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Predicted standard deviation of proficiency testing performance in animal nutrition methods

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Abstract. The empirical correlations of the results of over ten years of a proficiency testing of animal nutritional analysis conducted by Embrapa were evaluated. The results demonstrate the similarity of the Horwitz equation proposal, but it is desirable to use individual equations related to the different analyses performed by the laboratories. Using these equations enables a more realistic assessment of possible systematic errors and analytical processes, improving laboratory performance and providing the most reliable results.

Keywords. Horwitz equation, animal nutrition, proficiency testing, empirical analytes, reproducibility

1. Introduction

The Horwitz equation [1] is a practical and suitable exponential relationship of the variability of chemical measurements in the interlaboratory trials. It is one of the first empirical parameters applied in the evaluation of the quality of the results provided by a newly developed method, by laboratory control systems and by proficiency testing programs [2].

The Horwitz equation is based on more than 1000 interlaboratory comparisons. It is defined by a fixed relationship between analyte level and reproducibility standard deviation (RSD_R), more or less independent of the analyte, matrix, method, and time of published [3,4]. The curve was characterized by Hall and Selinger [5] as "one of the most intriguing relationships in modern analytical chemistry", and it is similar to other equations based on mathematical behavior found in different areas of nature, as described by mathematician I. Stewart [6].

The Horwitz curve has been used as the initial estimate of expected among-laboratory variability before the performance of an interlaboratory study [3]. The curve is also helpful in interpreting the results of the method- and laboratory-performance studies, and in setting initial limits for quality control purposes, defined by the "HorRat" ratio, as "acceptable" parameter, indicating the acceptability of methods of analysis concerning among-laboratory precision (reproducibility) [2,3]. On the other hand, as Horwitz and other authors emphasize [2,7,8,9], significant deviations from the values predicted by the original Horwitz relation are expected when analytical methods that express measurands as a mass concentration are used. It occurs mainly because of not all variations in the reproducibility in empirical analytes (such as moisture, ash, and fiber), indefinite analytes (such as enzymes, polymers and biomolecules), or physical properties (such as color, density, and viscosity)



can be explained only by the concentration levels, a fundamental prerequisite for using the HorRat as a performance criterion [2,9].

The assessment of the results of an interlaboratory study employing less subjective criteria is required. We adopted the statistical results generated by ten years of a proficiency testing of animal nutrition laboratories [10]. As initially proposed by Horwitz, based on the relative standard deviation of reproducibility (RSD_R) of our obtained results of fiber, crude protein, calcium, phosphorus, zinc, and iron mass fraction in samples of tropical forages and concentrates, we propose a set of new equations as acceptance criteria

2. Experimental

The ISO/IEC 17025 requires laboratory participation in a proficiency testing (PT) program for accreditation or qualification of a particular test with national and international regulatory bodies in the metrology field [11]. These programs are intended to demonstrate the laboratory's performance and competence in performing the tests for which it is planned to be accredited. Participation in PT is an essential quality assurance activity in routine testing laboratories, allowing the laboratory to detect poorly performing results and take corrective or preventive actions, achieving a minimum percentage of hits to achieve satisfactory performance levels.

The experimental procedure was based on the results provided by the Proficiency Testing on Animal Nutrition Laboratories [10]. The Analytical Laboratory Proficiency Testing organizational structure employs operational procedures based on the ABNT ISO/IEC GUIDE 43 [12] standards and the Harmonized International Protocol for Analytical (Chemical) Laboratory Proficiency Testing [13].

2.1 Samples

The evaluation was based on results provided by 37 samples of different forages (such as Panicum, Brachiaria, sugar cane, Cajanus Cajan) and 37 samples of feed and feed ingredients (such as fish meal, soybean meal, bean, poultry feed, sorghum, corn grains, cotton bran, wheat bran, citrus pulp). The forage samples were harvested from the Embrapa Pecuária Sudeste research unit's field experiments and furnished by other PT participants. The feeds and feed ingredients were commercial products purchased from the local markets in São Carlos, São Paulo State, Brazil and provided by other PT participants.

