

VALIDATION of Selenium (Se): XRF (X-Ray Fluorescence) Method

VALIDATION of Selenium (Se) by XRF in TMR e complete animal feed:

X-ray fluorescence (**XRF**) is widely used in the agri-feed sector for the determination of macro- and microelements in feeds and agricultural products (typically Ca, Mg, Na, K, P, Cl, S, Si, Mn, Fe, Cu, and Zn). A recent study conducted by our laboratory also demonstrated excellent performance for the determination of **Selenium**, a trace element normally present at very low concentrations and therefore not routinely investigated by XRF.

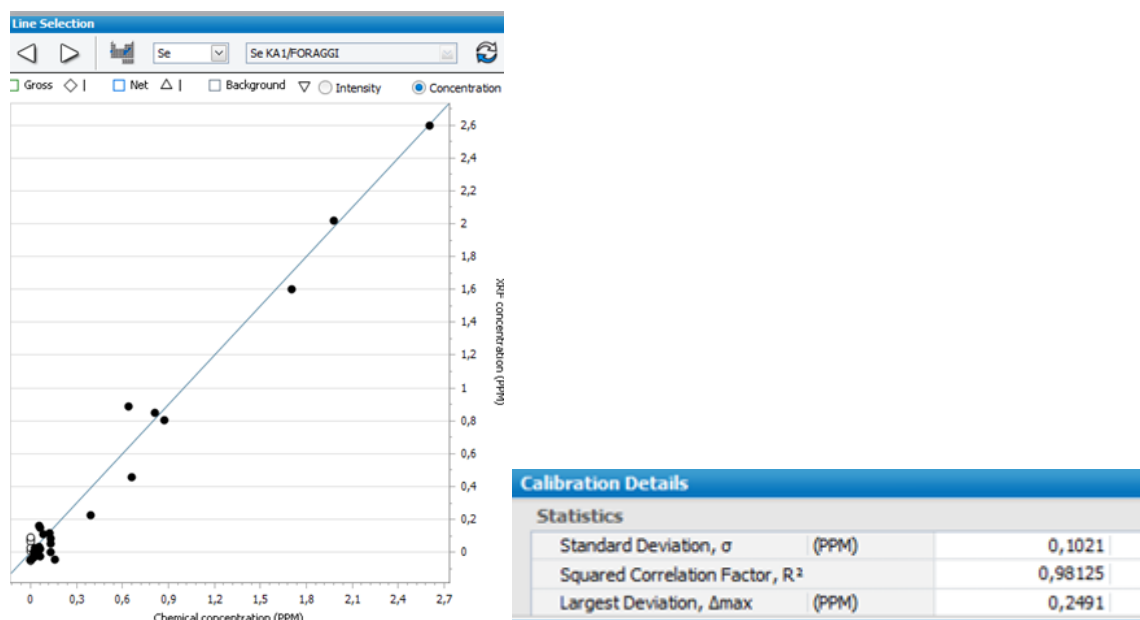
XRF results were compared with those obtained using reference analytical techniques, such as **Inductively Coupled Plasma (ICP) spectrometry**, for Unifeed samples, and with certified **Reference Materials** from interlaboratory Ring Tests for feeds.

Instrument Calibration

The XRF instrument (Bruker **Puma**, based on **Energy Dispersive X-ray Fluorescence, EDXRF**) was calibrated using certified reference materials supplied through the **WEPAL** interlaboratory proficiency testing scheme (Wageningen, The Netherlands) and **AAFCO** (USA).

The calibration included plant feed materials and feeds with selenium concentrations ranging from 0.06 to 2.6 mg/kg (together with the other macro- and microelements). Selenium fluorescence was measured after irradiation of the sample with 40 keV X-rays, using a 500 µm aluminum (Al) filter.

The selenium calibration data (**Intracal**) showed an excellent **correlation coefficient ($R = 0.98$)** with a **standard deviation of 0.102 mg/kg**. The calibration curve and its performance characteristics are shown below.



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These results encouraged us by demonstrating the feasibility of determining selenium using the XRF technique.

