



LIVESTOCK SYSTEMS – NEW EMERGING TECHNOLOGIES IN ANIMAL SCIENCE

breeds and populations (including 24 Italian breeds and populations, wild dogs and wolves).

By comparing the percentages of genomic background associated with one or more breeds, we obtained 3 types of results when assigning membership breeds to each of our 20 dogs:

- i. 'real mongrels': the first breed accounts for less 10% of their genomic background with no phenotype consistent for any breed of reference, with the exception of the size;
- ii. 'mixed breeds': the first breed accounts for 11 to 74% of their genomic variance with phenotype resemblance ranging from mild to high;
- iii. 'purebred dogs' without official pedigree: the first breed accounts for 75% or more of their genomic background.

We propose coining the word METIGREE, deriving from the Italian 'METIccio' (mongrel) and 'pediGREE', to reconstruct ancestral line for mongrels by using genomic analysis.

This scientific identification of mongrels would increase their chances of adoption and further our knowledge of their temperament and health issues, thus reducing medical costs while promoting the dogs' and their owners' happiness.

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Conventional culture, MALDI-TOF and 16S rRNA compared for test agreement in diagnosis of bacteria in bovine milk samples

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Comparison of culture, matrix-assisted laser desorption ionisation – time of flight (MALDI-TOF), and 16S rRNA genomic sequencing to identify mastitis pathogens was the objective. Milk (10 µl) from quarter samples submitted to The Dairy Authority (TDA) lab was streaked onto Columbia blood agar (CBA), MacConkey agar and

Modified Hayflick medium. Colonies isolated within 48 hr were subcultured onto CBA, plates were paraffin sealed and shipped overnight to the University of Missouri (MU). Culture at TDA identified bacteria to the genus level except for *Staphylococcus aureus* and *Escherichia coli* speciation, and grouping of streptococcal-like organisms, per standard mastitis diagnostics. Mycoplasma speciation PCR was available if mycoplasma were isolated. At MU colonies were tested in duplicate using a MALDI-TOF mass spectrometer (Bruker Daltonics). Comparison with the Biotyper known bacteria database produced identification scores from 1.7 to 1.99 for genus-level identification and ≥ 2.0 for species level. 16S rRNA colony lysate PCR products were Sanger sequenced and compared to GenBank data using nucleotide-BLAST at MU. Microbiologists were blind to other test results. Positive test agreement (same microbe identified by different tests) analysis used McNemar's test; overall test agreement (whether negative for same organism by different tests also included) used Kappa test. Culture and MALDI-TOF tested 181 isolates; 16S rRNA tested 179 (2 were lost in storage). No *Streptococcus agalactiae* or *Mycoplasma* spp. were detected. Overall agreement between all 3 diagnostic methods was 94% (169/179); agreement between 16S rRNA and MALDI-TOF was 98% (176/179), both good by McNemar's test. Culture agreement with each of the other 2 methods was 95% (170/179 with 16S rRNA, 171/181 with MALDI-TOF). For individual pathogens, positive agreement ranged from 90% to 100%, nearly all 'good' by McNemar's. Overall agreement (negative agreement included) was 97% to 100% among all 3 methods, all 'very good' by Kappa. For 22 isolates defined by culture as *S. aureus*, *Enterobacter* spp., *Klebsiella* spp., *Pasteurella* spp., or *T. pyogenes*, agreement among all methods was 100%. These results suggest that for usage in milk quality and udder health monitoring, any of the 3 methods are valuable tools for the dairy industry.

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Angiotensin Converting Enzyme-1 inhibitory activity of milk proteins evaluated after *in vitro* digestion and peptidomic analysis

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Milk proteins are relevant sources of bioactive peptides. Many hurdles still exist regarding the widespread utilisation of milk protein-derived bioactive peptides as they may be degraded during gastrointestinal digestion. A crucial issue in this field is the demonstration of a cause-effect relationship, from the ingested intact form to the bioactive form.

