with growth and body composition in an experimental broiler line. Poultry Science 80:839–843.

Liu, Y., Lyon, B.G. Windham, W.R. Lyon C.E. & Savage, E.M. 2004. Principal component analysis of physical, color and sensory characteristics of chicken breast deboned at two, four, six, and twenty-four hours postmortem. Poultry Science 83:101- 108.

Lonergan, S.M., Deeb, N., Fedler, C.A. & Lamont, S.J. 2003. Breast meat quality and composition in unique chicken populations. Poultry Science 82:1990–1994.

Minh, D.V. & Ogle, B. 2005. Effect of Scavenging and Supplementation of Lysine and Methionine on the Feed Intake, Performance and Carcase Quality of Improved Dual-Purpose Growing Chickens. Tropical Animal Health Production 37:573–587

Reiter, K. & Bessei, W. 1996. Effect of the distance between feeder and drinker on behaviour and leg disorders of broilers. In Proc. 30th Int. Congr. Applied Ethology, Guelph, Canada. p 131

Ruane, J. 1999. In: Genebanks and the conservation of farm animal genetic resouces. J.K. Oldenbroek ed. DLO institute for Animal Science and Health. The Netherlands, pp.59-73.

SAS Institute. 1997. SAS/STAT® User's Guide: Statistics, Version 6.12, SAS Institute Inc., Cary, NC.

Touraille, C., Kopp, J., Valin, C. & Ricard, F.H. 1981. Chicken meat quality. 1. Influence of age and growth rate on physicochemical and sensory characteristics of the meat. Archivs Geflügelkunde. 45:69–76.

Wattanachant S., Benjakul, S. & Ledward, D.A. 2005. Microstructure and Thermal Characteristics of Thai Indigenous and Broiler Chicken Muscles. Poultry Science 84:328–336.

Wattanachant, S., Benjakul, S. & Ledward, D.A. 2004. Composition, Color, and Texture of Thai Indigenous and Broiler Chicken Muscles. Poultry Science 83(1):123-128.

Westgren, R.E. 1999. Delivering food safety, food quality, and sustainable production practices: The Label Rouge poultry system in France. American. Journal of Agricultural. Economics. 81, 1107.

TABLES AND FIGURES

Table 1. *Carcass composition, pH values, breast chemical composition, shear force and cooking loss values of the three slow-growing chicken breeds at 190 days of age.*

Item	Breed			R^2	RMSE
	Padovana	Ermellinata	Pèpoi		
Weights, g					
- Live (LW)	2144[b]	2718[a]	1434[c]	0.89	187
- Carcass (CW)	1346[b]	1726[a]	879[c]	0.88	125
- Breast (BW)	225[a]	243[a]	140[b]	0.72	29
- Thigh (TW)	478[b]	667[a]	322[c]	0.89	50
Total dressing (%; CW/LW)	63[a]	64[a]	61[b]	0.27	0.02
Breast (%; BW/CW)	17[a]	14[b]	16[a]	0.27	0.02
Breast meat pH	6.18[a]	5.97[b]	5.99[b]	0.21	0.18
Thigh meat pH	6.19[b]	6.15[b]	6.30[a]	0.16	0.15
Breast muscle chemical composition, %					
- Dry matter	24.8[b]	26.2[a]	25.5[ab]	0.26	0.01
- Total proteins	23.2[b]	24.6[a]	23.8[b]	0.11	0.01
- Total lipids	0.6[a]	0.2[b]	0.5[ab]	0.37	0.01
- Ash	1.1	1.1	1.1	0.20	0.01
Shear Force, N	12.51[a]	16.76[b]	18.84[b]	0.19	3.82
Cooking loss, %	18[b]	19[b]	22[a]	0.29	0.03

a,b,c : Within a line, means with different letters significantly differ ($p<0.05$).

Table 2. *Breast and thigh color values of the three studied slow-growing chicken breeds*

Item	Breed			R^2	RMSE
	Padovana	Ermellinata	Pepoì		
Skin color					
Breast					
- Lightness (L*)	62.41[a]	56.96[b]	59.20[b]	0.32	3.27
- Redness (a*)	-2.15[a]	-2.42[a]	-3.07[b]	0.25	0.69
- Yellowness (b*)	1.91[a]	1.06[a]	-1.47[b]	0.21	2.84
Thigh					
- Lightness (L*)	62.08[b]	62.35[b]	63.81[a]	0.15	1.89
- Redness (a*)	-2.19	-1.99	-2.47	0.08	0.69
- Yellowness (b*)	-1.97[c]	3.94[a]	0.01[b]	0.62	1.94
Meat color					
Breast					
- Lightness (L*)	46.00[a]	48.41[b]	49.84[b]	0.35	2.38
- Redness (a*)	-1.42	-1.72	-1.39	0.11	0.42
- Yellowness (b*)	-0.45[a]	-1.64[b]	0.04[a]	0.14	1.75