External Validation (Extracal): Lactating and Dry Cow TMRs

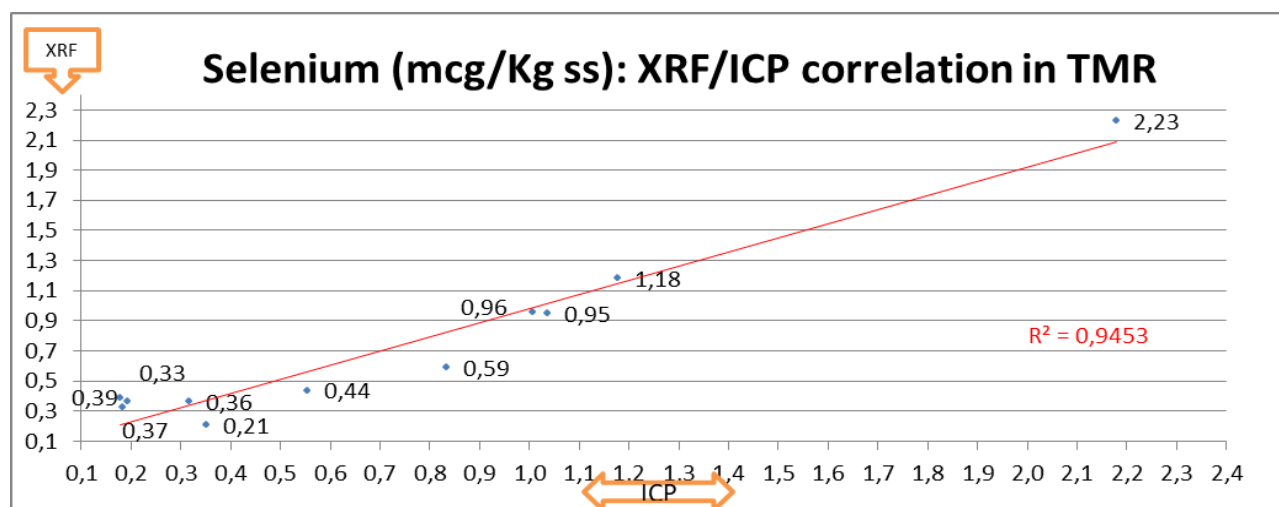
The reliability of the new parameter was further assessed through a correlation study performed on **11 TMR** samples for lactating and dry cows. The samples were analyzed in parallel by XRF and by the reference **Inductively Coupled Plasma (ICP) spectrometry** method at the **Università Cattolica del Sacro Cuore**, under the supervision of **Gian Maria Beone**, Faculty of Agricultural, Food and Environmental Sciences.

Experimental Results

The comparison between the two analytical methods confirmed the robustness of the XRF technique, particularly within the selenium concentration ranges of greatest nutritional relevance for dairy cattle feeding:

- **Excellent precision within the critical range (0.5–2.3 mg/kg):** An excellent **coefficient of variation (CV)** of **9.1%** was achieved, with a **standard deviation of 0.10 mg/kg (ppm)**, ensuring high analytical precision precisely within the selenium concentration range where monitoring is most critical for animal health.
- **Overall performance:** Across the full analyzed range (0.2–2.3 mg/kg), the mean CV is 19.6% (standard deviation 0.14 ppm).

The dispersion of the XRF data is shown below, both as a graph and in tabular form.

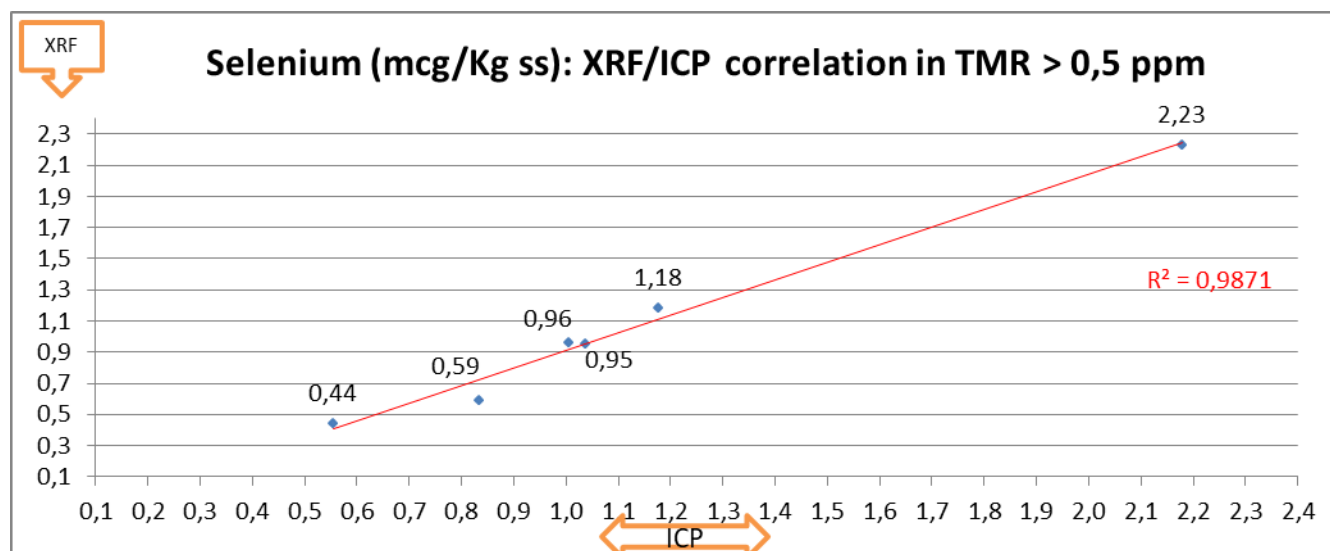


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In the table below, the observed differences for the validation of TMR are reported.