2.2 Analytical determinations and Statistical analysis

The 130 laboratories participants of the EPLNA provide the results. The operational scheme has been previously described elsewhere [10]. As an example of the proposed procedure, we will discuss the results obtained for the mass fraction of the following analytes: crude protein (CP), acid detergent fiber (ADF) [14], the macronutrients calcium and phosphorus, and the micronutrients copper and iron. Crude protein was based on the Kjeldahl method's nitrogen determination; phosphorus was determined by colorimetry or inductively coupled optical emission spectrometry (ICP OES), and the others were determined either by ICP OES or by flame atomic absorption after dry or wet samples digestion.

The participants are free to use independent methods of analysis following the applicable protocols and available equipment. Therefore, the analytical methods and the procedures can differ among the participants.

The statistical model was the one recommended by the ABNT ISO/IEC GUIA 43 [11] and ISO 13528:2015 [15]. The laboratory performance was obtained by using the z score (Eq. 1).

$$z = \frac{(x_i - \bar{X})}{\sigma_p} \quad (1)$$

In this equation, x_i the analyte's concentration is obtained by the laboratory, \bar{X} the assigned value, and σ_p the robust standard deviation of analyte concentration.

3. Results

Similar to others based on mathematical behavior found in different areas of nature, as described by mathematician I. Stewart [15], the Horwitz equation is an empirically based relationship and can be applied to the relative standard deviation (RSD) of reproducibility (RSD_R) of the method and the concentration of the analyte.

The Horwitz relationship can be expressed as Eq. 2 [3,8]:

$$RSD_R = 2C^{-0.1505} \quad (2)$$

where the RSD_R is the SD of reproducibility of interlaboratory data and C is the concentration of the analyte.

Table 1 gives the Horwitz equation's values at different mass fractions, and the standard deviation of reproducibility of PT obtained amounts of crude protein (CP), acid detergent acid (ADF), Ca, P, Zn, and Fe. The data were calculated, given the range of obtained values.

Table 1. Values of Horwitz equation at different mass fractions and the standard deviation of reproducibility of PT of animal nutrition laboratories (EPLNA) of crude protein (CP), acid detergent acid (ADF), Ca, P, Zn, and Fe.

			CP	ADF	Ca	P	Zn	Fe
		Horwitz	EPLNA					
Analyte, %	Mass fraction C	RSD_R %	RSD_R %	RSD_R %	RSD_R %	RSD_R %	RSD_R %	RSD_R %
100	1	2.0	2.0	3.5	0.7	0.5	0.3	0.7
50	0.5	2.2	2.7	5.3	1.0	0.7	0.3	0.9
10	0.1	2.8	6.0	14.1	2.4	1.7	0.7	1.6
5	0.05	3.1	8.4	21.6	3.6	2.4	0.9	2.1
1	0.01	4.0	18.5	57.6	8.8	5.3	1.7	3.9
0.1	0.001	5.6	56.8		32.3	17.2	4.3	9.5
0.01	0.0001	8.0			118.5	55.3	11.1	23.1
0.001	0.00001	11.2					28.5	55.8
0.0001	0.000001	15.9					73.3	134.9
0.00001	0.0000001	22.4						
0.000001	0.00000001	31.7						
0.0000001	0.000000001	44.8						

In Figure 1, the RSD_R models represent by CP (a), ADF (b), Ca(c), P (d), Fe (e), and Zn (f) plotted by a normalized mass fraction with the results presented in Table 1. Figure 2 presents the predicted RSD_R of the new models, compared with the Horwitz model, and Figure 3 presents the similarity trend among CP and ADF (a), Ca and P (b), and Zn and Fe (c). As can be seen in Figure 2, independent of the analyte the mathematical function proposed in this work presents behavior similar to the Horwitz model. However, the coefficients are significantly higher, as presented in Table 2.