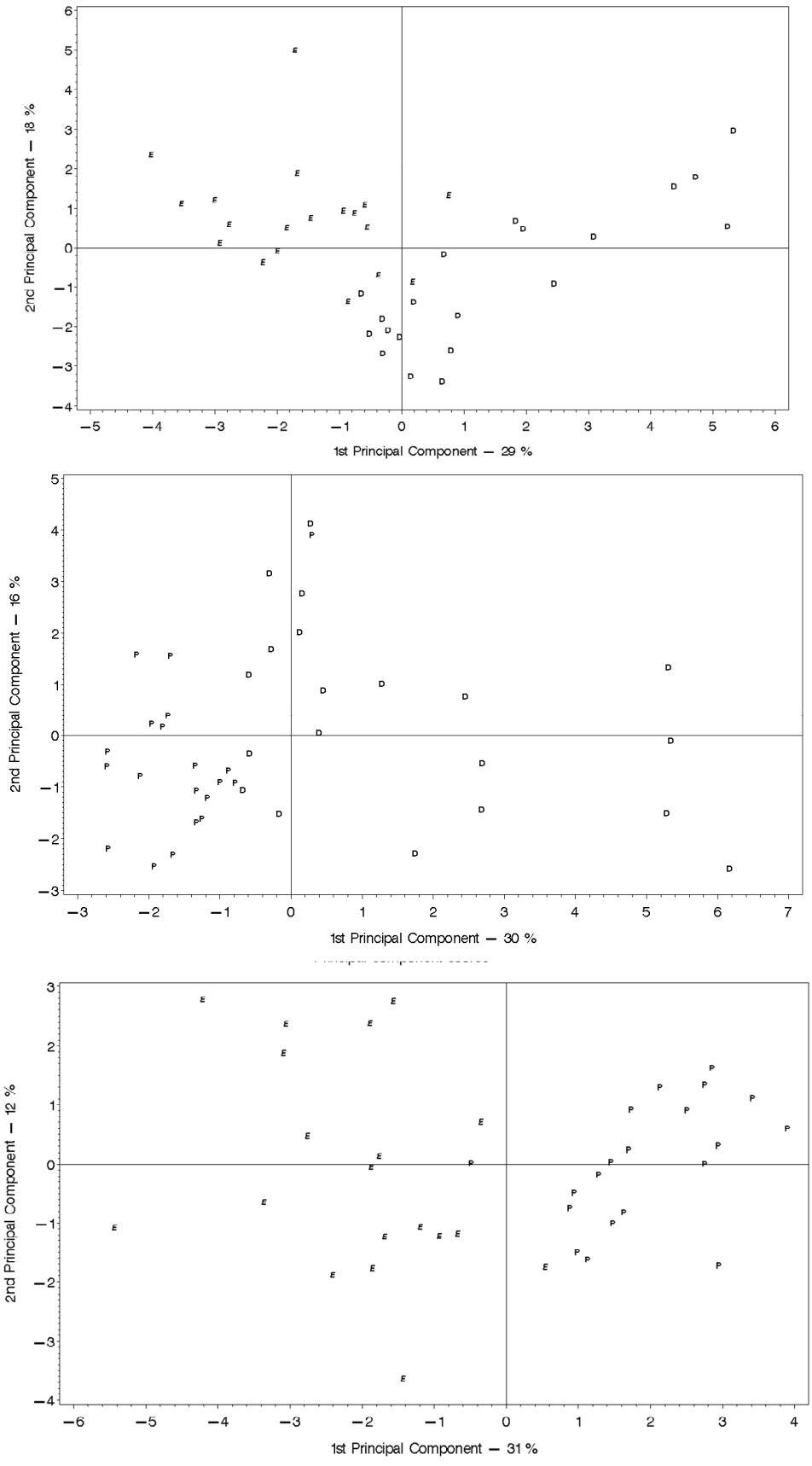
a,b,c : Within a line, means with different letters significantly differ ($p<0.05$).

Table 3. *Raw breast (without skin) fatty acid composition of the three slow-growing chicken breeds at 190 days of age.*

Item	Breed			R^2	RMSE
	Padovana	Ermellinata	Pépoi		
Breast muscle	% of total analyzed fatty acid				
C6:0	0.02	0.02	0.01	0.18	0.02
C8:0	0.06^{b}	0.10^{a}	0.11^{a}	0.40	0.03
C10:0	0.06	0.08	0.07	0.08	0.02
C12:0	0.03	0.02	0.02	0.08	0.02
C14:0	0.44^{a}	0.29^{b}	0.28^{b}	0.28	0.12
C15:0	0.09	0.08	0.07	0.08	0.02
C16:0	20.65^{a}	21.40^{a}	19.50^{b}	0.34	1.13
C17:0	0.28^{a}	0.25^{ab}	0.24^{b}	0.16	0.04
C18:0	11.51^{b}	12.64^{a}	11.86^{b}	0.27	0.81
C20:0	0.08^{ab}	0.06^{b}	0.09^{a}	0.15	0.04
C21:0	0.01	0.01	0.01	0.10	0.01
C22:0	0.01^{b}	0.01^{b}	0.02^{a}	0.21	0.02
C10:1 n-1	0.05	0.04	0.04	0.05	0.02
C14:1 n-1	0.01	0.01	0.01	0.01	0.01
C16:1 n-9	0.20	0.16	0.18	0.09	0.05
C16:1 n-7	0.39	0.34	0.46	0.08	0.17
C17:1 n-9	0.03	0.07	0.11	0.09	0.10
C17:1 n-7	0.01^{b}	0.01^{ab}	0.02^{a}	0.38	0.01
C18:1 n-7 trans	0.04	0.03	0.02	0.13	0.02
C18:1 n-9 trans	0.01^{b}	0.02^{ab}	0.02^{a}	0.17	0.01
C18:1 n-9 cis	20.69^{a}	17.79^{b}	21.53^{a}	0.33	2.38
C18:1 n-7 cis	1.73^{b}	1.46^{c}	2.18^{a}	0.68	0.21
C20:1 n-9	0.19^{a}	0.13^{b}	0.22^{a}	0.34	0.05
C18:2 n-6 trans	0.01	0.01	0.01	0.04	0.01
C18:2 n-6 cis	25.25^{a}	21.79^{b}	21.84^{b}	0.18	3.52
C18:3 n-6	0.08^{a}	0.04^{b}	0.05^{b}	0.32	42.23
C18:3 n-3	0.93^{a}	0.56^{b}	0.61^{b}	0.26	0.28
CLA	0.01	0.01	0.01	0.06	0.01
C20:2 n-6	0.45^{a}	0.39^{b}	0.43^{ab}	0.15	0.05
C20:3 n-6 cis	0.41^{b}	0.73^{a}	0.54^{b}	0.43	0.16
C20:4 n-6	9.06^{b}	13.11^{a}	11.78^{ab}	0.19	3.64
C22:1 n-9 cis	0.01	0.01	0.01	0.03	0.01
C20:5 n-3 cis	0.06	0.05	0.08	0.03	0.06
C22:2 n-6	0.17	0.29	0.22	0.10	0.16
C22:4 n-6	0.01	0.03	0.01	0.14	0.04
C22:5 n-6	1.19	1.33	1.36	0.04	0.34
C24:1 n-9 cis	0.30	0.29	0.34	0.02	0.13
C22:5 n-3 DPA	1.19	1.37	1.26	0.04	0.41
C22:5 n-3 DHA	1.42^{b}	1.66^{ab}	1.90^{a}	0.13	0.53
Total SFA	33.88^{b}	35.59^{a}	32.82^{b}	0.46	1.29
Total MUFA	23.45^{a}	20.21^{b}	24.92^{a}	0.39	2.55
Total PUFA	40.24	41.38	40.09	0.05	2.71
Total n-6 FA	36.63	37.74	36.23	0.08	2.27
Total n-3 FA	3.60	3.64	3.85	0.02	0.73
n-6/n-3	10.51	10.93	9.66	0.06	2.19

a,b,c : Within a line, means with different letters significantly differ ($p<0.05$).

