Determination of contaminants in samples of organic and inorganic raw materials and mineral mixture: collaborative study

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Abstract. An inter-laboratory comparison was running focused on determining As, Cd, Cr, Hg, and Se in raw materials and mineral mixture. The test materials were commercially available calcitic limestone, copper sulfate, premix mineral supplement, and sodium selenite, which were processed, bottled, labeled, and dispatched to participants, together with the details of the analytical procedure. The exercise enabled the establishment of an adequate analytical protocol for measuring undesirable trace elements in the evaluated matrices, defining the analytical precision parameters about repeatability (r) and reproducibility (R) in the laboratory results. Regarding the Hg content, it was not possible to establish the precision parameters since the mass fraction for that measurand was below the limit of detection of the method used.

1. Introduction

The Analytical Methods Commission (CMA) of the National Union of the Animal Feed Industry (Sindirações) has the participation of animal feed industries, commercial laboratories, universities, and research centers that develop and validate methods of analysis for evaluating the quality of raw materials and finished products. This committee is responsible for preparing and updating the analytical methods of the Brazilian Animal Feed Compendium (CBAA).

The selection, development, and validation of analysis methods are crucial for physicochemical testing laboratories. In this context, interlaboratory comparisons have been used as a tool to evaluate the performance of assay results [1]. In addition to this role, interlaboratory programs are also used as an alternative to assess quality requirements, such as analytical precision, mainly regarding repeatability and reproducibility [2,3]. During the annual workshops organized by the CMA for animal nutrition methods, the participants' laboratories have recurrently asked the CMA to schedule a collaborative test to determine inorganic contaminants in feed mineral mixtures (premixes) and other raw materials. These matrices are highly challenging from an analytical point of view.

Directive 2002/32/EC describes 'premixtures' as the 'mixtures of additives or mixtures of one or more additives with substances used as carriers, intended to manufacture feedingstuffs. The several starting materials as well as the diversity of production, may lead to contaminated and/or dangerous end products, introducing undesirable substances in the food chain [4]. The directive and its

amendments set maximum levels for undesirable substances (organic and inorganic) in animal feed. For inorganic contaminants in feed premixes, the maximum limits set by UE are 200 mg kg^{-1} for lead and 15 mg kg^{-1} for cadmium.

The Brazilian animal feed legislation does not explicitly deal with the maximum levels of inorganic contaminants in mineral mixtures. Still, administrative reference limits are adopted for contaminants in products intended for animal feeding in the raw material. For example, for sodium selenite, the maximum levels allowed for Pb, Cd, As, and Hg are 100, 50, 100, and 1.5 mg kg^{-1}, respectively [5]. Likewise, there are no official methods for analyzing inorganic contaminants in animal products or animal feed. Selected methods must be validated/verified according to the Analytical Quality Assurance Manual and must meet the intended use [6,7].

Based on the interlaboratory comparison model and the guidelines for collaborative interlaboratory study procedures recommended in Appendix D of the AOAC – Official Methods of Analysis [8], a collaborative interlaboratory test was carried out to evaluate the repeatability (r) and reproducibility (R) indices for the determination of inorganic constituents and contaminants in mineral ingredient matrices used in animal feed formulation.

2. Procedure

Calcitic limestone, copper sulfate, premix mineral supplement, and sodium selenite were the selected matrices for the interlaboratory collaborative study. The samples were prepared in line with the sample preparation criteria defined and specified in the Brazilian Compendium of Animal Feed, 2023 edition [5].

Participation was voluntary, and a total of 10 laboratories agreed to participate. They received 4 vials containing 100 g of each sample in a single lot by mail.

Among the norms of the collaborative essay for the determination of the values of the r and R indices, the following items were defined: code for each laboratory; schedule for sending the results; requirement of six replicates for sample; description of matrices and methods of preparation and determination, including analytical blanks, addition and recovery experiments to trueness evaluation, and inductively coupled plasma mass spectrometry (ICP-MS) performance requirements. A standard spreadsheet in Excel® for sending the results to the coordination was also sent.

2.1 Sample Preparation

Two alternatives were presented for sample preparation, one in a closed system using microwave radiation and the other in an open system using a heating plate.

Briefly, the following procedure was suggested: 0.5000 ± 0.0050 g of sample (6 replicates) were weighed directly into the digestion flask or into the beaker in the open system. Two blanks were also prepared.

Trueness were accessed by addition and recovery test. Another 3 replicates of the samples were weighed, and 150 µL of a multielement standard solution containing 10 mg L^{-1} of As, Cd, Cr, Pb, and Se and 1.0 mg L^{-1} of Hg were added directly to the digestion flasks.