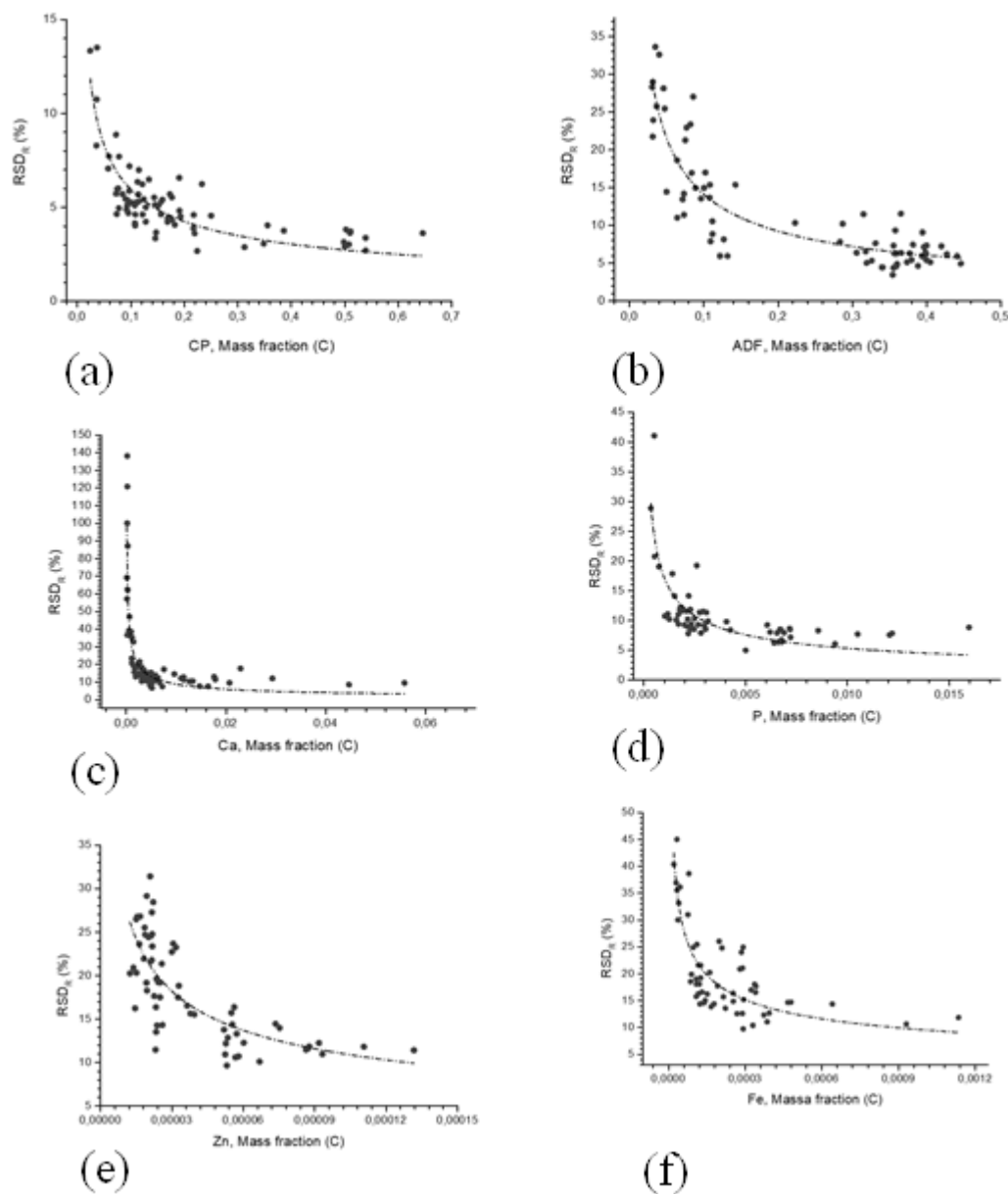


Figure 1. Plots of RSD_R (%) by normalized mass fraction for new models of crude protein (CP) (a), acid detergent fiber (ADF) (b), Ca (c), P (d), Zn (e), and Fe (f).

