

**STATISTICAL EVALUATION OF THE  
DETERMINATION OF NA, K, CA, FE, P IN FEED AND  
PREMIXES BY ENERGY-DISPERSIVE X-RAY  
FLORESCENCE SPECTROMETRY**

— research paper —

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**Abstract:** For development, maintenance and normal growth, reproduction, animals need in their diet a number of dietary minerals such as calcium (Ca), Phosphorus (P), potassium (K), sodium (Na) and iron (Fe). For the animals to be more productive, an appropriate nutritional composition of feed has to be chosen. Have to consider that quality of feed depends on many factors such as area of origin, plant type, soil. To assess accurately the quality of feed, with which animals must to be fed, it needs to establish a definite program of testing. For this purpose, laboratory tests are performed. Results obtained in the laboratory are a good indicator to determine the exact feed ratio for animals, before being fed. The aim of this study was to investigate the use of the energy-dispersive X-ray fluorescence spectrometry (EDXRF) for the measurement of calcium, phosphorus, potassium, sodium and iron concentration in feed and premixes.

**Keywords:** energy-dispersive X-ray fluorescence spectrometry (EDXRF), dietary minerals, feed, premixes.

## INTRODUCTION

The quality of the feeding stuff reflects the ability of a given feed to meet the nutrient needs of the consuming animal. The quality of the feed depends on many factors such as plant species, climatic conditions and soil fertility. For

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the animal body maintenance, growth, good immune system and reproductive performance a balanced mineral program should be done. The chemical composition of the feeding stuff is a useful tool in assessing its quality.

Calcium is used in the formation and maintenance of bones and teeth. It also functions in transmission of nerve impulses and contraction of muscle tissue. Because of its importance in bone structure, deficiency of calcium in young animals leads to skeletal deformities. Phosphorus works in conjunction with calcium in the formation of bone. In addition, phosphorus is a component of deoxyribonucleic acid (DNA), the molecules that make up chromosomes and control genetic inheritance. Phosphorus is also involved in the chemical reactions of energy metabolism. Phosphorus-containing compounds like adenosine triphosphate and creatine monophosphate are the body's major storage depots of readily available energy. Deficiency of phosphorus results in decreased animal performance, including reduced weight gains, poor reproductive efficiency, and for cows low milk production. Because of their mutual role in bone metabolism, calcium supplementation and phosphorus supplementation are usually considered simultaneously. Sodium is involved in muscle and nerve function. Potassium is ubiquitous in the body of mammals because it is required in large amounts by most organ systems for normal functioning. Thus, a deficiency of potassium results in nonspecific symptoms such as poor appetite, followed by thinness, and reduced performance. Iron functions in oxidative enzyme systems involved in energy metabolism. It also enables the hemoglobin in red blood cells to carry oxygen to the tissues of the body (Hale and Olson, 2012).

Accurate methods are available for mineral analysis, by using instrumentation such as inductively coupled plasma atomic emission spectrometry (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS). The sample preparation for the analysis on ICP-OES and ICP-MS involves the total destruction of the matrix by chemical treatment. These methods are very laborious, time consuming and expensive.

Energy Dispersive X-ray Fluorescence (EDXRF) analysis techniques provide major, minor and trace element quantification across the broadest range of samples, including bulk solids, granules, powders, thin films and all manner of liquids with extreme ease of sample preparation (Thermo Scientific, 2012).

XRF is a physical phenomena involving the interaction of X-rays with matter. Most of the applications of XRF are in areas outside of physics:

chemistry, environmental sciences, food and product quality monitoring, etc.. In figure 1 is presented the schematic diagram of XRF instrument.

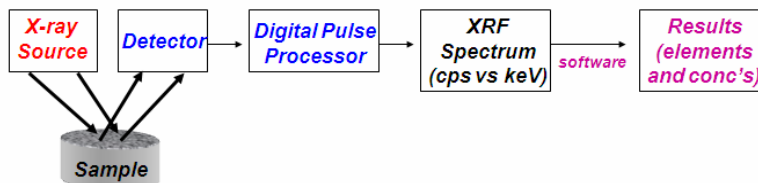


Figure 1. Schematic diagram of EDXRF instrument (Palmer, 2012)

The aim of this study is to present and to evaluate the performances of the quantitative determination of calcium (Ca), phosphorus (P), potassium (K), sodium (Na) and iron (Fe) concentration in feed and premixes by energy-dispersive X-ray fluorescence spectrometry technique as an alternative to the conventional and expensive methods. The evaluation of the method's performance was performed by using certified reference materials.