Digestions were carried out in 3:1 HNO$_3$:HCl medium (4.5:1.5 mL). The mixture was microwave-assisted according to the following heating program: (1) 25 min ramp to 200 °C, and (2) 20 min hold at 200 ºC. Digestions carried out on the heating plate were made at the temperature of 210 °C for at least 10 min, avoiding drying the samples inside the beakers. After cooling down to room temperature, the digested samples were transferred to volumetric flasks and made up to 50 mL with previously purified water.

2.2 Determination

The digested solutions were 10 times diluted before determination by ICP-MS. For the ICP-MS determinations, the Kinetic Energy Discrimination (KED) analysis mode was used to minimize analytical interferences. The final matrix had about 0.9% HNO_3 + 0.3% HCl, and the analytical standards were prepared in this medium. Calibration curves ranged from 0.5 to 10 µg L^{-1} for As, Cd, Pb, and Se, and 0.05 to 1.0 µg L^{-1} for Hg.

2.3. Statistical Analysis

For the statistical evaluation, the Grubbs test was initially applied to the raw data to verify the presence of discrepant values, and the Cochran test to assess the homogeneity of variances. Then, a single-factor analysis of variance was performed to obtain repeatability and reproducibility indices.

The r values were obtained from the variance value calculated for each laboratory under repeatability conditions. For the calculation of R, a single-factor analysis of variance (ANOVA) was applied to estimate the between-laboratory and within-laboratory variances, which are used to calculate the reproducibility variance [3,9].

3. Results

The results are presented by matrix, considering the particularities and intrinsic difficulties concerning the sample preparation and determination. Thus, table 1 presents the results of calcitic limestone, table 2, the copper sulfate matrix, table 3, the mineral mix (mineral salt for swine), and table 4, the sodium selenite matrix. In the tables, N° refers to the number of laboratories participating in the collaborative interlaboratory trial; sr: repeatability standard deviation of repeatability; r: repeatability limit (sr x 2.8); RSDr: relative repeatability standard deviation; s_R: reproducibility standard deviation; R: reproducibility limit (sr x 2.8); RSDR: relative reproducibility standard deviation; HorRat: value obtained from the RSDR ratio and the relative standard deviation obtained from the Horwitz equation (RSDH = $2^{(1-0.5logC)}$); Rec: the analytical recovery.

Table 1. Collaborative results obtained with the calcitic limestone matrix

Calcitic Limestone

Analyte	N°	Mean	s_r	r	RSD$_r$ (%)	s_R	R	RSD$_R$ (%)	RSD$_H$	HorRat	Rec (%)
Ca[a]	10	36.9	0.45	1.25	1.2	1.54	4.31	4.2	2.3	1.8	*
Mg[a]	10	1.5	0.04	0.10	2.4	0.15	0.43	10.0	3.8	2.7	*
As[b]	7	643.2	28.19	78.94	4.4	419.55	1174.73	65.2	24.2	2.7	97
Cd[b]	6	98.4	7.49	20.98	7.6	23.78	66.58	24.2	32.1	0.8	92
Pb[b]	7	4117.7	102.45	286.85	2.5	555.58	1555.62	13.5	18.3	0.7	92
Cr[b]	5	1705.6	76.83	215.13	4.5	429.57	1202.80	25.2	20.9	1.2	93
Se[b]	1	3.1	0.29	0.80	9.2	*	*	*	53.9	*	95

[a] %
[b] µg kg^{-1}

Table 2. Collaborative results obtained with copper sulfate matrix

Copper Sulfate

Analyte	N°	Mean	s_r	r	RSD$_r$ (%)	s_R	R	RSD$_R$ (%)	RSD$_H$	HorRat	Rec (%)
Cu[a]	8	25.9	0.50	1.41	2.0	1.30	3.65	5.0	2.5	2.1	*
As[b]	5	147.0	13.03	36.47	8.9	28.53	79.89	19.4	30.2	0.6	99
Cd[b]	8	206.3	11.47	32.11	5.6	15.45	43.25	7.5	28.7	0.3	100
Pb[b]	8	23513.9	1083.52	3033.87	4.6	4160.60	11649.68	17.7	14.1	1.3	95
Cr[b]	3	2254.9	103.10	288.68	4.6	274.58	768.81	12.2	20.0	0.6	97
Se[b]	1	203.4	73.84	206.75	36.3	*	*	*	28.8	*	101

[a] %
[b] µg kg^{-1}

Table 3. Collaborative results obtained with mineral mix (mineral salt for swine) matrix

Mineral Mix (mineral salt for swine)