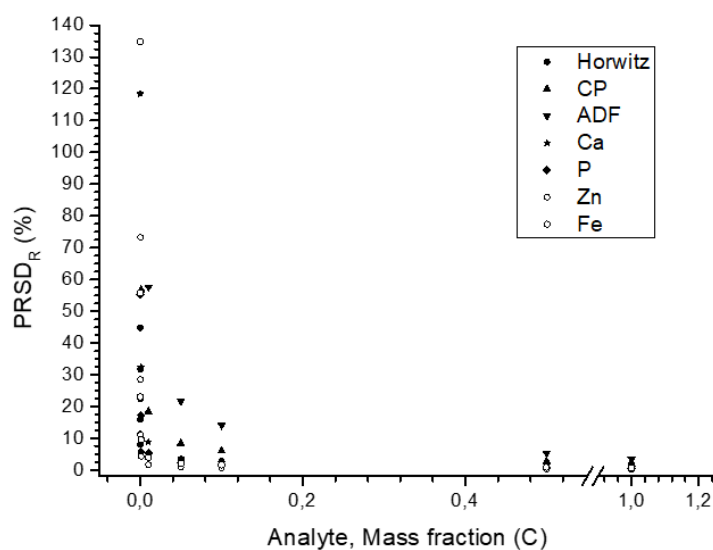
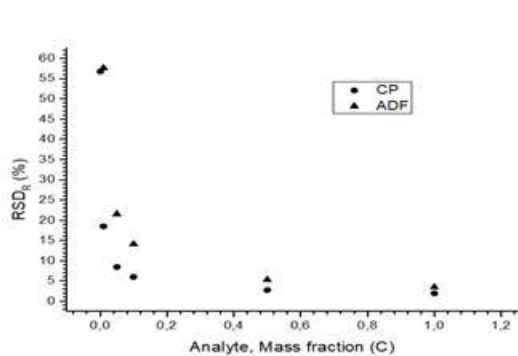
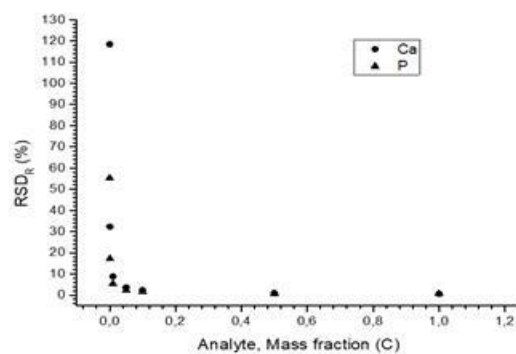


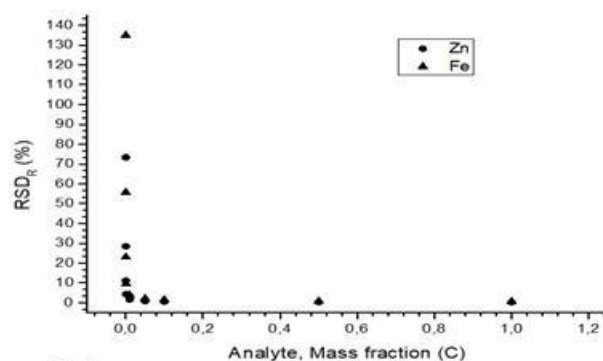
Figure 2. Predicted RSD_R (%) by normalized mass fraction trend for the crude protein (CP), acid detergent fiber (ADF), Ca, P, Zn, and Fe with Horwitz added for comparison.



(a)



(b)



(c)

Figure 3. Plots of RSD_R (%) by normalized mass fraction of CP and ADF (a), Ca and P (b), and Zn and Fe (c).

In Table 2 is presenting a summary of equations regressions statistics, including the number of points, degrees of freedom, and R-square adjust.

Table 2. Equation regression statistics for the results from PT animal nutrition laboratories.

$y = ax^b$	CP	ADF	Ca	P	Zn	Fe
A	1.955	3.457	0.657	0.516	0.255	0.675
B	-0.483	-0.611	-0.564	-0.507	-0.410	-0.383
Number of Points	73	71	73	56	64	57
Degrees of Freedom	71	69	71	54	62	55
R-Square adjust	0.70	0.78	0.73	0.64	0.55	0.68

Wehling and DeVries [8] discussed statistical anomalies for dietary fiber methods and the original developers' acknowledgment regarding the limitation of the Horwitz curve in this kind of analytes. Our results confirm the need for specific equations, more adequate to model variations. Table 2 can be used to model reproducibility SD as a function of the studied analytes' concentration.