Figure 1. *Principal Component scores for Padovana (P) and Ermellinata (E); Pépoi (P) and Padovana (P); Pépoi (P) and Ermellinata (E).*



A PROTEOMIC APPROACH TO STUDY LOCAL CHICKEN BREEDS CHARACTERIZATION

Enrico Zanetti[1], Chiara Dalvit[1], Caroline Molette[2,3], Christophe Chambon[4], Jeremy Pinguet[4]
Hervè Remignon[2,3], Martino Cassandro[1]

[1] Dipartimento di Scienze Animali, Università di Padova, Italy

[2]Ecole Nationale Agronomique de Toulouse, France

[3]INRA, UMR 1289 Tandem, France

[4]INRA, Plate-Forme d'Exploration du Métabolisme, composante Protéomique, Theix, 63122 Saint-Genès-Champanelle, France

ABSTRACT

Aim of this study is to apply a proteomic approach for characterization of local chicken breeds. The experiment involved a total of 29 males of Pépoi, Padovana, and Ermellinata local chicken breeds. Sarcoplasmic protein fractions of breast muscle were analysed by two-dimensional electrophoresis. Image analysis followed by statistical analysis enabled to differentiate groups of individuals on the similarities of protein expression. Individuals were distinguished into clusters and groups, corresponding to the breed of origin. SAM analysis enabled identification of the most relevant spots; 10 of these were identified by Mass Spectrometry revealing preliminary evidences on the mechanics of the breed differentiation process. Results evidenced a possible utilisation of proteomic approach in the field of breed characterization studies as an alternative to genomic analyses performed using molecular markers, both for breed and product traceability purposes.

Key words: Chicken, Proteomic, Local Breeds, Characterization

INTRODUCTION

The FAO Global Databank for Farm Animal Genetic Resources (DAD-IS) contains information on 6,379 breeds of 30 mammalian and bird species. Estimates show that 18% of the breeds existing in the early 20th century have already been lost and a total of 1,491 breeds (20%) are classified as being "at risk" (FAO, 2007). Since no complete surveys have been yet extended to all the breeds, an estimate of 35% of all breeds has an unknown risk status and their productive and reproductive traits, and the traits related to disease resistance or to the ability to live in a particular environment giving favourable economic outputs have not been studied and recorded. That situation motivates the need for a detailed breed characterisation, where different aspects and approaches should be considered.

Part of the characterisation efforts could be directed toward the analysis of the proteome expressed by the different species or, within species, breeds or populations. Proteomic analyses describe identity, relative quantity, and state of proteins in a cell, under a specific set of conditions. Proteomics complements and extends study of genomic and transcript data, reflecting true biochemical outcome of genetic information (Doherty *et al.*, 2007). In proteomics expression, the relative abundances of proteins are measured and compared and it is conceptually equivalent to differential gene expression experiments using cDNA

microarrays (Burgess, 2004). At the present, avian proteome studies have been limited and no study used proteomic technique for local poultry breed characterization.

The aim of this study is to propose a proteomic approach to characterize local chicken breeds.

MATERIAL AND METHODS

Breeds

In the Veneto region of Italy, Padovana (PD), Ermellinata di Rovigo (ER), and Pèpoi (PP), which are typically reared in free range systems, provide an interesting alternative to commercial lines. These local breeds were previously described by De Marchi *et al.* (2005a, 2005b). The trial made use of day-old chicks reared at the Agricultural High School "Duca degli Abruzzi" (Padova). The experiment consisted of 29 males (PD=10, PP=10, and ER=9) slaughtered at 190 d of age. At hatch, chicks were placed together in an indoor pen with access to a grass paddock. Rearing and feeding conditions and veterinary treatments were the same for all animals during the whole rearing period.

Samples collection and protein extraction

About 15 min post mortem, 5 grams samples of muscle (Pectoralis superficialis) were collected from the left breast and frozen in liquid Nitrogen for the analysis. The extraction of sarcoplasmic proteins was performed using a procedure modified from Rathgeber et al. (1999). One-gram samples of previously ground in liquid Nitrogen breast meat (Pectoralis superficialis) were homogenized in 20 mL of low ionic strength (LIS) buffer (0.05 M potassium phosphate, 1 mM NaN3, 2 mM EDTA, pH 7.3, 2°C) for 10 s, and placed on ice for 30 min. These samples were centrifuged at 17,500g for 15 min at 2°C. Ten ml of supernatant (the sarcoplasmic protein extract) were removed at a level 2 cm from the bottom of the tube. The remaining supernatant was discarded and the pellet was resuspended in an additional 20 mL of LIS buffer, homogenized and centrifuged as previously described. The protein content in the sarcoplasmic samples was determined using the Bradford reagent (Pierce).

Sarcoplasmic protein fraction represent about 30-35% of the muscular proteins. Despite the great diversity of the proteins contained in this fraction, they share common characteristics such as a relatively low molecular weight, a relatively high isoelectric point and globular structure.

Two-dimensional electrophoresis

Two-dimensional electrophoresis was made on a total of 58 samples (2 repetitions per animal). Proteases Inhibitor (80-6501-23, GE Healthcare, Uppsala, Sweden) were added to the LIS protein extraction in an Amicon Ultra 4 Millipore and centrifuged at 7,500g for 15 min at 3°C. Two ml of UHQ water containing protease inhibitors were added to the concentrate and the centrifugation step was repeated. Protein concentration in LIS fraction was quantified using the Bradford assay (Bio-Rad Laboratories Inc., Hercules, CA). The Isoelectric focusing (IEF) was carried out using a Protean IEF cell (Bio-Rad Laboratories Inc., Hercules, CA). 300 µg of protein were loaded onto immobilised pH gradient (IPG, Bio-Rad Laboratories Inc., Hercules, CA, 17 cm, pH 4–7 linear). Proteins were loaded by inclusion of an adequate volume of extract in a buffer consisting of 7 M Urea, 2 M Thiourea, 2% (w/v) CHAPS, 0.2% (w/v) DTT and 0.2% carrier ampholytes. Strips were rehydrated 12 hours applying a voltage of 50 V. For the subsequent IEF, voltage was increased gradually to 10,000 V until a total of 60,000 Vh was reached. Strips were immediately frozen and stored at -20°C until further use. Prior to SDS–PAGE, strips were equilibrated for 15 min in a reducing solution containing 2% DTT, 6 M Urea, 30% Glycerol, 2% SDS and 50 mM Tris-Cl, pH 8,8 followed by a 15 min step in an alkylation solution made of 5% (w/v) Iodoacetamide, 6 M Urea, 30% (v/v) Glycerol, 2% (w/v) SDS and 50 mM Tris-Cl pH 8.8 and bromophenol blue as a dye. SDS–PAGE was performed in a Protean XL cell (Bio-Rad Laboratories Inc., Hercules, CA) on 12% polyacrylamide gels (2.6% bisacrylamide) at 35 mA/gel at 8°C, until the dye track reached the end of the gels. Gels were silver stained following the protocol of Shevchenko *et al.* (1996).