Analyte	N°	Mean	s_r	r	RSD$_r$ (%)	s_R	R	RSD$_R$ (%)	RSD$_H$	HorRat	Rec (%)
Ca[a]	8	100.0	2.31	6.46	2.3	3.50	9.79	3.5	2.8	1.2	-
Mg[a]	9	1.9	0.10	0.27	5.0	0.17	0.47	8.8	5.1	1.7	-
Na[a]	6	3.4	0.21	0.59	6.2	0.74	2.08	21.7	4.7	4.6	-
K[a]	5	2.4	0.23	0.64	9.6	0.26	0.73	10.9	5.0	2.2	-
P[a]	5	4.4	0.18	0.49	4.0	0.92	2.57	20.9	4.5	4.6	-
Cu[b]	5	29633.9	502.86	1408.0	1.7	3005.27	8414.76	10.1	3.4	3.0	-
Co[b]	6	1482.4	57.05	159.74	3.8	145.68	407.90	9.8	5.3	1.8	-
Fe[b]	5	74844.7	1184.30	3316.0	1.6	6760.78	18930.18	9.0	3.0	3.1	-
Mn[b]	5	48216.1	756.91	2119.3	1.6	793.66	2222.26	1.6	3.2	0.5	-
Zn[b]	6	144945.4	3614.49	10120.6	2.5	11160.88	31250.46	7.7	2.7	2.9	-
Se[b]	6	495.2	21.00	58.79	4.2	57.68	161.49	11.6	6.3	1.9	-
As[c]	6	848.8	56.69	158.733	6.7	93.03	260.48	11.0	23.2	0.5	99
Cd[c]	5	743.1	53.75	150.502	7.2	129.07	361.41	17.4	23.7	0.7	103
Cr[c]	5	25979.1	677.35	1896.57	2.6	4428.39	12399.50	17.0	13.9	1.2	100
Pb[c]	5	7072.4	288.25	807.098	4.1	1990.22	5572.62	28.1	16.9	1.7	100

[a] g kg^{-1}
[b] mg kg^{-1}
[c] µg kg^{-1}

Table 4. Collaborative results obtained with Sodium Selenite matrix

Sodium Selenite

Analyte	N°	Mean	s_r	r	RSD_r (%)	s_R	R	RSD_R (%)	RSD_H	HorRat	Rec (%)
Se[a]	6	453.9	7.74	21.67	1.7	11.74	32.88	2.6	2.3	1.1	*
As[b]	6	1478.6	83.56	233.96	5.7	265.49	743.39	18.0	21.3	0.8	96
Cd[b]	6	*	*	*	*	*	*	*	*	*	92
Cr[b]	2	570.3	53.49	149.77	9.4	53.49	149.77	9.4	24.6	0.4	103
Pb[b]	2	75.8	3.26	9.12	4.3	32.57	91.19	43.0	33.4	1.3	101

[a] $g\ kg^{-1}$
[b] $\mu g\ kg^{-1}$

Five participants reported results for total Hg, although the mass fraction for that measurement was below the limit of detection of the method used (Hg < 10 μg kg^{-1}). For Se in the calcitic limestone and copper sulfate matrices, it was not possible to calculate the reproducibility (R) since only 1 laboratory sent the result. Although not the focus of the exercise, the results indicated Mg as the analyte that presented the lowest values of both repeatability (r) and reproducibility (R), probably because it is a well-established method and is present in higher concentrations in the samples.

The relative standard deviation of reproducibility calculated from the Horwitz equation (RSD_H) and the relative standard deviation calculated from the standard deviation of reproducibility obtained experimentally (RSD_R) make it possible to establish the HorRat ratio. The HorRat ratio is helpful in analytical precision studies, in which, as mentioned in Annex F of the AOAC [10], the limits between 0,5 and 2.0 for the HorRat ratio are the acceptability values of the method's precision established in terms of reproducibility.

In the current experiment, most of the analytes presented HorRat ratio values at acceptable levels of analytical precision. However, the Mg and As analytes for calcitic limestone; Cu for copper sulfate; and Na, K, P, Cu, Fe, and Zn for the mineral supplement showed values > 2.0 for the HorRat ratio, which indicates the need for adjustment or standardization of analytical procedures.

Regarding the recovery (Rec %) evaluation, the experiment was carried out for the analytes considered inorganic contaminants. For these analytes, satisfactory values were obtained within the 91% and 103% limits, indicating compliance with the values established in Table A5 of Appendix F of the AOAC [10].

4. Conclusion

Through the interlaboratory collaborative study, it was possible to establish an analytical protocol for the determination of inorganic contaminants in the evaluated matrices and, consequently, to establish the analytical precision parameters about repeatability and reproducibility. Regarding the Hg content, it was not possible to establish the precision parameters since the results were lower than the quantification limits in the evaluated matrices.

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Acknowledgments