Se Validation by XRF 20/2/26			
Se (ICP)	Se (XRF) Ric	Error Standard	Bias
0,35	0,21	0,14	0,14
0,18	0,33	0,15	-0,15
0,32	0,36	0,04	-0,04
0,19	0,37	0,17	-0,17
0,18	0,39	0,21	-0,21
0,56	0,44	0,12	0,12
0,83	0,59	0,24	0,24
1,04	0,95	0,09	0,09
1,01	0,96	0,05	0,05
1,18	1,18	0,01	-0,01
2,18	2,23	0,05	-0,05
Averages	0,73	0,11	0,00
Correl	0,972	standard deviation	0,14
		CV%=	19,6%

Excluding data below 0.5 ppm, a better correlation is obtained (CV = 9.1%, SD = 0.102 ppm).



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External Validation (Extracal): Feed Samples

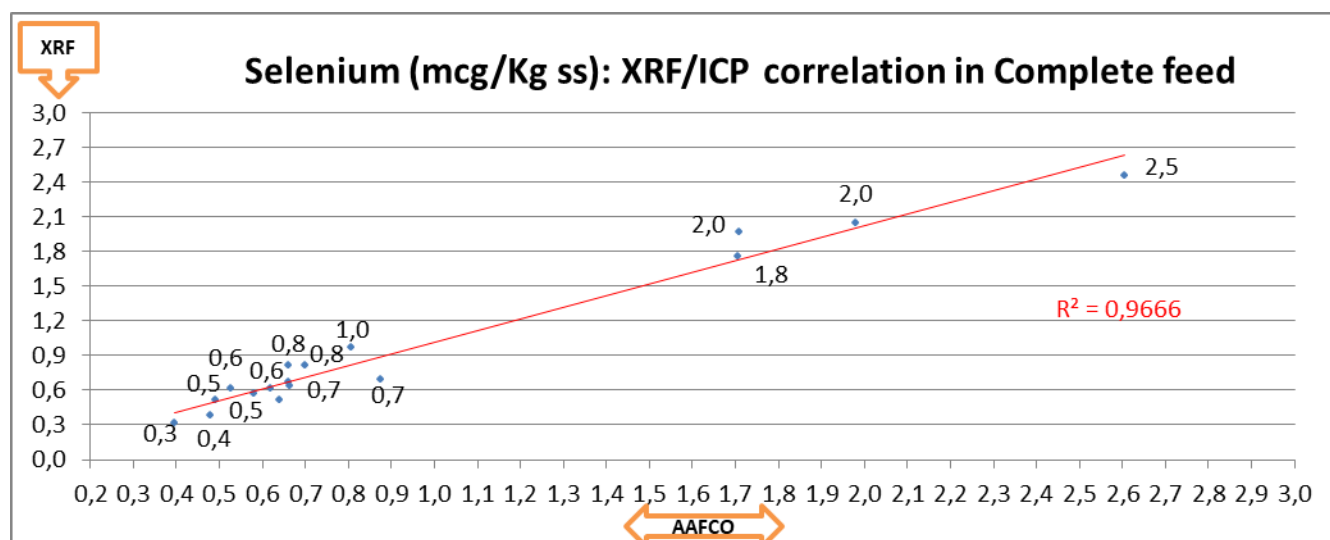
The correlation study was conducted on **17 complete feed samples** obtained from the interlaboratory proficiency testing scheme organized by the **AAFCO**, which were not included in the calibration set.

Experimental Results

Even for compound feeds, the XRF technique shows excellent correlation with reference data:

• **Overall performance:** Across the full analyzed range (0.2–2.5 mg/kg), the mean CV is 12.7%, with a standard deviation of 0.12 ppm. In compound feeds, dispersion across different concentration levels appears more linear compared to TMR samples.

The following section shows the graphical distribution of selenium measured by XRF versus the reference results obtained from the **AAFCO** interlaboratory data.



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In the table below, the observed differences for the validation of feed samples are reported.

Se Validation by XRF 25/3/26: Comp. Feed			
Se (AAFCO)	Se (XRF)	Error Standard	Bias
0,40	0,32	0,08	0,08
0,48	0,38	0,10	0,10
0,49	0,52	0,03	-0,03
0,64	0,52	0,12	0,12
0,58	0,57	0,01	0,01
0,53	0,62	0,09	-0,09
0,62	0,62	0,00	0,00
0,66	0,64	0,02	0,02
0,66	0,67	0,01	-0,01
0,88	0,69	0,18	0,18
0,66	0,82	0,16	-0,16
0,70	0,82	0,12	-0,12
0,81	0,97	0,16	-0,16
1,71	1,76	0,05	-0,05
1,71	1,97	0,26	-0,26
1,98	2,04	0,06	-0,06
2,61	2,46	0,15	0,15
Averages	0,95	0,96	0,09
	Correl	0,983	standard deviation
		CV%=	0,12
			12,7%

Conclusions

In both validation studies, the error increases as concentration decreases (as expected), showing higher correlation at **values above 0.3–0.5 ppm**, with CV% around 10–12%. On average, the CV% remains below 20% across the full analytical range (0.2–2.5 ppm).

These results confirm XRF as a highly reliable rapid screening tool, especially when compared to the long turnaround times of conventional wet chemistry methods.

Gonzaga, 25/03/2026
Marco Mancinelli