Image analysis

Gels images were acquired through a GS-800 densitometer and analysed with a computerized image analysis: Image Master 2D Platinum (GE Healthcare, Uppsala, Sweden). On each gel (replicate), spot detection was first automatically performed by the software. Automatic spot detection was validated by manual spot editing. Then, one master gel per breed was finally obtained from the 2 replicates of the 9 or 10 animals. After a comparison of master gels, the program gave us the possibility to localize on the individual gels (replicates), the spots (or proteins) of interest that were differentially expressed between the 3 different breeds

Statistical analysis

Data on protein extracted in the LIS fraction, expressed as percentage of the total proteins, were subjected to ANOVA by the GLM procedure, considering breed as a fixed effect using SAS® software (1996, SAS Institute, Cary, NC). For breed effect a multiple comparison of means was performed using the Bonferroni's test ($P < 0.001$).

All spots detected were included for the statistical analysis. Comparisons among breeds were performed two by two. Cluster analysis was performed using the PROC CLUSTER of SAS (1997) and the Ward's minimum variance method. Dendrograms were plotted using PROC TREE procedure of SAS. Principal component analysis was performed using PROC PRINCOMP of SAS.

The statistical differences in protein expression among groups were tested using the Significance Analysis of Microarrays (SAM) method as described by Meunier et al, (2005). Spots with a Fold Change greater than 2 were retained and considered for the identification.

PROTEIN IDENTIFICATION

Prior to matrix-assisted laser desorption/ionization-time-of-flight (MALDI- TOF) mass spectrometry analysis, the spots where prepared like described by Laville *et al*, (2009). Peptide mass fingerprint (PMF) of trypsin-digested spots was determined in positive-ion reflector mode using a Voyager DE Pro MALDI-TOF-MS (PerSeptive Biosystems, Framingham, MA). PMFs were compared to *Aves* nrNCBI (12/2008, 102 448 seq) protein sequence databases (http://www.ncbi.nlm.nih.gov/Database/) using MASCOT 2.2 software [http://www.matrixscience.com]. The initial search parameters allowed a single trypsin missed cleavage, partial carbamidomethylation of cysteine, partial oxidation of methionine, and mass deviation under 30 ppm. We required at least five matched peptides per protein for identification and used MASCOT probabilistic scores, accuracy of the experimental-to-theoretical pI, and molecular weight (MW).

RESULTS

LIS fraction quantification evidenced a significant lower extractability in Padovana (33.7% of total protein) than in Ermellinata and Pépoi (37.44 and 37.22%, respectively) ($p<0.001$). Extractability of this fraction has been correlated, in Turkey, to an higher post-mortem glycolysis (Rathgeber et al., 1999). In the Padovana was probably due to an higher stress susceptibility to pre slaughter stress.

Image analysis detected 246, 275, and 226 different spots for the comparisons PP-PD, PP-ER, and PD-ER, respectively (Figure 3). For each spot, expression results were averaged to obtain a single value within individual. Figure 1 (a, b, and c) represent the cluster plot obtained using Ward's minimum distance option. For each comparison, individual results always well divided into two groups, corresponding to the breeds analysed. Within each sub-cluster, individuals are differently grouped based on similarity on protein expression. Principal component analysis was used as alternative statistical approach to study individual grouping. Figure 2 (d, f, and g) outline the bidimensional plot of the first and second principal component scores for PP-PD, PP-ER, and PD-ER comparisons, respectively. First principal component can fully distinguish the analysed animals into two groups, corresponding to the different breeds, even thought only a small amount of variability is explained by this component (13.1%, 16.0%, and 17.3% for PP-PD, PP-ER, and PD-ER, respectively).

The Significance Analysis of Microarrays (SAM) method was adopted to discriminate, among all "statistically" significant spots, those witch retain a "biological" significance. This was performed choosing only the spots presenting a volume ratio greater than a predefined Fold Change level. This method was studied to minimize false positive and to avoid loosing information with false negative, expecially when few replicates are available. SAM analysis detected 16 significant spots for the confrontation Pépoi vs Padovana, 18 for Pépoi vs Ermellinata, and 13 for Padovana vs Ermellinata (Figure 3). Of these, 10 were identified by mass spectrometry. The list of the identified proteins is reported in Table 1. Identified proteins can be divided in two categories: breed specific spots, i.e. spots that are expressed only in a particular breed, and spots that are declared up or down expressed respect to a predefined Fold Change level (fixed to a value of 2) (Table 2). Identified proteins appear heterogeneous in their function. Enzymes, transport, contractile and motile, regulatory and scaffold proteins have been identified and seem hence to play a function in breed differentiation. APOA1, a protein participating to the transport of cholesterol from the tissues to the liver, was up expressed in the Padovana compared to the other breeds. GLO1, a 184 aa long protein of the glyoxalase I family, resulted up expressed in Pépoi compared to Ermellinata. BRD4 and PGP, enzymes respectively involved in the process of cellular mitosis and carbohydrates metabolism, were expressed in the Ermellinata breed and could contribute to explain the differences in terms of growth rates shown by this breed respect to the other. HSPB1 instead, a protein involved in stress resistance and actin organization, is up-expressed in the Pépoi breed, and could help in explaining the marked aptitude to environmental adaptation and stress resistance. Anyway, since just a small part of the proteome has been analyzed and

identified, such differences in protein expression, successful in enabling breed differentiation, can not be used univocally to explain factors involved in this phenomenon.

CONCLUSIONS

The obtained results evidence a possible utilisation of proteomic approach in the field of breed characterization studies. This approach is alternative or complementary to genomic analyses using molecular markers, both for breed and product traceability purposes. Advantages of this technique include lesser instruments equipment necessity for the analysis, even if it is a more time consuming technique. Moreover, mass-spectrometry identification of all the most relevant spots could lead to understand and explain qualitative/quantitative differences existing among breeds and their products.

Figure 1. Ward minimum distance cluster plot for a) Pèpoi (PP) and Padovana (PD) individuals; b) Pèpoi (PP) and Ermellinata (ER); c) Padovana (PD) and Ermellinata (ER).