## MATERIALS AND METHODS

The study involves the use of energy-dispersive X-ray fluorescence spectrometry technique. A high performance Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometer, with element coverage from Na to U, with a Peltier cooled Si(Li) detector of <155 eV resolution and ten-position automated sample changer was used. The instrument uses an X-ray tube excitation source and a solid state detector to provide simultaneous spectroscopic analysis of elements ranging from a few ppm to 100 percent (Thermo Electron). For satisfying the performance criteria according to the studied matrix, elements to be analyzed and their content, appropriate measuring conditions were set. The conditions of the measurements are presented in Table 1.

Table 1. EDXRF's measurement conditions

Conditions	Na	K	Ca	Fe	P
Atmosphere	Vacuum	Vacuum	Vacuum	Air	Vacuum
Filter	No filter	Cellulose	Cellulose	Pd Thin	No filter
Voltage	4 kv	7 kv	7 kv	14 kv	4 kv
Live time	120 sec	90 sec	90 sec	90 sec	120 sec

Four certified reference materials (CRM1, CRM2, CRM3, and CRM4) were used in this study for creating the calibration curves. An independent certified material (CRM5) was used to check the precision and the accuracy of the method and to determine the expanded uncertainty. The certified reference materials were in the form of fine powder. The certified concentration of Na, K, Ca, P and Fe are presented in Table 2. Also, in Table 2 are presented the uncertainties of the certified concentrations, corresponding to a coverage factor  $k=2$ .

Table 2. Certified reference materials concentrations

Element	CRM1	CRM2	CRM3	CRM4	CRM5
	Concentration, %				
Na	0.2954±0.0312	0.6708±0.032	0.065±0.0021	0.157±0.0014	0.1360±0.014
K	0.6523±0.0219	0.1833±0.009	0.5131±0.0295	0.5795±0.0218	0.2071±0.010
Ca	0.608±0.0246	0.0576±0.003	0.8147±0.0362	0.4251±0.0279	0.4706±0.029
Fe	0.0084±0.0004	0.001±0.0001	0.015±0.00158	0.0083±0.00051	0.005±0.0004
P	0.4205±0.027	0.2952 ±0.02	0.5893±0.0419	0.3522±0.0258	0.3437±0.03

For the sample preparation, the certified reference materials were pressed as pellets under 15 tones using a mechanical hydraulic press. The sample is pressed into a compressible pressed powder sample holder (aluminium cup) of 32 mm.

Prior to be prepared in order to be analyzed on EDXRF the certified reference materials were dried at 135°C for 2 hours in an electric oven to remove the water content. After drying the materials were let to cool in desiccator for 30 minutes. In order to prepare the certified reference materials for analysis, 2 g were weighed in a grinding mortar and 0.2 g of binder (Ethylenediaminetetraacetic acid) was added to the materials. The certified reference materials and the binder were homogenized for 2 minutes in the grinding mill. The certified reference materials well mixed with the binder were transferred with a palette into the pressed powder samples holder (aluminum cups). The mixtures were leveled with the palette in the cups and pellets were pressed under 15 tones for 2 minutes. The pellets were analyzed on EDXRF spectrometer.

The above procedure is also followed in order to analyze real samples. After drying the animal feed the test portion is grinded in a grinding mill for 2 minutes in order to be transformed in powder form.

## RESULTS AND DISCUSSIONS

In order to evaluate the method's performance the calibration range, standard deviation, repeatability limit, reproducibility limit, uncertainty and limits of detection and quantification have been considered. The statistical evaluation of the method was made using the results obtained from the analysis of certified reference material CRM5.

For the accurate analysis of Na, P, K, Ca, Fe it is necessary to set up individual calibrations on the EDXRF for each element because the response between line intensity and concentration is only relative (Bremser and Hässelbarth, 1997).

The equation described by the calibration curve represents the relationship between the outlet value and the inlet value of the process. Equation (1) represents the mathematical model which describes the dependence between the peak intensity (Y) expressed in cps/mA (outlet value) and the amount (X) of the certified reference material expressed in wt% (inlet value).

$$Y = a \cdot X - b \quad (1)$$

In Table 3 are presented the coefficients of equation (1) corresponding for each element and the accuracy indicators (R2) of the mathematical models.

