Brief technical notes relating to statistics

The Laboratory has developed the NIR calibration curves through its own traditional chemistry laboratory using the most suitable methods (official, more precise, robust); so the choice of the method influences the result of the NIR prediction. Collaborating with Dairyland - Arcadia, WI-USA (through the standardization procedure for instrumental alignment) it was sometimes highlighted an average discrepancy in the results due precisely to the methods used.

The NIR readings performed over the years by the Zootechnical Analysis Laboratory have been subject to periodic recalibrations, method changes and possible equipment drifts. To overcome this, the results used to perform the statistics come from the reprocessing of the old NIR spectra with our most recent calibration curves, thus obtaining more accurate but above all homogeneous data.

In the statistical processing of the Dairyland results, corrections were applied to harmonize the two systems where the calibration parameters envisage the same method and which refer to the same reference framework, i.e. the RING TEST of the National Forage Testing Association (NFTA) USA. This also mitigated any LAZ-Dairyland standardization errors.

Clarifications on some methods used in the calibration of the NIR curves:

PROTEINS	Laboratorio Analisi Zootecniche -IT	Dairyland Laboratories Inc. -USA
Method:	Kjeldahl (Official Method UE)	Dumas (Official Method USA)
Assayed analytes:	- protein nitrogen - urea nitrogen - ammonia nitrogen	- protein nitrogen - urea nitrogen - ammonia nitrogen -nitric nitrogen (nitrate)
Theoretical/practical implications:	Nitric nitrogen is not a nutrient and it should be considered toxic-undesirable	
Analytical implications:	Regardless of the presence of N-nitric acid, the Dumas method however presents a better recovery of Nitrogen than the Kjeldahl, which remains the official method in Europe (Reg. CE 152/2009). The Protein values entered in our calibrations come mainly from Kjeldahl but they have been updated with validation sets obtained from the Dumas method.	

VOLATILE FATTY ACIDS	Laboratorio Analisi Zootecniche -IT	Dairyland Laboratories Inc. -USA
Method:	Wiegner for fractional distillation	HPLC chromatography
Assayed analytes:	-Lactic acid -Acetic acid -Butyric acid	-Lactic acid -Acetic acid -Butyric acid -Propionic acid -Valeric acid and others
Theoretical/practical implications:	The fractional distillation method is more approximate but it has performed the important function of allowing in past years an evaluation of the correctness of the fermentations very close to reality. The chromatographic determination method is more accurate and punctual but requires a specific reference framework, different from the traditional one. However, Dairyland does not report all volatile fatty acids.	
Analytical implications:	The chromatographic method accurately determines all the individual organic acids originating from the fermentation process, while fractional distillation calculates only the three organic acids by combining the total acidity and the distilled volatile acids as a function of the length of the molecular chain in a calculation. With this method, the propionic acid partially flows into the lactic acid and certainly the values obtained are not made up only of the volatile fatty acids (Lactic, Acetic and Butyric).	

SOLUBLE CARBOHYDRATES - SUGAR	Laboratorio Analisi Zootecniche -IT ZUCCHERI	Dairyland Laboratories Inc. -USA CARBOIDRATI SOLUBILI
Method:	IPRA method with Dinitrosalicylic Acid in aqueous extract: -direct (Reducing Sugars) -after inversion (Total sugars - only for Unifeeds and sorghum silages). The results for both procedures are comparable to those obtained with the official method EEC 152/2009 (Luff Shoorl).	Dairyland distinguishes between Water Soluble Carbohydrates (WSC) and Ethanol Soluble Carbohydrates (ESC). The determination takes place with the Phenol-Sulfuric Acid Method.
Assayed analytes:	The method determines the reducing sugars (glucose, fructose, maltose, etc.). The inversion process also frees the reducers from sucrose and presumably also some oligosaccharides.	The method detects virtually all soluble carbohydrates, including mono-, di-, oligo- and polysaccharides. The Ethanol extract is more selective and contains only mono-di-oligosaccharides and a portion of fructosans.
Theoretical implications:	The theoretical difference between WSC and ESC is that ESCs under-detect compared to WSCs which detect even the healthy fruits and part and all of the oligosaccharides. Empirically, by comparing the statistics of our and Dairyland curves, we have found that the results obtained using the Dairyland curves are on average: -WSC higher than 3 points of ours for grasses; -ESC plus 2 points of ours in medical. -WSC of medics and ESC of grasses can be superimposed on ours.	
Analytical implications:	The sugars (present in the hay profiles of NIR L.A.Z.) have been calibrated with methods that only detect simple sugars and also do not provide for the WSC and ESC distinction, typical of the CNCPS system. In conclusion, Zuccheri and WSC-ESC are hardly comparable.	

Statistical division into subgroups of the individual forage classes: choices and reasons

In the diagram below we have made a further division into subgroups which was necessary due to the excessive lack of homogeneity within the individual forage classes which would have made the averages insignificant and in fact useless. On the contrary, the identification of homogeneous subclasses for an "index" parameter makes it possible to compare the profile of a given forage with the statistics presented here with reasonable precision.

Classes of forage subject to division into subclasses

1) **Graminaceous hays** divided by protein content. The protein content is a function of the vegetative stage of the plant at the time of cutting, which determines the coherent evolution of the other composition parameters in cascade.

2) **Alfalfa hays** according to the cut (1st, 2nd, 3rd, etc.) Sufficient homogeneity was found within the individual cuts, making a further subdivision by protein content unnecessary.

3) **Corn silages** as a function of Dry Matter. The dry substance of corn silage is directly proportional to the cutting time, i.e. the vegetative stage, and it affects its composition and fermentation profile.

4) **Silages** of other fodder for Dry Matter. In this case, the DM is an indicator of the ensiling procedure, i.e. with pre-wilting in the field or with direct ensiling. The differences between the 2 subclasses essentially concern the fermentation parameters, those of soluble protein and sugars, parameters influenced by the fermentation intensity.

5) **Sorghum silages** distinguished by Dry Matter and Starch. The DM follows the dynamics of the ensiling mode and the Starch that of the vegetative stage.

6) In the **XRF statistics** of grass hays we have distinguished two subgroups based on the Anion/Cation balance as a function of the inclusion of the hays in the dry rations. Dry Unifeeds, on the other hand, were grouped according to the chlorine content reasonably present as a physiological endowment of the forage. In fact, the widespread and necessary practice of adding Anionic supplements would make the statistics of the mineral composition insignificant. The class of greatest interest could be that with a low chlorine content, i.e. that of unifeeds without specific integration.

7) For the contaminants (**NO_3 and Mycotoxins**) we have opted for the number of concentration ranges. Also in this case an average concentration would have no meaning.

Reference Ring Test

Our analytical "System" has its centrality in the RING Tests to which we are anchored and whose samples represent the internal standards of the main analyses, constantly monitored by control charts.

The circuits we join are:

- American **NFTA** but with global significance, for which we have had certification for 10 years and which covers the key parameters of the CNCPS system.

- French **BIPEA (INRA)** is the reference tool for the official European methods of food chemistry.

- **University of Piacenza** is the only Italian circuit. It allows us to relate to the results of the Italian laboratories with which we collaborate and compare ourselves.

- Austrian **ROMER** with which we maintain the quality of Mycotoxin results.

- Dutch **WEPAL** is the circuit for the quality of XRF analysis results for minerals.