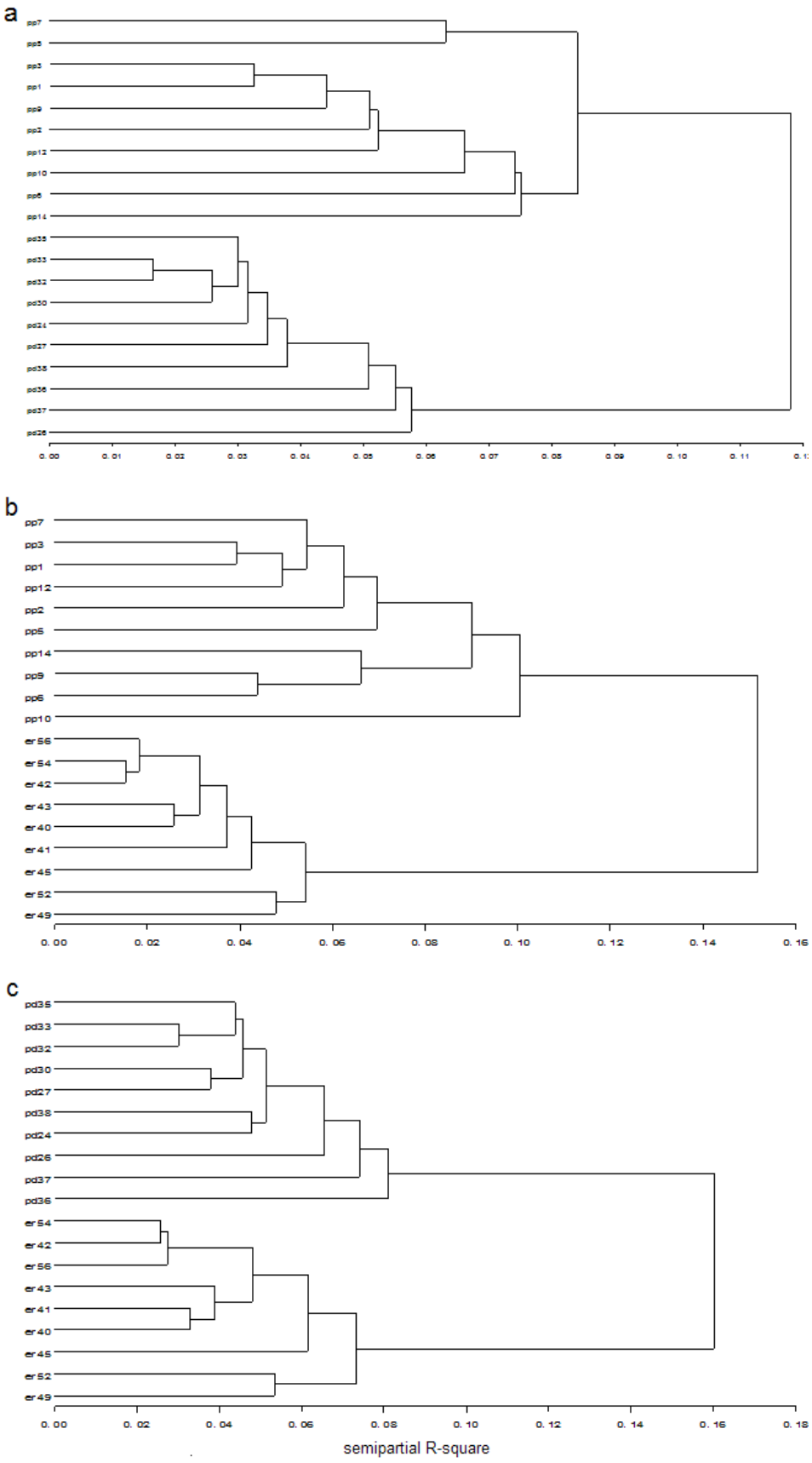


Figure 2. Principal component scores for d) Pépoi (P) and Padovana (P); f) Pépoi (P) and Ermellinata (E); g) Padovana (P) and Ermellinata (E).