Table 3. Equation's (1) coefficients and the accuracy indicators

Case	Equation's coefficients		Accuracy indicator
	a	b	R2
Na	18.204	0.0851	0.9959
K	1741.5	27.37	0.9995
Ca	2436.5	22.84	0.9963
Fe	10688	21.605	0.9863
P	1959.9	5.0201	0.9886

The calibration curve for Na was linear in the range 0.0650 - 0.6708 %, as presented in Figure 2. The calibration curve for P was linear in the range 0.2952 - 0.5843 %, as presented in Figure 3. The calibration curve for K was linear in the range 0.1833 - 0.6523 %, as presented in Figure 4. The calibration curve for Ca was linear in the range 0.0576 - 0.8147 %, as presented in Figure 5. The calibration curve for Fe was linear in the range 0.001 - 0.015 %, as presented in Figure 6.

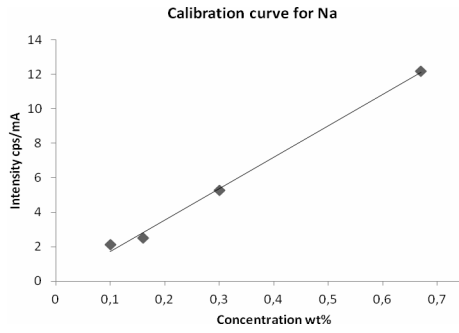


Figure 2. Intensity vs. given concentration for Na

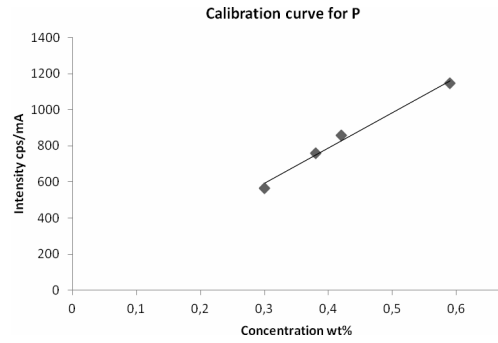


Figure 3. Intensity vs. given concentration for P

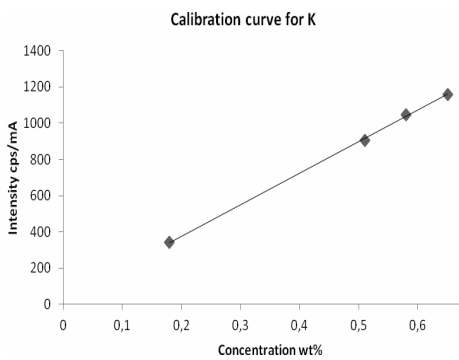


Figure 4. Intensity vs. given concentration for K

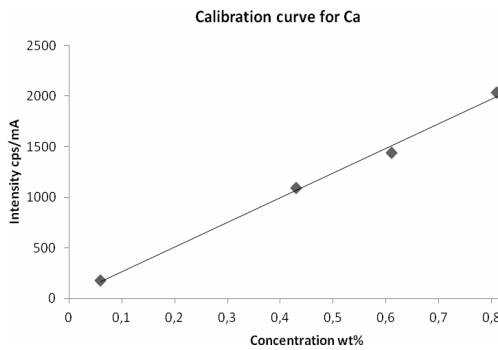


Figure 5. Intensity vs. given concentration for Ca

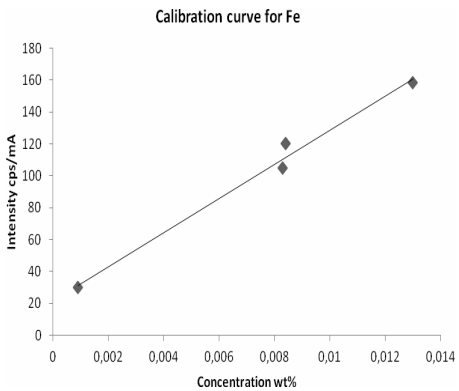


Figure 6. Intensity vs. given concentration for Fe

Based on the dependences presented in the figures 2-6 and their correlation coefficients the developed method has acceptable linear ranges for all the elements and the range can be considered as working range.

Potassium was found to have the best calibration performance compared with the others elements, with  $R^2=0.9995$ . The lowest calibration performance was met by iron, with  $R^2=0.9863$ .

For the evaluation of the accuracy and precision of the method it was prepared the CRM5 pellet as quality control sample, having a composition within the calibration range for each element to be analyzed.

The accuracy is represented by the closeness agreement between the result of a measurement and the true value of the measurand.

The precision represents the closeness agreement between independent test results obtained under prescribed conditions. The precision was examined under repeatability and reproducibility conditions.

