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PRACTITIONER'S REPORT

Uncertainty from sample preparation in the laboratory on the example of various feeds

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Abstract A way of uncertainty calculation from test sample preparation in the laboratory was presented on the example of feeds. The essence of the proposal lies in separating two components of results' variability expressed as coefficient of variation CV_p: analytical variability CV_a (repeatability) and technical variability CV_h corresponding with the inhomogeneity of a component. The law of Gauss's error propagation was used. Analytical variability CV_a was calculated from the range of duplicate analyses, following Nordtest Handbook. It was assumed that the coefficient of technical variability CV_h is the measure of the standard uncertainty from sample preparation in the uncertainty budget of a method u_{s+h}, and expanded uncertainty U_{s+h} for $k = 2$ ($P = 95$ %) can be easily calculated as $U_{s+h} = 2\ u_{s+h}$. Calculated uncertainties with uncertainties from sample preparation for loose feed and no ground premixture have increased from 4 to 125 % and were higher than analytical uncertainties calculated acc. to GUM during validation of methods. In the case of granulated feed mixture, the obtained uncertainties were similar. Grinding the premix results in lower uncertainties. Uncertainty from sample preparation should be taken into account in the uncertainty budget of a test method, especially in the case of inhomogeneity of tested materials.

Keywords Sample preparation · Analytical variability · Technical variability · Uncertainty · Feedingstuffs

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Introduction

The result with measurement uncertainty of the sample tested in laboratory is often used for conformity assessment [1]. Total measurement uncertainty should cover the uncertainty of (1) sampling, (2) uncertainty of test sample preparation in the laboratory and (3) the analytical uncertainty [2]. Official laboratories receive laboratory (final) samples from the authorized inspection units. In the case of feedingstuffs, these are often products of which ingredients tend to segregate, and sample preparation errors may importantly affect the measurement uncertainty.

In case of the official feed control, the official method was introduced by the Commission Regulation 691/2013 [3] in order to reduce errors involved in sampling as well as the uncertainty of the procedure. A sample taken in conformity with the regulation is regarded as representative of the tested batch. The regulation does not require that the uncertainty from sampling should be determined. However, due to inhomogeneity characterizing numerous feed products and their tendency to segregation, an official laboratory is supposed to divide a laboratory (final) sample weighing minimum 0.5 kg into test samples weighing in most cases ca. 100 g, depending on determined analytes. One of test samples is randomly chosen and ground. The degree of sample grinding depends on the type of analyte to be determined and its stability. From the completely ground and homogenized test sample, the test portions are weighed. The stage of sample preparation, the errors occurring at this stage of the procedure and related uncertainties of sample preparation may be significant. Therefore, this stage of the procedure should be appropriately carried out and monitored. The guidelines for preparing feed samples are presented accurately in the recently published standard EN-ISO 6498 [4].

The uncertainty of sample preparation is affected mainly by the inhomogeneity of the tested material. In case of the tested analyte, uncertainty is measured by the variability of analyte concentration in the test samples obtained from the laboratory sample, expressed as standard deviation or coefficient of variation (%). However, variability of the tested analyte includes two components: variability of the method used for determination of the analyte (repeatability) and variability of distribution (inhomogeneity) of the analyzed component in test samples separated from the laboratory samples. Analyzing the test sample in at least two replications, it is possible to calculate the method variability (repeatability) from the range of duplicate analyses, following Nordtest Handbook [5].

The aim of the paper was to show a simple way of uncertainty calculation from test sample preparation in the laboratory and to include this uncertainty into the uncertainty budget. The results of uncertainty calculation of some basic nutrients, minerals and feed additives on the example of various feeds of different homogeneity characteristics were presented.

Materials and methods

Materials

Different kinds of feeds characterized by various inhomogeneity were investigated. In the case of feed mixtures, samples of loose supplementary feed mixtures (high heterogeneity) and granulated compound feeds (low heterogeneity) were chosen. In the case of premixtures, test samples with mean particle size of 416 μm and test samples after grinding with mean particle size of 260 μm were investigated. It should be underlined that feed premixture without grinding filled the criteria of homogeneity for chlorides according to IUPAC Technical Report [6], for the test portion equal to 1 g.

Sample preparation

Laboratory samples of about 800 g were divided by riffle divider in eight test samples of about 100 g each. Six samples from eight were chosen randomly and ground. Test samples of feed mixtures for basic nutrients and minerals were ground in the ultra centrifugal mill with 0.5-mm sieve (Retsch ZM 200). Test samples for vitamins A and E were ground in the same mill with 1.0-mm sieve just before the testing. Premixture samples were tested without grinding (particle diameter about 416 μm) and after grinding in the planetary ball mill (Retsch PM 100) to obtain particle diameter of 260 μm.