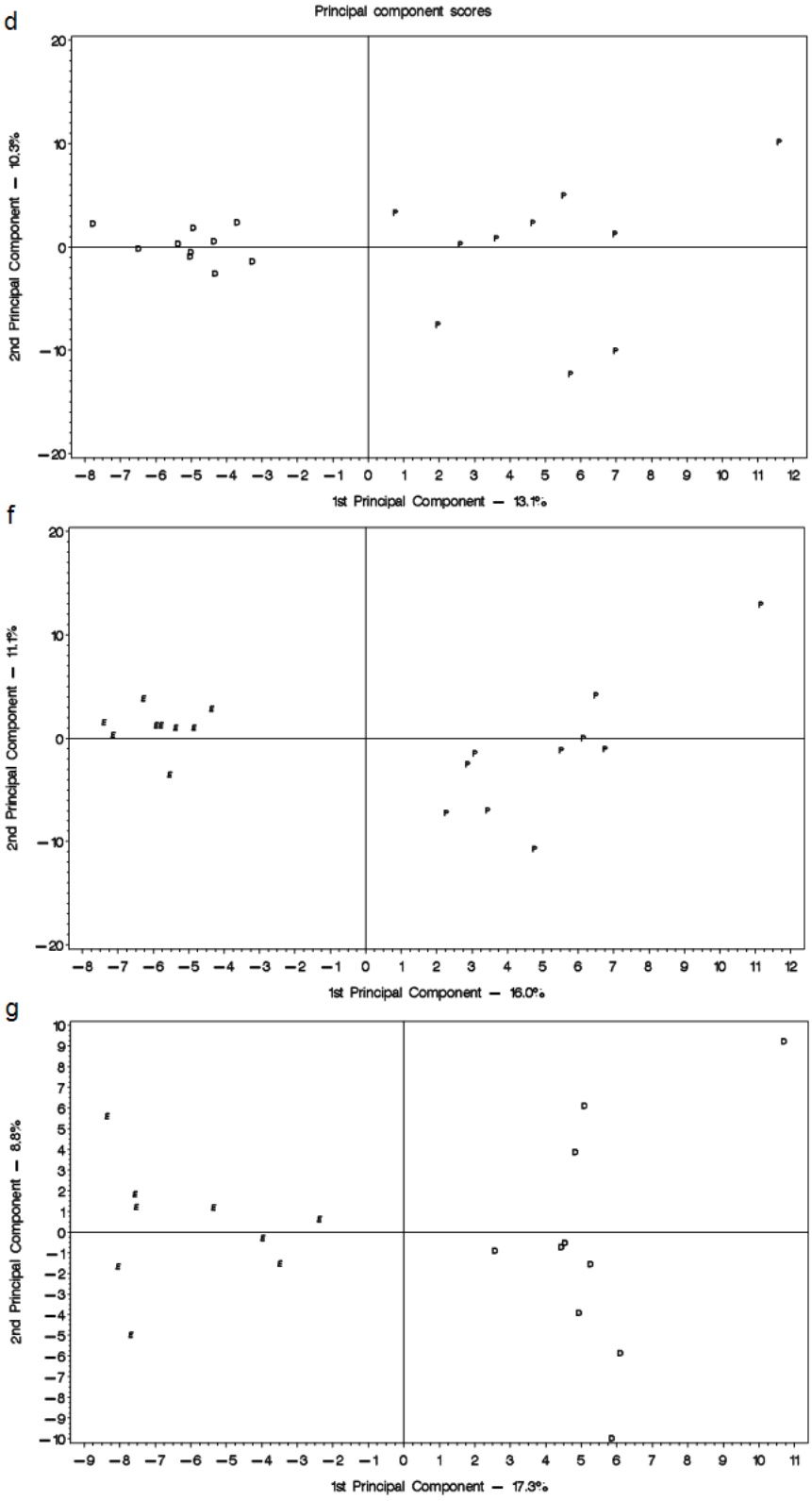


Figure 3. 2-D gels images for a) Pèpoi, b) Ermellinata di Rovigo and c)Padovana. Up and down expressed spots are evidenced.

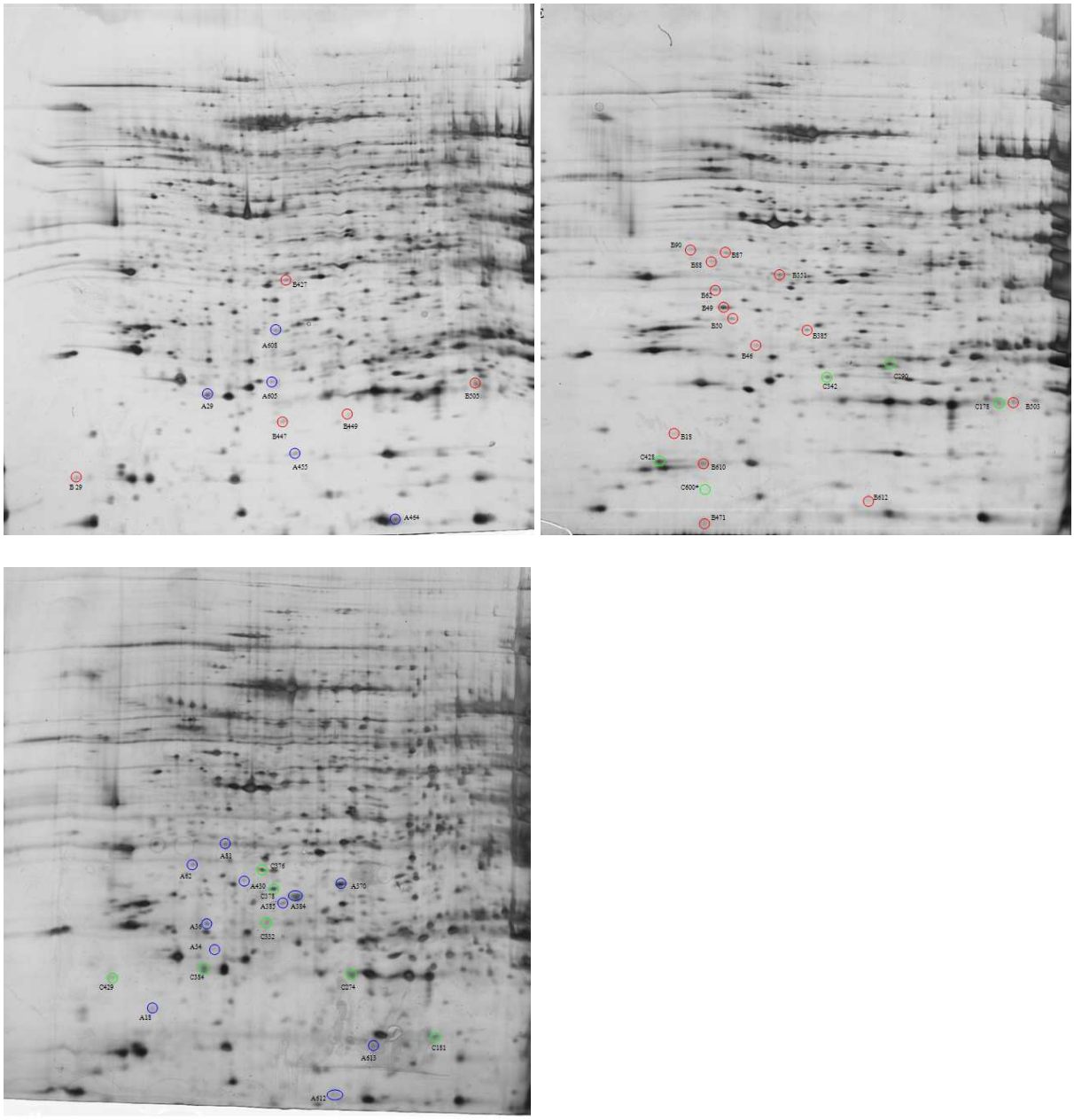


Table 1. List of identified proteins by mass spectrometry

Spot n°	Database	Taxonomy	Sequence ref.	Protein name	Mascot Score*	Sequence coverage	Number of aligned peptide	Theoritical MW (Da)	Theoritical pI
C181	Aves	Unknown species	gi|78100779	Chain A, Solution Structure Of Chick Cofilin	77	57%	7	18650	7.66
C274	Aves	Unknown species	gi|50740506	PREDICTED: similar to Glyoxylase 1	87	40%	8	20540	6.10
A370	Aves	Unknown species	gi|62738642	Chain A, Crystal Structures Of Chicken Annexin V In Complex With Zn2+	141	45%	14	36045	5.61
C290	Aves	Unknown species	gi|124110120	growth factor receptor-bound protein 2	74	34%	8	25161	5.78
C290	Aves	Unknown species	gi|71896147	bromodomain containing 4	67	43%	6	26197	5.56
A34	Aves	Unknown species	gi|227016	apolipoprotein AI	71	28%	7	28790	5.45
C384	Aves	Unknown species	gi|212347	myosin a1 light chain (partial)	112	59%	10	19468	4.72
B351	Aves	Unknown species	gi|71894743	phosphoglycolate phosphatase	90	40%	10	32975	5.53
C342	Aves	Gallus gallus	gi|227016	apolipoprotein AI	69	27%	7	28790	5.45
B18	Aves	Unknown species	gi|56605896	leucine zipper and CTNNBIP1 domain containing	70	34%	7	21457	4.80
B612	Aves	Unknown species	gi|118099124	PREDICTED: hypothetical protein	74	57%	6	13822	5.71
A29BIS	Aves	Unknown species	gi|55584149	Myosin light chain 1, skeletal muscle isoform	88	52%	8	20886	4.96
B449	Aves	Unknown species	gi|48374049	heat shock 27kDa protein 2	69	31%	4	19719	5.80

* Protein scores greater than 63 are significant ($p<0.05$).