4. Conclusions

The results of the PT of animal nutrition laboratories (EPLNA) allow us to draw up a profile of participants and learn about the main analytical problems related to the different employed methods. These methods involve since gravimetric procedures, like ADF, as spectrometric procedures as minerals. Besides, the differences between the sample matrices, which have different concentration ranges in the different analytes (e.g., fiber, minerals, protein) should be emphasized. The characteristic equations for the different analytes obtained from the RSD_R results achieved by the PT are present in Table 1. These equations allow us to include the interlaboratory analytical deviations characteristic of the different analytical processes. The equations were obtained with the results of crude protein, acid detergent fiber, calcium, phosphorus, zinc, and iron. They can represent the trend of SD with statistical rigor more realistically because it was obtained from an average number of 65 samples and data collection concerning 10 years of the EPLNA PT program. These results provide robustness to the proposed equations and suggest that the nonlinear behavior of the correlation curves between the RSD_R and the analyte concentration follows the same mathematical equation regardless of the nature of the analytes, the matrix and the principle of the measurement method. The proposed procedure can also be applied to the other parameters assessed in the PT, that, as observed by Horwitz and Albert [6], the Horwitz curve is not adequate.

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References

- [1] Horwitz W, Kamps LR, Boyer KW 1980 Quality Assurance in the Analysis of Foods for Trace Constituents, *J Assoc Off Anal Chem* **63** 6 1344-1354.

- [2] Rivera C, Rodríguez R, Horwitz Equation as Quality Benchmark in ISO/IEC 17025 Testing Laboratory. https://pdfs.semanticscholar.org/d6d6/a38d1a9e01e526ca4e2b5b8d804670e5414f.pdf?_ga=2.149818485.2108515611.1564572314-1074601812.1564572314. Accessed July 30, 2019.
- [3] Albert R, Horwitz W, A Heuristic Derivation of the Horwitz Curve 1997 *Anal Chem* **69** 789-790.
- [4] Horwitz W 1982 Evaluation of Analytical Methods Used for Regulation of Foods and Drugs *Anal Chem* **54** 67-76.
- [5] Hall P, Selinger B, 1989 A Statistical Justification to Relating Interlaboratory Coefficients of Variation with Concentration Levels, *Anal Chem* **61**, 13, 1465-1466.
- [6] Stewart, I. From Here to Infinity. Oxford University Press: New York, 1996. P. 242.
- [7] Horwitz W, Albert RJ 2006 The Horwitz Ratio (HorRat): A Useful Index of Method Performance with Respect to Precision. *Assoc Off Anal Chem* **89**, 4, 1095-1109.
- [8] Wehling P, DeVries W 2012 On the Use of the Horwitz Ratio (HorRat) as an Acceptance Criterion for Dietary Fiber Collaborative Studies *JAOAC Int* **95** 5 1541-1546.
- [9] Linsinger TPJ Josephs RD 2006 Limitations of the Application of the Horwitz Equation *TRAC* **25** 11, 1125-1130.
- [10] Souza GB, Nogueira ARA, Del-Santo V Picchi MCC, Gimaraes ES, Barione Jr W 2009 Proficiency Testing of Animal Nutrition Laboratories *Accred Qual Assur* **14** 455-460.
- [11] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
- [12] Associação Brasileira de Normas Técnicas ABNT ISO/IEC GUIA 43 (2017) Ensaios de Proficiência por Comparações Interlaboratoriais-Parte 1: Desenvolvimento e Operação de Programas de Ensaios de Proficiência. Rio de Janeiro, Brazil.
- [13] Thompson M, Ellison SLR, Wood R 2006 International harmonized protocol for the proficiency testing of analytical chemistry laboratories. *Pure Appl Chem* **78** 145-196.
- [14] Van Soest PJ 1963 Use of detergents in the analysis of fibrous feeds. A rapid method for the determination of fiber and lignin. *J Assoc Official Agr Chem* **46** 5, 829-835.
- [15] ISO 13528:2015 Statistical methods for use in proficiency testing by interlaboratory comparison