The statistical indicators of the measurements under repeatability and reproducibility conditions, for each element, were calculated using the following formulas (Magnusson et al., 2003):

Arithmetic mean value of a sample of n results ( $\bar{x}$ ):

$$\bar{x} = \frac{\sum_{i=1}^n x_i}{n} \quad (2)$$

Standard deviation ( $\sigma$ ):

$$\sigma = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}} \quad (3)$$

Standard deviation of the mean (standard error of the mean) ( $S_{\bar{x}}$ ):

$$S_{\bar{x}} = \frac{\sigma}{\sqrt{n}} \quad (4)$$

Relative Standard Deviation (RSD):

$$RSD(\%) = \frac{\sigma}{\bar{x}} \cdot 100 \quad (5)$$

In order to verify the repeatability of the measurements the same pellet CRM5 was measured 10 times. Repeatability refers to the degree of agreement of results when conditions are maintained as constant as possible with the same analyst, reagents, equipment, and instruments performed within a short period of time (AOAC, 2002). The repeatability limit r was calculated. The values are presented in table 4.

Repeatability limit (r), for 95% confidence interval was calculated with equation (6) (Magnusson et al., 2003):

$$r = 1.96 \cdot \sqrt{2} \cdot \sigma_r \quad (6)$$

where:  $\sigma_r$  – standard deviation under repeatability conditions.

The repeatability limit represents the value less than or equal to which the absolute difference between two test results obtained under repeatability conditions may be expected to be with a probability of 95%.

Also, in table 4 are presented the statistical parameters which describe the measurements performed under repeatability conditions: mean value ( $\bar{x}$ ), standard deviation ( $\sigma_r$ ), standard error of the mean ( $S_x$ ) and relative standard deviation (RSD %).

Table 4. Evaluation of precision under repeatability conditions

Element	n	x mean	$\sigma_r$	$S_x$	RSDr%	r
Na	10	0.1359	2.25 x 10 <sup>-3</sup>	7.11 x 10 <sup>-4</sup>	1.65	0.00623
K	10	0.2080	0.98 x 10 <sup>-3</sup>	3.09 x 10 <sup>-4</sup>	0.47	0.00270
Ca	10	0.4707	0.58 x 10 <sup>-3</sup>	1.82 x 10 <sup>-4</sup>	0.13	0.00159
Fe	10	0.00535	0.15 x 10 <sup>-3</sup>	0.48 x 10 <sup>-4</sup>	2.81	0.00041
P	10	0.3441	1.11 x 10 <sup>-3</sup>	3.49 x 10 <sup>-4</sup>	0.32	0.00306

For the reproducibility examination, different analysts have prepared in different days, the quality control sample (pellet) from the same CRM5. The statistical indicators which describe the measurements under reproducibility conditions were calculated and are presented in Table 5: mean value ( $\bar{x}$ ), standard deviation ( $\sigma_R$ ), standard error of the mean ( $S_x$ ), relative standard deviation (RSD %) and reproducibility limit (R). The reproducibility limit is the value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions may be expected to be with a probability of 95%.

Reproducibility limit (R), for 95% confidence interval was calculated with equation (7) (Magnusson et al., 2003):

$$R = 1.96 \cdot \sqrt{2} \cdot \sigma_R \quad (7)$$

In Table 6 are presented acceptable values, according literature (AOAC, 2002), for relative standard deviation (RSD) under repeatability (r) and reproducibility (R) conditions.

For all the studied elements the RSDr and RSDR were found to be acceptable, less than 3% and less than 4% respectively.



Table 5. Evaluation of precision under reproducibility conditions

Element	n	x mean	$\sigma$	Sx	RSD%	R
Na	10	0.1391	3.05 x 10 <sup>-3</sup>	9.65 x 10 <sup>-4</sup>	2.19	0.008463
K	10	0.2067	2.35x 10 <sup>-3</sup>	7.43 x 10 <sup>-4</sup>	1.14	0.006509
Ca	10	0.4708	0.85 x 10 <sup>-3</sup>	2.67 x 10 <sup>-4</sup>	0.18	0.002345
Fe	10	0.0052	0.19 x 10 <sup>-3</sup>	0.61 x 10 <sup>-4</sup>	3.73	0.000536
P	10	0.3393	6.77 x 10 <sup>-3</sup>	21.41 x 10 <sup>-4</sup>	1.99	0.018763

Table 6. Acceptable values for RSD (AOAC, 2002)

Concentration	RSDr	RSDR
100 %	1 %	2 %
10 %	1.5%	3%
1 %	2 %	4%
0.1 %	3 %	6%
0.01%	4 %	8%
10 ug/g (ppm)	6 %	11 %
1 ug/g	8%	16%
10 ug/kg (ppb)	15 %	32%

Based on the reproducibility measurements the expanded uncertainty was calculated. In Table 7 are presented the bias, the combined uncertainty and the expanded uncertainty of the measurements.