Table 2. List of identified specifically expressed, up or down regulated genes in the analysed breeds and function of these proteins

Breed	Spot n°	Gene name	Function	AA
All		GLO1	Catalyzes the conversion of hemimercaptal, formed from methylglyoxal and glutathione, to S-lactoylglutathione. Belongs to the glyoxalase I family.	184
All		APOA1	Participates in the reverse transport of cholesterol from tissues to the liver for excretion by promoting cholesterol efflux from tissues and by acting as a cofactor for the lecithin cholesterol acyltransferase (LCAT). Belongs to the apolipoprotein A1/A4/E family.	264
All		HSPB1	Involved in stress resistance and actin organization. Detected in all tissues tested, is expressed in response to environmental stresses such as heat shock, or estrogen stimulation in MCF-7 cells. Belongs to the small heat shock protein (HSP20) family.	205
PD		CFL2	Controls reversibly actin polymerization and depolymerization in a pH-sensitive manner. Belongs to the actin-binding proteins ADF family.	166
PD		ANXA5	Collagen-binding protein Belongs to the annexin family.	321
ER		BRD4	Plays a role in a process governing chromosomal dynamics during mitosis.	1362
ER		PGP	Hydrolase playing function in the Carbohydrate metabolism. Catalytic activity: 2-phosphoglycolate + H2O = glycolate + phosphate. Belongs to the HAD-like hydrolase superfamily.	312
ER		B4E2N0	Beta-catenin binding	211
ER		GRB2	growth factor receptor-bound protein 2. Adapter protein that provides a critical link between cell surface growth factor receptors and the Ras signaling pathway. It also seems to interact with RAS in the signaling pathway leading to DNA synthesis.	217

REFERENCES

Bendixen, E. 2005. The use of proteomics in meat science. Meat Sci. 71:138-149.

Borderies, G., E. Jamet, C. Lafite, M. Rossignol, A. Jauneau, G. Boudart, B. Montsarrat, E. Esquerré-Tugayé, A. Boudet et R. Pont-Lezica. 2003. Proteomics of loosely bound cell wall proteins of Arabidopsis thaliana cell suspension cultures: a critical analysis. Electrophoresis 24: 3421-3432.

Burgess, S.C., 2004. Proteomics in the chicken: tools for understanding immune responses to avian diseases. Poult. Sci. 83:552-573.

DAD-IS. 2006. Domestic Animal Diversity Information System (DAD-IS). FAO (available at www.fao.org/dad-is/).

De Marchi, M., Cassandro, M., Targhetta, C., Baruchello, M., Notter, D.R., 2005a. Conservation of poultry genetic resource in the Veneto region of Italy. Anim. Genet. Res. Inf. 37:63-74.

De Marchi, M., Dalvit, C., Targhetta, C., Cassandro, M., 2005b. Assessing genetic diversity in indigenous Veneto chicken breeds using AFLP markers. Anim. Genet. 37:101-105.

Doherty, M.K., McLean, L., Beynon, R.J., 2007. Avian proteomics: advances, challenges and new technologies. Cytogenet. Genome Res. 117:358-369.

FAO (Food and Agriculture Organisation of the United Nations). 2007. The State of the World's Animal Genetic Resources for Food and Agriculture, edited by Barbara Rischkowsky & Dafydd Pilling. Rome.

Hollung, K., Grove, H., Mosleth Færgestad, E., Singh Sidhu, M., Berg, P. 2009. Comparison of muscle proteome profiles in pure breeds of Norvegian Landrace and Duroc at three different ages. Meat Sci. 81:487-492.

Meunier,B, Bouley, J., Piec, I., Bernard, C., Picard, B., Hocquette, J.F. 2005. Data analysis methods for detection of differential protein expression in two-dimensional gel electrophoresis Anal. Biochem. 340:226–230

Rathgeber, B.M, Boles, J.A., Shand, P.J., 1999. Rapid post mortem ph decline and delayed chilling reduce quality of turkey breast meat. Poultry Sci. 78:477-484.

SAS, 1997. SAS Institute: SAS/STAT® User's Guide: Statistics, Version 6.12, SAS Institute Inc., Cary, NC.

Shevchenko, A., Wilm, M., Vorm, O., Mann, M., 1996. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal. Chem. 68:850-858.

Ingenuity Systems. http//:www.ingenuity.com.

GENERAL DISCUSSION AND CONCLUSION

The contributes presented shared the objective to study and characterise the Italian local chicken breeds of the Veneto region. Different approaches have been developed and exploited to understand the different aspects that contribute to breed differentiation and to study the typical products that derive from them.

In Particular, the first contribute, dealing with the genetic molecular characterisation performed by means of microsatellites analysis, highlighted the high level of genetic diversity among the Italian local breeds. Whatever the method used to analyse genetic differentiation (i.e. genetic distances, structure clustering), breeds resulted well distinct while the populations belonging to the same breed, reared in distinct conservation nuclei, appeared homogeneous, with the single exception of the Polverara breed which presents complicate population substructures. Sampling for molecular analysis may be combined with surveying and/or monitoring of productive and phenotypic traits, as molecular information on its own cannot be used for utilization and conservation decisions. Once decision about conservation have already been undertaken, molecular markers remain an useful tool to perform chicken characterisation, to monitor the conservation scheme and to arrange matings.

Future perspectives this conservation scheme and other implemented in the territory include the identification of gaps and assessment of factors limiting the optimum utilization, development and conservation of these AnGR; and the need for follow-up action, including financial and technical assistance, policy development and awareness raising and education. Improving the understanding of the status and characteristics of AnGR could enable and stimutate their sustainable use, development and conservation. Conserving AnGR will therefore ensure their availability for future use and development in all production systems and to achieve the successful implementation of national programmes for AnGR there is need to enhance institutional development. The access to a wide range of AnGR is necessary for producing under diverse environments and under changing environmental conditions. Genetic resources could be used for cross breeding and development of new genotypes.

