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# A proteomic approach to study local chicken breeds characterization

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**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 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. 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** - 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.

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

**Material and methods** - In the Veneto region of Italy, Padovana (PD), Ermellinata di Rovigo (ER), and Pépoi (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) hatched at May 17, 2007 and 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. About 15 min *post mortem*, 5 grams samples of muscle (*Pectoralis major*) 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 major*) were homogenized in 20 mL of low ionic strength (LIS) buffer (0.05 M potassium phosphate, 1 mM NaN<sub>3</sub>, 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

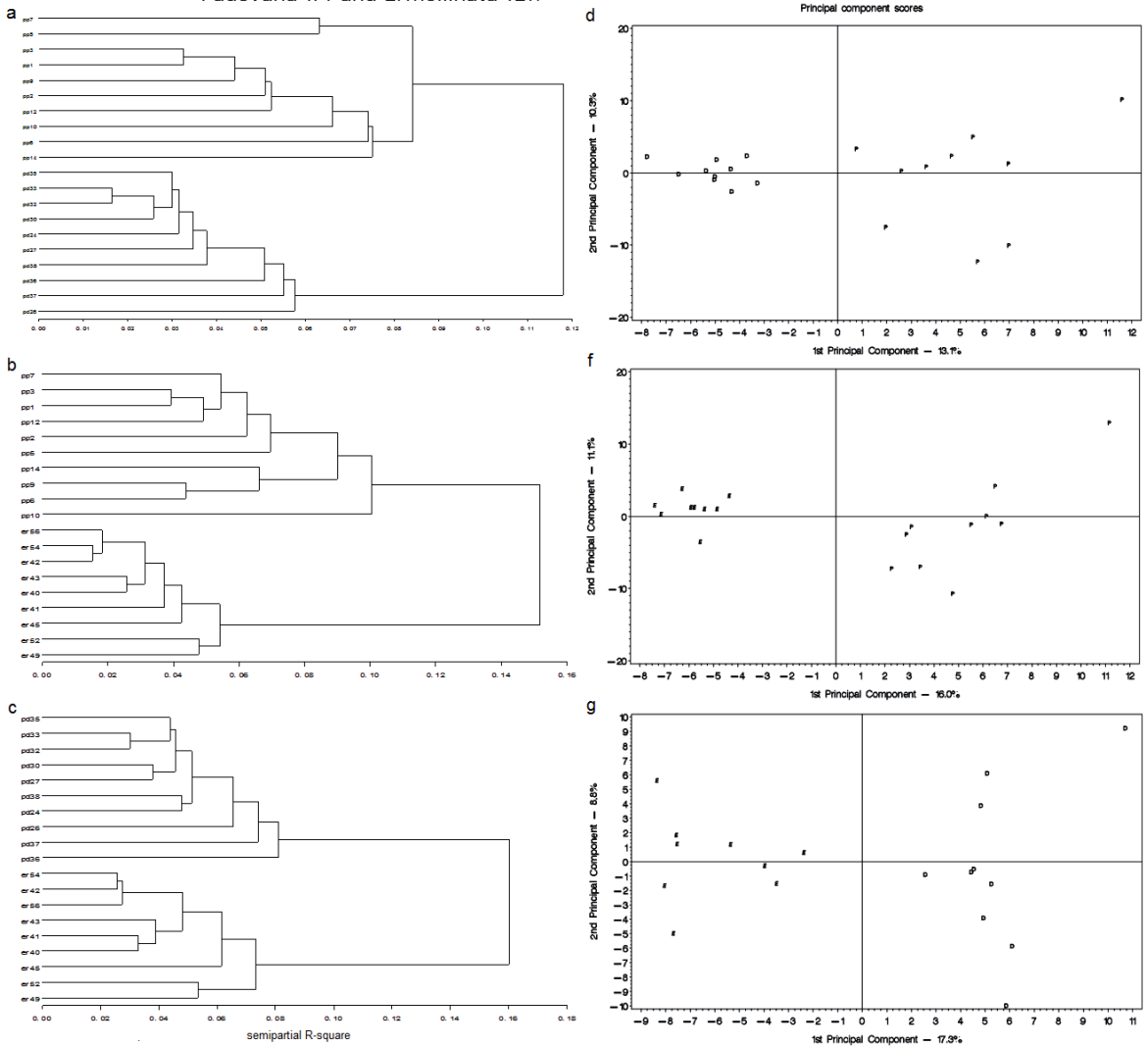
mL of LIS buffer, homogenized and centrifuged as previously described. The protein content in the sarcoplasmic samples was determined using the Bradford reagent (Pierce). Bidimensional electrophoresis was made on a total of 58 samples (2 repetitions each animal). Twenty  $\mu$ l Proteases Inhibitor were added to 2 ml whole 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. LIS fraction was quantified using the Bradford assay (Bio-Rad). Immobilised pH gradient (IPG) isoelectric focusing (IEF) was carried out in a Protean IEF cell (Bio-Rad), using Bio-Rad ReadyStrip, 17 cm, pH 4–7 linear, 300  $\mu$ g of protein were loaded onto the strips. 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. 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) 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). Gels were produced in multiple copies, and the two gels of best quality were retained for further analysis. Gels images were acquired through a GS-800 densitometer and analysed using the Image Master 2D Platinum (GE Healthcare). Relative integrated spot intensities in the individual gels were estimated. Then, the 2DE images were matched by comparing the relative positions and integrated intensities of the individual spots on each gel. For comparative image analysis, the images were grouped, after which the relative levels of expression of individual spots were analysed and compared within and between the image groups. The matches suggested by automated image analysis were finally individually inspected and corrected when necessary.

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.

**Results and conclusions** - Image analysis detected 246, 275, and 226 different spots for the comparisons PP-PD, PP-ER, and PD-ER, respectively. 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 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 1 (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 though 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 obtained results evidence a possible utilisation of proteomic approach in the field of breed characterization studies. This approach provides an alternative 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 the most relevant spots (i.e., spots that statistically and biologically differ among groups) 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). Principal component scores for d) Pèpoi (P) and Padovana (P); f) Pèpoi (P) and Ermellinata (E); g) Padovana (P) and Ermellinata (E).



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