The aim of this study was to evaluate *in vitro* digestion, digestibility (IVD, using two different hydrolysis methods) and Angiotensin Converting Enzyme-1 inhibitory activity (ACE-Ii) of milk and plant proteins (used as control). Based on ACE-Ii activity, a peptidomic and proteomic profile analysis was performed on permeate and retentate samples.

In particular, milk and plant protein samples were *in vitro* digested, and the total digest was filtered using a 3 KDa membrane. A permeate fraction (<3 KDa) and retentate fraction (>3 KDa) were obtained. ACE-Ii activity was measured as the ability of protein fractions (pre-digested, permeate and retentate) to decrease the hydrolysis of furanacrololyl-Phe-Glu-Glu (FAPGG) synthetic substrate for ACE enzyme. Furthermore, permeate was characterised by LC-nano ESI MS/MS using a shotgun-peptidomic approach, whereas retentate was further trypsin-digested prior the analysis with mass spectrometry using a shotgun-proteomic approach.

We found a positive correlation among the IVD methods tested ($p < .05$; $r = 0.85$). Milk proteins exhibited higher values of IVD (>82.5%) with both methods used, compared with plant proteins. Milk proteins after *in vitro* digestion exhibited a significant increase in ACE-Ii ($p < .05$) ($> 23.91 \pm 0.64\%$) compared with plant protein tested ($10.40 \pm 1.07\%$). Based on proteomic and peptidomic analysis performed, specific peptides associated with anti-hypertensive and ACE-Ii effect have been identified in permeate and retentate fractions of milk proteins. Our results demonstrated that milk and plant proteins are highly digestible and, in particular, milk proteins may represent valuable sources of ACE-Ii and anti-hypertensive peptides which may confer the ability to decrease blood pressure *in vivo*.

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The ability of the partial least square regression to predict the number of bites in Sarda dairy sheep by using acceleration data

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The number of bites taken by a grazing sheep is an important component of the equation that estimates the herbage intake. Recent development in technologies has promoted the use of tri-axial accelerometer devices to study the behaviour of grazing animals. Such instruments can produce a set of variables in the three dimensions that, combined each other, can accurately discriminate between grazing, ruminating and other activities. Number of bites can be also estimated with a moderate accuracy from the sum of accelerations recorded in X-axis with linear regression models. The aim of this study was to predict number of bites using all accelerometer variables with a multivariate approach, the partial least square regression (PLSR), that has become an established tool for modelling linear relations when a set of dependent variables has to be predicted from a set of independent variables highly correlated. For the scope, an experiment has been conducted under controlled conditions to test if the number of bites can be predicted from accelerometer data. Ten dairy Sarda sheep were fitted with a halter equipped with an accelerometer (BEHARUM device) and subjected to short term tests (6 min) using micro-swards of Italian ryegrass (*Lolium multiflorum* L.), alfalfa (*Medicago sativa* L.), oat (*Avena sativa* L.), chicory (*Cichorium intibus* L.) and a mixture (Italian ryegrass and alfalfa). Each animal was video recorded during the test to detect the number of bites. Accelerometer data sum, mean, variance and inverse of coefficient of variation calculated for the X-, Y- and Z-axes and the resultant were summarised with an epoch setting of one minute. A database inclusive of the acceleration variables and the number of bites detected was created. To verify if the acceleration variables could be used as predictors of the number of bites, the partial PLSR model was used. The precision and accuracy of PLSR predictions were evaluated implementing the Model Evaluation System, in which the predicted values were regressed against the observed ones, based on r^2 , root-mean-square error of prediction (RMSEP) and Dent & Blackie test. The PLSR showed an overall good accuracy (Dent & Blackie test $P = 1$) and was proven precise for the estimation of number of bites ($r^2 = 0.77$, RMSEP = 0.21).

To conclude, PLSR procedure can accurately estimate with high precision the number of bites of sheep equipped with the BEHARUM device and short term grazing Mediterranean forages.

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