The second contribute focused on a comparative study about qualitative parameters; evidencing the differences in meat quality traits among the studied local chicken breeds. Meat quality characteristics are very important for the consumer point of view. Usually meat eating experience is one that associates meat with being tender, juicy and flavorsome. A wide range of other attributes, however, determinate the acceptability of meat. Color and visual appearance is very important in determining the likelihood of purchase, but also perceived

nutritional value, the amount of fat, freshness and microbiological safety are extremely important. Extrinsic quality attributes also influence acceptability of meat. These include elements such as animal welfare and the impact of production on the environment.
The breeds clearly differed each other for some aspects such as carcass yield, colour, tenderness, and fatty acid composition. Each breed presented unique features. Beyond a more incisive traditional interest and an high historical and cultural value, Padovana has the highest tenderness, a particular color, and, with Pépoi, the lower content in saturated fatty acids. However Ermellinata di Rovigo, with a good carcass weight seems to better meet the preference of the modern consumer, even those looking for chicken meat in a niche market.

The third contribute, focusing on a comparative expression proteomic study among three chicken breeds, evidenced a possible utilisation of proteomic approach in the field of breed characterization studies and confirmed the genetic variation, also at protein level, among the local chicken breeds analysed. This approach provides an alternative to genomic analyses using molecular markers, both for breed and product traceability purposes. Mass-spectrometry identification of the most relevant spots has finally set the basis to understand and explain the qualitative/quantitative differences existing among breeds and their products. Anyway the most remarkable result remains the demonstration that, even if just a small part of the whole proteome is analysed (proteome that for its own nature remains not analyzable with the present techniques), in a given controlled environment, the genetic differences among the three local chicken breeds are sufficient to distinguish the animals belonging to the different breeds. Despite the complexity of the biological system represented by a muscle coming from a growing animal, proteomics could be successfully used to distinguish the three breeds. This technique, in addition, could enable the identification of breed specific protein markers leading to an easy and cheap method for breed and product traceability, although many breeds, animals and populations should be analysed and compared to determine breed specific expressed proteins.

On the whole the contributes evidenced, in different ways, the great diversity existing among the studied breeds and their products, and different approaches have been developed to study the different aspects involved in the study and characterisation of these breeds, drawing the basis for their utilization and valorisation as local animal genetic resources.

LIST OF PUBLICATIONS

1. **Zanetti E.**, De Marchi M., Dalvit C., Cassandro M.. Genetic Characterization and Population Structure of Italian Local Chicken Breeds Undergoing In-Situ Conservation. Submitted to POULTRY SCIENCE (June 2009)

2. **Zanetti E.,** De Marchi M., Dalvit C., Molette C., Remignon H., Cassandro M.. Carcass characteristics and qualitative meat traits of three Italian local chicken breeds. Submitted to BRITISH POULTRY SCIENCE (July 2009)

3. **Zanetti E**., Dalvit C., Molette C., Remignon H., Cassandro M. (2009). A proteomic approach to study local chicken breeds characterisation. ITALIAN JOURNAL OF ANIMAL SCIENCE vol. 8 – Supplement 2, pp 174-176.

4. Dalvit C., **Zanetti E**., Cassandro M. (2009). Estimation of genetic diversity over time in an in-situ marker assisted conservation scheme of local chicken breeds. ITALIAN JOURNAL OF ANIMAL SCIENCE vol. 8 – Supplement 2, pp 63-65.

5. Dalvit C., M. De Marchi, R. Dal Zotto, **E. Zanetti**, T. Meuwissen, M. Cassandro. (2008). Genetic characterization of the Burlina cattle breed using microsatellites markers. JOURNAL OF ANIMAL BREEDING AND GENETICS 125:137–144.

6. **Zanetti E.**, Gervaso M., Dalvit C., Cassandro M. (2007). Genetic diversity in some local chicken breeds using microsatellite markers. ITALIAN JOURNAL OF ANIMAL SCIENCE. vol. 6 - Supplement 1, pp. 225-227 ISSN: 1594-4077.

7. Cecchinato A., Maretto F., **Zanetti E**., Carnier, P. (2007). Survival analysis of piglet pre-weaning mortality. ITALIAN JOURNAL OF ANIMAL SCIENCE. vol. 6 - Supplement 1, pp. 67-69 ISSN: 1594-4077.

8. Cassandro M., Dalvit C., **Zanetti E.**, De Marchi M., Dal Zotto R. (2007). Genetic aspects of milk coagulation properties in dairy cattle. AGRICULTURE. Vol. 13 – number 1, pp. 30-34 ISSN: 1330-7142.

9. **Zanetti E.**, Dalvit C., De Marchi M., Dal Zotto R., Cassandro M. (2007). Genetic characterisation of Italian chicken breeds using a panel of twenty microsatellite markers. AGRICULTURE. Vol. 13 – number 1, pp. 197-200 ISSN: 1330-7142.

10. **Zanetti E**., Dalvit C., De Marchi M., Cassandro M. (2007). Genetic diversity of six Italian local chicken breeds. In: Book of Abstract of the 58th Annual Meeting of the European Association for Animal Production. Annual Meeting of the European Association for Animal Production. 26-29 August 2007. (vol. 13, pp. 76). ISBN/ISSN: 978-90-8686-045-6/1382-6077. WAGENINGEN: Academic Publishers (NETHERLANDS).

11. **Zanetti E.**, Dalvit C., De Marchi M., Baruchello M., Cassandro M. (2007). Monitoring genetic diversity in Italian local chicken populations involved in a marker assisted conservation plan. 5th European Poultry Genetic Symposium. 26-28 September 2007, Braedstrup, Denmark**.**

12. **Zanetti E.**, De Marchi M., Dal Zotto R., Dalvit C., Cassandro M. (2008). An application of an *in situ* marker assisted conservation scheme in poultry. In proceedings of the 23th World Poultry Congress. 30 June – 4 July, Brisbane, Australia

13. **Zanetti E.**, De Marchi M., Molette C., Remignon H., Cassandro M. (2008). Meat quality traits and proteomic characterisation of three Italian local breeds of chicken. In proceedings of the 23th World Poultry Congress. 30 June – 4 July, Brisbane, Australia

14. **Zanetti E.**, De Marchi M, Dalvit C., Dal Zotto R., Cassandro M. (2006). Productive and reproductive traits of three different Italian poultry species involved in an in-situ conservation program. ACTA AGRARIA KAPOSVÁRIENSIS. vol. 10 No 2, pp. 215-222 ISSN: 1418-1789.

15. De Marchi M., Dalvit C., **Zanetti E.**, Cassandro M. (2006). Analysis of performance and reproductive traits of the Padovana chicken breed involved in a conservation programme. WORLD'S POULTRY SCIENCE JOURNAL. Vol. 62 Supplement, pp. 205 ISSN: 0043-9339.