The Book of Analysis

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Introduction:

The Book of Analysis has been enriched in its third edition with a significant increase in sample sizes and a new layout for more complete and rapid consultation.
The animal feed statistics we publish are intended to provide a framework for the composition of forage: they include the food rationing for dairy cow, the agricultural and livestock sector in general.
Each feed is defined in terms of its nutritional composition (using Near-Infrared Spectroscopy (NIR)), indicating the mean value and standard deviation for each parameter: the "NIR Profile" contains the results obtained with our calibrations, while the "NIR CNCPS Profile" contains the data from the Dairyland calibrations, which adopt the CNCPS (Cornell Net Carbohydrate and Protein System) parameters. Along with our NIR results, we also include the Mineral Profile (X-ray Fluorescence (XRF)), also analyzed by us. We have also included the nitrate contamination found in the last 7 years as an indication of the average nitrate nitrogen load.
In the following scheme, we further divided the data into subgroups: was necessary due to the excessive non-uniformity within the individual forage classes, which would have rendered the averages meaningless and, in effect, useless. Conversely, identifying homogeneous subclasses for an "index" parameter allows us to compare the profile of a specific forage analyzed with the statistics presented here with reasonable accuracy.

Classes of forage subject to subdivision into subclasses

1. **Gramineae hays** fractionated by protein content. Protein content depends on the plant's vegetative stage at the time of cutting, which determines the evolution of the other compositional parameters.
2. **Alfalfa hays** (1st, 2nd, 3rd cut etc.). Sufficient homogeneity was found within the individual cuts, making further subdivision by protein content unnecessary.
3. **Corn silages** divided in function of dry matter content. The dry matter content of corn silages is directly proportional to the cutting time, or vegetative stage and it affects the composition and fermentation profile.
4. **Haylages** of other forage crops in function of Dry Matter. In this case, the DM indicates the ensiling process, the pre-wilting in the field or the direct ensiling. The difference between the two subclasses essentially concern the fermentation parameters, those of soluble protein and sugars, which are influenced by the intensity of fermentation.
5. **Sorghum haylages** are classified by Dry Matter and Starch. Dry Matter follows the dynamics of the ensiling method, while Starch follows the dynamics of the vegetative stage and the cultivated variety (grain/sugar).

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6. **In the XRF statistics** of grass hays, we distinguished two subgroups based on the anion/cation balance depending on the hay inclusion in the dry rations. Dry Unifeeds, on the other hand, were grouped based on the chloride content present as a physiological endowment of the forage. In fact, the widespread and necessary practice of adding anion supplements would make the mineral composition statistics non-significant. The class of greatest interest may be the low-chlorine content, for example, the Unifeeds without specific supplementation. Dry Unifeeds were also divided into four other statistical groups based on the average DCAD values to highlight their impact on the remaining nutritional and mineral components.
7. **Lactation Unifeeds** have been divided into two types of rationing: Parmigiano-Reggiano and silage-based unifeeds (e.g., Grana Padano, which is the most common type). Indeed, due to the strict specifications for Parmigiano-Reggiano, its nutritional composition differs from other unifeeds.

In some cases, the sample number was not statistically representative; in these cases, two warnings have been included to encourage critical use of these data: **"ns" = not significant** when the population size is less than 20 samples and **"ls" = less significant** when the population size is between 20 and 50.

1. Standard Profile Feeds: NIR-XRF-NO3

Grass hay 1st cut	Oat Silage	Corn silage x 4
Oat Hay	Grass Silage x 2	Earlage
Wheat Hay	Wheat Silage x 2	Snaplage
Ryegrass Hay	Sunflower Silage	
Alfalfa Hay x4	Ryegrass Silage x 2	
Panic Hay	Alfalfa Silage x 4	Dry TMR x 7
Mixed Gramineae Hay x4	Millet Silage	Beef Cattle TMR
Sorghum Hay	Barley Silage x 2	Lactation TMR x 2
Sulla Hay	Panic Silage	Heifer TMR
Clover Hay	Pea Silage	Pre-Calving TMR
Triticale Hay	Polyphyte Grass Silage x 2	
Straw	Rye Silage x 2	
	Soybean Silage x 2	
	Triticale Silage x 2	Beef Cattle Feces
	Mixed Legume and Grass Silage x 2	Lactation Feces

2. CNCPS Profile Feeds: NIR Dairyland

Dehydrated Alfalfa x 5	Grass Silage x 2	Dry TMR
Grass hay 1st cut	Wheat Silage x 2	Beef Cattle TMR
Oat Hay	Ryegrass Silage x 2	Lactation TMR x 2
Wheat Hay	Alfalfa Silage x 3	Heifer TMR
Ryegrass Hay	Mixed Legume and Grass Silage	Pre-Calving TMR
Alfalfa Hay x 4	Barley Silage x 2	
Polyphyte Grass Hay x 4	Polyphyte Silage x 2	
Clover Hay	Rye Silage x 2	Corn grain
Straw	Sorghum Silage x 3	
	Clover Silage	
	Triticale Silage x 2	
	BMR Corn Silage	
	Corn silage x 4	
	Earlage	
	Snaplage	
	Fresh Corn silage	

3. Clarifications on some methods used in the different NIR calibrations

The Laboratory developed the NIR calibration curves through its traditional chemistry laboratory using the most suitable methods (official or more precise, robust); in this sense, the choice of method influences the NIR prediction results. Collaboration with Dairyland - Arcadia, WI-USA (through an appropriate instrument alignment procedure) has occasionally highlighted average discrepancies in results due to the methods used (but also to the aforementioned 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**	**Dairyland Laboratories Inc. - USA**
Calibration method	Wiegner per distillazione frazionata	Cromatografia in HPLC
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.	
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).		

4. Ring Reference Test

Our analytical "System" is centered on the Interlaboratory Circuits (RING Tests), whose samples serve as internal standards for the main analyses, constantly monitored by control charts. We participate in the following circuits:

- **NFTA**, an American circuit with global relevance, which we have been certified for 14 years and which covers the key parameters of the CNCPS system.
- **BIPEA** (French: INRA), the reference tool for official European food chemistry methods, is also the circuit for the quality of mycotoxin results.
- **WEPAL** (Dutch: WEPAL), the circuit for the quality of XRF analysis results for minerals.
- **ROMER** (Austria: ROMER), the Ring Test we use for the quality of mycotoxin results.

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