Test range

In each test sample of loose supplementary feed mixture and granulated compound feed, some basic nutrients including crude protein, crude ash and minerals including calcium, chlorides, sodium, iron, manganese, zinc, copper, cobalt, selenium and molybdenum as well as vitamins A and E were tested. In the case of premixtures, some minerals like calcium, iron, manganese, copper and zinc were analyzed.

Methods

Basic nutrients (crude ash, crude protein) were tested by official methods given in regulation 152/2009 [7]. Macro- and microelements like calcium, sodium, iron, manganese, copper and zinc were analyzed by flame atomic absorption spectrometry according to ISO 6869 [8]. Selenium was tested by atomic absorption spectrometry with hydride generation HGAAS [9] and molybdenum by electrothermal atomic absorption spectrometry (ETAAS). Vitamins A and E were analyzed by HPLC method according to regulation 152/2009 [7]. Chlorides soluble in water were tested by titrimetric method with amperometric detection of final point of titration [10]. The average size of premixture particles was measured prior to and following grinding by means of the optical-electronic particle-size analyzer, AWK 3D System.

Calculating uncertainty from sample preparation

The essence of the proposal lies in separating two components of the variability of results expressed as coefficient of variation CV_p: analytical variability CV_a (repeatability) and technical variability CV_h corresponding with the inhomogeneity of a feed component. The law of Gauss's error propagation was used. Analytical variability CV_a was calculated from the range of duplicate analyses on the basis of the Nordtest TR 537 [5]. For calculation, the following formulas were used:

$$CV_p = \sqrt{CV_h^2 + CV_a^2} \quad (1)$$

$$CV_h = \sqrt{CV_p^2 - CV_a^2} \quad (2)$$

It was assumed that technical variability CV_h is the component of standard measurement uncertainty u_h from sample preparation in the uncertainty budget u_{s+h}, which is calculated from the formula (3),

$$u_{s+h} = \sqrt{u_a^2 + u_h^2} \quad (3)$$

where u_a is uncertainty of analytical procedure and measurement. Expanded uncertainty U_{s+h} for coverage factor

$k = 2$ ($P = 95$ %) was calculated in the simple way: $U_{s+h} = 2\ u_{s+h}$.

Measurements of each of the six samples obtained by dividing the laboratory sample were performed in two replications. The differences between the replications (the range) were used to calculate analytical variability (repeatability), in conformity with the formula (4) following the Nordtest Handbook [5]:

$$CV_a = \frac{X_1 - X_2}{d_2} \quad (4)$$

where d_2 is a factor dependent on the number of replications. In case of measurements performed in two replications, $d_2 = 1.128$.

Examples of calculating uncertainty from sample preparation

Table 1 presents an example of calculating measurement repeatability for calcium content tested by FAAS method in a granulated compound feed, expressed as the coefficient of variation, CV_a. The analyses were done on six test samples separated from the laboratory sample. Each test sample, after grinding, was used to prepare two test portions in which the content of calcium was determined, following mineralization. Coefficient of variation CV_p was calculated from all values X_1 and X_2 (12 measurements). Calculations were carried out with the use of the Excel sheet. Table 2 presents, using the same example of determining calcium content in a granulated compound feed and for comparison in a loose feed mixture, the successive stages of the procedure, resulting in calculating the coefficient of technical variability, CV_h, and the standard uncertainty of calcium calculation, u_{s+h}, taking into account the uncertainty from sample preparation and expanded uncertainty, U_{s+h}.

Results and discussion

The ways presented in Tables 1 and 2 were used to calculate the coefficients of analytical variability (repeatability), the coefficients of technical variation, standard uncertainties of the measurement including the uncertainty of sample preparation and expanded uncertainties for the mass fractions of all the tested analytes. The results of analyzing the components in loose feed mixture and granulated compound feed are presented in Table 3, while the results of analyzing non-ground and ground feed premixtures are shown in Table 4.