The expanded uncertainty  $U$  is obtained by multiplying the combined standard uncertainty  $u_c$  by a coverage factor  $k$  (Magnusson et al., 2003):

$$U = k \cdot u_c \quad (8)$$

For an approximate level of confidence of 95%, the coverage factor  $k$  is 2.

Combined Standard uncertainty  $u_c$  is calculated with equation (9) [12]:

$$u_c(\%) = \sqrt{u(R_w)^2 + (U_{bias})^2} \quad (9)$$

where:  $u(R_w)$  – reproducibility within-laboratory (%);  $U_{bias}$  – uncertainty component for bias (%).

For every estimation of the uncertainty from the method and laboratory bias, two components have to be estimated to obtain  $U_{bias}$ : the bias (as % difference from the certified value or nominal value) and the uncertainty of the certified value  $u(C_{ref})$ .  $U_{bias}$ , if only one certified reference material is used, can be estimated by equation (10) (Magnusson et al., 2003):

$$U_{\text{bias}}(\%) = \sqrt{(\text{bias})^2 + \left(\frac{S_{\text{bias}}}{\sqrt{n}}\right)^2} + u(C_{\text{ref}})^2 \quad (10)$$

where: bias – difference between mean measured value from a large series of n test results and an accepted reference value (a certified value);  $S_{\text{bias}}$  – standard deviation of bias (%);  $u(C_{\text{ref}})$  – uncertainty component from the certified value (%).

The quantification of the bias is performed with equation (11) (Magnusson et al., 2003):

$$\text{bias}(\%) = \frac{\bar{x} - \mu}{\mu} \cdot 100 \quad (11)$$

where:  $\bar{x}$  – arithmetic mean value of a sample of n results, analyzed under reproducibility conditions;  $\mu$  – certified value of the reference material.

The uncertainty component  $u(C_{\text{ref}})$  from the certified value for a 95% confidence level, in (%), can be estimated with equation (12) (Magnusson et al., 2003):

$$u(C_{\text{ref}}) = \frac{c \cdot 96}{\mu} \cdot 100 \quad (12)$$

where: c – confidence interval of the reference material;  $\mu$  – certified value of the reference material.

Table 7. Calculated Uncertainties for CRM5

Element	$u(C_{\text{ref}})\%$	bias %	$U_{\text{bias}}\%$	$u(R_w)\%$	uc %	U %
Na	5.33	2.29	5.84	2.19	6.24	12.48
K	2.66	-0.22	2.69	1.14	2.92	5.85
Ca	3.14	0.034	3.14	0.18	3.15	6.30
Fe	4.08	3.60	5.58	3.73	6.71	13.42
P	4.57	-1.28	4.79	1.99	5.19	10.37

The limit of detection is the lower content of an analytical parameter that can be detected with a specifically defined level of confidence (e.g. 95, 99 or 99.7%) using a given method of analysis (Irish National Accreditation Board, 2007). The method's limit of detection was determined by measuring ten times in the same day the lowest standard for each element. The limits of quantification is the lowest concentration that can be determined with acceptable laboratory reproducibility and trueness. The limits of quantitation

represent the lower level for the measuring range (Irish National Accreditation Board, 2007). The method quantification limit was determined using the lowest standard measured ten times in the same day. The limits of detection (LOD) and the limits of quantification (LOQ) are presented in Table 8.

Table 8. Limits of detection and quantification

Element	LOD, %	LOQ, %
Na	0.10	0.34
K	0.003131	0.010
Ca	0.001581	0.0052
Fe	0.000127	0.00042
P	0.004439	0.015

## CONCLUSIONS

The statistical evaluation of the determination of Na, K, Ca, Fe, P in feed and premixes by energy-dispersive X-ray fluorescence spectrometry, represent a model for the calculation of repeatability, reproducibility and uncertainty when using a certified reference material. The method used above, for determination of the Na, K, Ca, Fe, P in feed and premixes by energy-dispersive X-ray fluorescence spectrometry, is a simple, quick and cheap method of analysis. The (EDXRF) technique for screening and determination of the concentration of calcium, phosphorus, potassium, sodium and iron in feed and premixes, presented in this study, was found to be suitable for its intended use.

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