

Clarifications on some methods used in the Mancinelli and Dairyland NIR calibrations

Below, we explore the observable causes of the (unavoidable) differences in analytical results from the **NIR spectroscopy** used by our laboratory compared to that of the Dairyland Laboratory (Arcadia, WI, USA).

The Mancinelli Laboratory and the Dairyland Laboratory developed the NIR calibration curves through their traditional chemistry lab using the most suitable methods (official or more precise, robust); in this sense, the choice of method influences the NIR calibration. **Collaborating with Dairyland has occasionally highlighted a medium discrepancy in results due to the methods used** (but also to the instrument alignment procedure: in fact, a small source of differences is inherent in the Shenk & Westerhaus procedure used to standardize our instruments with those of Dairyland).

The comparison of average data highlights greater critical issues for haylages with significant differences in **protein** (with variations of up to 1.2 points), **protein solubility** (5-9 points), and **ammonia** (from 1000 to 2000 ppm, but not for Alfalfa haylages, which are approximately aligned). Various parameters exhibit significant deviations across all feeds in the statistics: **fats** (with differences of approximately 0.9 points), **NDFom** (with 2.5 points), **ADF** (with 2 points) and **Volatile Fatty Acids** (with notable variations). The **pH of the only Alfalfa haylages** is anomalous, showing an average difference of one point.

In the tables below we indicate the analytical differences underlying the NIR curves of the two laboratories. (Dairyland method information was taken from <https://dairylandlabs.com/>)

PROTEIN	NIR Laboratorio Analisi Zootecniche -IT	NIR Dairyland Laboratories Inc. –USA
Calibration method:	Kjeldahl (Met Uff. UE)	Dumas (Met Uff. USA)
Analytes measured:	-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 should be considered toxic and undesirable	
Analytical implications	Regardless of the presence of nitric N, the Dumas method offers better nitrogen recovery than the Kjeldahl method, which remains the official method in Europe (EC Regulation 152/2009). The protein values included in our calibrations are primarily from the Kjeldahl method but have been updated with validation sets obtained from the Dumas method.	
VOLATILE FATTY ACIDS	NIR Laboratorio Analisi Zootecniche -IT	NIR Dairyland Laboratories Inc. –USA
Calibration method:	Wiegner for fractional distillation	HPLC chromatography (mainly)
Analytes measured:	-Lactic acid -Acetic acid -Butyric acid	-Lactic acid -Acetic acid -Butyric acid -Propionic acid
Theoretical/practical implications	The fractional distillation method is more approximate but it has played an important role in past years of allowing a very realistic assessment of the accuracy of fermentations. The chromatographic determination method is more accurate and precise but requires a specific reference frame, different from the traditional one. However, Dairyland does not report all volatile fatty acids.	

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Analytical implications	The chromatographic method precisely determines all the individual organic acids resulting from the fermentation process. Fractional distillation, on the other hand, calculates only the three organic acids by combining the total acidity and the distilled volatile acids depending on the length of the molecular chain. With this last method, the propionic acid partially flows into the lactic acid and therefore the values obtained didn't consist only of the lactic, acetic and butyric VFAs.	
SOLUBLE CARBOHYDRATES - SUGARS	NIR Laboratorio Analisi Zootecniche – IT: SUGARS	NIR Dairyland Laboratories Inc. – USA: SOLUBLE CARBOHYDRATES
Calibration method:	IPRA method with dinitrosalicylic acid in aqueous extract: -direct (reducing sugars) -after inversion (total sugars - for Unifeed and Silosorgo only). The results for both procedures are comparable to those obtained with the official EEC method 152/2009 (Luff Shoorl).	Dairyland distinguishes between water-soluble carbohydrates (WSC) and ethanol-soluble carbohydrates (ESC) . Determination is performed using the Phenol-Sulfuric Acid Method.
Analytes measured:	The method determines reducing sugars (glucose, fructose, maltose, etc.). The inversion process also releases reducing sugars from sucrose and presumably also some oligosaccharides.	The method detects virtually all soluble carbohydrates, including mono-, di-, oligo-, and polysaccharides. The ethanolic extract is more selective and contains only mono-, di-oligosaccharides and a portion of fructosans.
Theoretical/practical implications	The theoretical difference between the WSC and ESC methods is that the ESC underestimates sugars compared to the WSC, which also detects fructosans and some or all of the oligosaccharides. Empirically, comparing the statistics of our curves and those of Dairyland, we found that the average results obtained using the Dairyland curves are: -WSC 3 points higher than ours for grasses; -ESC 2 points higher than ours for alfalfa. -WSC for alfalfa and ESC for grasses are comparable to ours.	
Analytical implications	The sugars present in the NIR L.A.Z. hay profiles were calibrated with methods that detect only simple sugars and they don't include the distinction between WSC and ESC, typical of the CNCPS system. In conclusion, Sugars and WSC-ESC are difficult to compare.	
AMMONIA	Laboratorio Analisi Zootecniche – IT:	Dairyland Laboratories Inc. –USA:
Ammonia is a volatile base that presents the greatest challenges during NIR calibration. Various tests between different laboratories can reveal these NIR-on-NIR differences (the calibration chemistry is performed on the as-is product while the subsequent NIR analysis is performed on the dry sample).		