In case of a loose feed mixture, the coefficients of technical variation, CV_h, of the analyzed parameters ranged from 1.96 % to 11.8 %, the average of 5.77 %; they were nearly twice as high as the coefficients of variation for the analyzed components in the granulated compound feed, from 0.92 % to 6.16 %, respectively, the average of 3.09 % (Table 3). In the tests, there were used a loose supplementary feed mixture of specific composition in which the mass fraction of minerals was high, ca. 360 g/kg (Table 3). The remaining components of the mixture were plant- and animal feed materials of lower bulk density, as compared with minerals, which fostered their segregation not only at the stage of manufacturing the mixture but also in the laboratory during the preparation of the sample for tests. That is why a chemist-analyst should try to learn about the physical and chemical properties of the tested materials and, in justified cases, pay special attention to preparing the sample in the laboratory and, particularly, to its division in order to obtain a test sample and its proper grinding. Some useful information regarding this issue can be found in the standard EN-ISO 6498 [4], whose guidelines may be used not only in testing feedingstuffs, but also in testing all types of loose materials and materials which are difficult at the stage of test sample

Table 1 Calculation of repeatability from the range on the example of calcium testing in a granulated compound feed according to Nordtest TR [5]; mass of test portion equals 5 g

No of test sample	X_1 g/kg	X_2 g/kg	X_{mean} g/kg	Difference $d = X_1 - X_2$ g/kg	Relative difference r (%)
1	8.27	8.68	8.475	−0.41	4.84
2	8.51	8.31	8.41	0.20	2.38
3	8.20	8.36	8.28	−0.16	1.93
4	8.33	8.26	8.295	0.07	0.84
5	8.70	8.51	8.605	0.19	2.21
6	8.13	8.10	8.115	0.03	0.37
		X_{mean} =	8.363		
		SD =	0.198		
		CV_p =	2.37 %	r_{mean} =	2.095
		d_2 =	1.128		
				CV_a =	1.86 %

SD standard deviation

Table 2 Comparison of uncertainty calculation from sample preparation in laboratory on the example of calcium in loose and granulated compound feed according to Nordtest TR [5]; mass of test portion equals 5 g

Item	Loose feed mixture[a]	Granulated compound feed
Coefficient of variation	Laboratory data	Laboratory data
	$CV_p = 4.96$ %	$CV_p = 2.37$ %
Repeatability calculated from the range [5]	Laboratory data—excel sheet	Laboratory data—excel sheet
	$CV_a = 1.67$ %	$CV_a = 1.86$ %
Coefficient of technical variation	Formula 2:	Formula 2:
	$CV_h = 4.67$ % $= u_h$	$CV_h = 1.47$ % $= u_h$
Standard uncertainty for calcium measurement calculated acc. to GUM [11]	Validation data	Validation data
	$u = 4.8$ %; $U = 9.6$ % ($k = 2$)	$u = 4.8$ %; $U = 9.6$ % ($k = 2$)
Standard uncertainty for calcium measurement calculated with uncertainty of sample preparation	Formula 3:	Formula 3:
	$u_{s+h} = 6.0$ %	$u_{s+h} = 5.0$ %
Expanded uncertainty	$U_{s+h} = 12.0$ % ($k = 2$)	$U_{s+h} = 10.0$ % ($k = 2$)

[a] Supplementary feed

Table 3 Results of expanded uncertainty calculation for some basic nutrients, minerals and feed additives including uncertainty of sample preparation in laboratory, $n = 6$

Feed component	Test portion g	Loose feed mixture[a]				Granulated compound feed			
		Mass fraction	CV_h %	U %	U_{s+h} %	Mass fraction	CV_h %	U %	U_{s+h} %
Crude protein, g/kg	0.5	288	1.53	3.0	4.3	196	1.33	4.0	4.8
Crude ash, g/kg	5	362	2.67	4.0	6.7	46.9	0.98	4.2	4.6
Calcium, g/kg	5	125	3.59	9.6	12.0	8.36	1.47	9.6	10.0
Sodium, g/kg	5	4.71	4.65	11.6	14.9	1.50	1.53	11.6	12.0
Chloride, g/kg	2	6.59	3.45	8.4	10.9	3.16	0.92	9.7	9.9
Iron, mg/kg	5	nd	nd	nd	nd	223	3.09	15.0	16.2
Manganese, mg/kg	5	nd	nd	nd	nd	99.3	3.07	12.2	13.7
Zinc, mg/kg	5	233	5.02	10.4	14.5	198	2.52	10.4	11.6
Copper, mg/kg	5	nd	nd	nd	nd	10.1	6.16	18.0	21.8
Cobalt, mg/kg	0.5	nd	nd	nd	nd	0.47	4.41	24.0	25.6
Selenium, mg/kg	0.5	0.89	11.5	15.0	27.4	0.28	3.55	15.0	16.6
Molybdenum, mg/kg	0.5	2.40	11.5	20.0	30.5	1.86	5.29	20.0	22.6
Vitamin A[b], mg/kg	20	27.0	11.8	19.4	30.6	2.61	3.39	27.5	28.3
Vitamin E, mg/kg	20	372	1.96	13.1	13.6	61.5	5.50	17.1	20.3

U expanded uncertainty ($k = 2$) according to GUM; CV_h coefficient of technical variation; U_{s+h} expanded uncertainty ($k = 2$) with uncertainty from sample preparation; *nd* not determined

[a] Supplementary feed; [b] Calculated as retinol

preparation. For comparison, the studies of inhomogeneity of components in a granulated compound feed, with limited component segregation, were characterized by lower by nearly 50 % values of technical variation coefficients (Table 3). The results suggested that the variability of the analyzed components related to their inhomogeneity in the feeds may significantly affect the uncertainty of sample preparation and that it should be considered in the budget of method uncertainty. The presented way of calculating the coefficients of technical variability, consisting of separating analytical variability from technical variability is fit for purpose. The application of this way was feasible due to using the possibility to calculate analytical variability (repeatability) from the range, following Nordtest Handbook [5].

The results of studying inhomogeneity of calcium, iron, manganese, zinc and copper in a non-ground premixture with the average particle size of 416 μm suggested high variability of the results, from 5.76 % to 10.1 %, the average of 7.76 % (Table 4). Grinding the test samples separated from the laboratory sample of the premixture in the planetary ball mill to the average particle size of

Table 4 Results of expanded uncertainty calculation for some minerals in premixtures with and without grinding, $n = 6$

Feed component	Test portion g	Premixture without grinding[a], particle size 416 μm				Premixture ground[a], particle size 260 μm			
		Mass fraction	CV_h %	U %	U_{s+h} %	Mass fraction	CV_h %	U %	U_{s+h} %
Calcium, g/kg	1	175	5.77	7.4	13.7	170	0.70	7.4	7.6
Iron, g/kg	1	13.2	5.76	9.4	14.9	13.2	1.67	9.4	10.0
Manganese, g/kg	1	14.2	7.07	10.4	17.6	14.0	2.56	10.4	11.6
Zinc, g/kg	1	9.22	10.1	10.0	22.5	9.85	3.88	10.0	12.7
Copper, g/kg	1	1.42	10.1	10.6	22.8	1.44	3.17	10.6	12.3

U expanded uncertainty ($k = 2$) according to GUM; CV_h coefficient of technical variation; U_{s+h} expanded uncertainty ($k = 2$) with uncertainty from sample preparation; *nd* not determined

[a] Premixtures with homogeneity confirmed by chloride testing

260 μm led to its enhanced homogeneity, from 0.70 % to 3.88 %, the average of 2.40 %, although in case of non-ground premixture, its homogeneity was confirmed on the basis of testing chloride content [6]. Grinding significantly enhanced homogeneity and made the expanded uncertainty including the uncertainty of sample preparation differ only slightly from expanded uncertainty calculated during validation. In case of the non-ground premixture, the difference was from 58 % to 125 %, the average of 90 % (Table 4). The results confirmed the recommendation in the standard EN-ISO 6498 [4] stating that the samples of some mineral feeds (premixtures, mineral mixtures) for stable analytes like minerals should be ground to the particle size <0.5 mm, preferably 0.25 mm.

Conclusion

Calculated uncertainties with uncertainties from sample preparation for loose feed and unground premixture were higher by 4 % up to 83 % (44 % on average) than uncertainties of the analytical procedure and measurement, calculated according to GUM [11] during validation of a method. Higher differences were obtained for unground premixture (90 %), but in this case, average particle diameter was too high (0.416 mm), however, it was consistent with general requirements. Generally, in the case of the granulated compound feed, the obtained uncertainties were similar. Grinding the premixture resulted in decreasing measurement uncertainties. Hence, the laboratory should check the characteristics of tested materials (especially their homogeneity) and use proper divider and grinding mill for sample preparation, as this step has been shown to be one of the largest sources of laboratory errors, in some cases much larger than the analytical procedure [4]. In conclusion, uncertainty from sample preparation should be taken into account in the uncertainty budget of a test method, especially in the case of inhomogeneity of the materials analyzed. It is necessary to take into account measurement uncertainty with the uncertainty from sample preparation in the laboratory in order to assess correctly the conformity of the declared content of feed additives in feedingstuffs on the label and the assessment of conformity of measurement result uncertainty in the laboratory with the permitted tolerances of nutrients [12, 13].

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