Session 45.

Indicators, hi-tech solutions, and tools to effectively manage cattle

Room: Valdostana – Palazzo Affari 3rd Floor
Chair: Aernouts

Effectiveness of energy dispersive X-ray fluorescence (ED-XRF) for the prediction of mineral elements in individual cow urine

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HEAT STRESS

Physiological condition that occurs when an animal is unable to effectively dissipate excess body heat.

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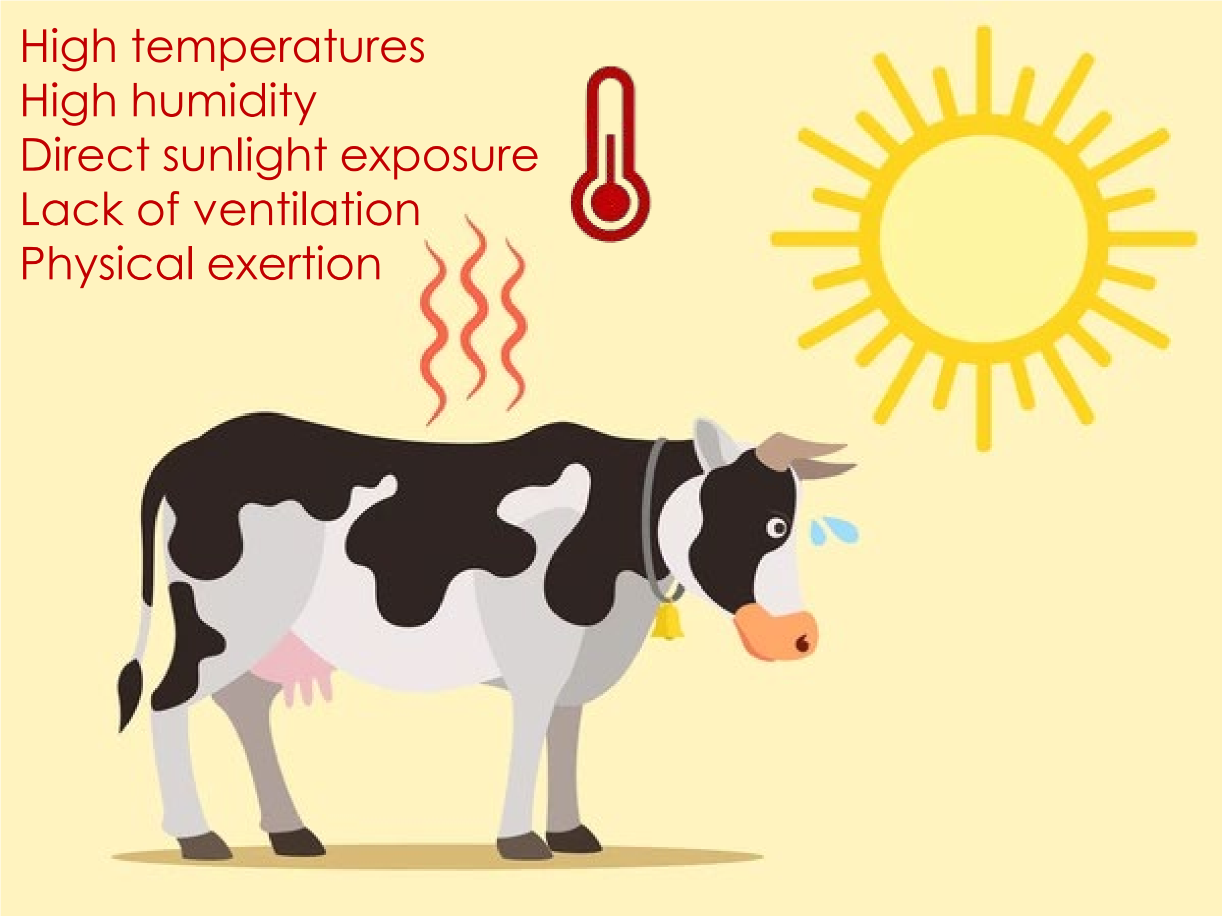
Respiratory rate and body temperature
Incidence of mastitis and ketosis

Milk production

Dehydration and electrolyte imbalances
Alteration in milk quality
Reproductive problems



INTRODUCTION

HEAT STRESS INDICATORS IN URINE

- MINERAL ELEMENTS
- pH
- URIC ACID
- UREA
- ALLANTOIN
- SUGARS
- CREATININE
- FREE AMINOACIDS

INTRODUCTION

HEAT STRESS INDICATORS IN URINE

- **MINERAL ELEMENTS**
- pH
- UREA
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- FREE AMINOACIDS
- CREATININE
- ALLANTOIN
- URIC ACID

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REFERENCE METHOD

PREDICTIVE METHOD

INTRODUCTION

REFERENCE METHOD

↓

Inductively coupled plasma optical emission spectrometry (**ICP-OES**)

↓

- ✓ High precision and sensitivity
- ✓ Expensive equipment
- ✓ Specialized personnel
- ✓ Time-compsuming

PREDICTIVE METHOD

INTRODUCTION

REFERENCE METHOD

Inductively coupled plasma optical emission spectrometry (**ICP-OES**)

- ✓ High precision and sensitivity
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- ✓ Time-compsuming

PREDICTIVE METHOD

Energy dispersive X-ray fluorescence (**ED-XRF**)
Mid-infrared spectroscopy (**MIRS**)

- ✓ Non-destructive technique
- ✓ Non-specialized personnel
- ✓ Cost-effective
- ✓ Rapid analysis

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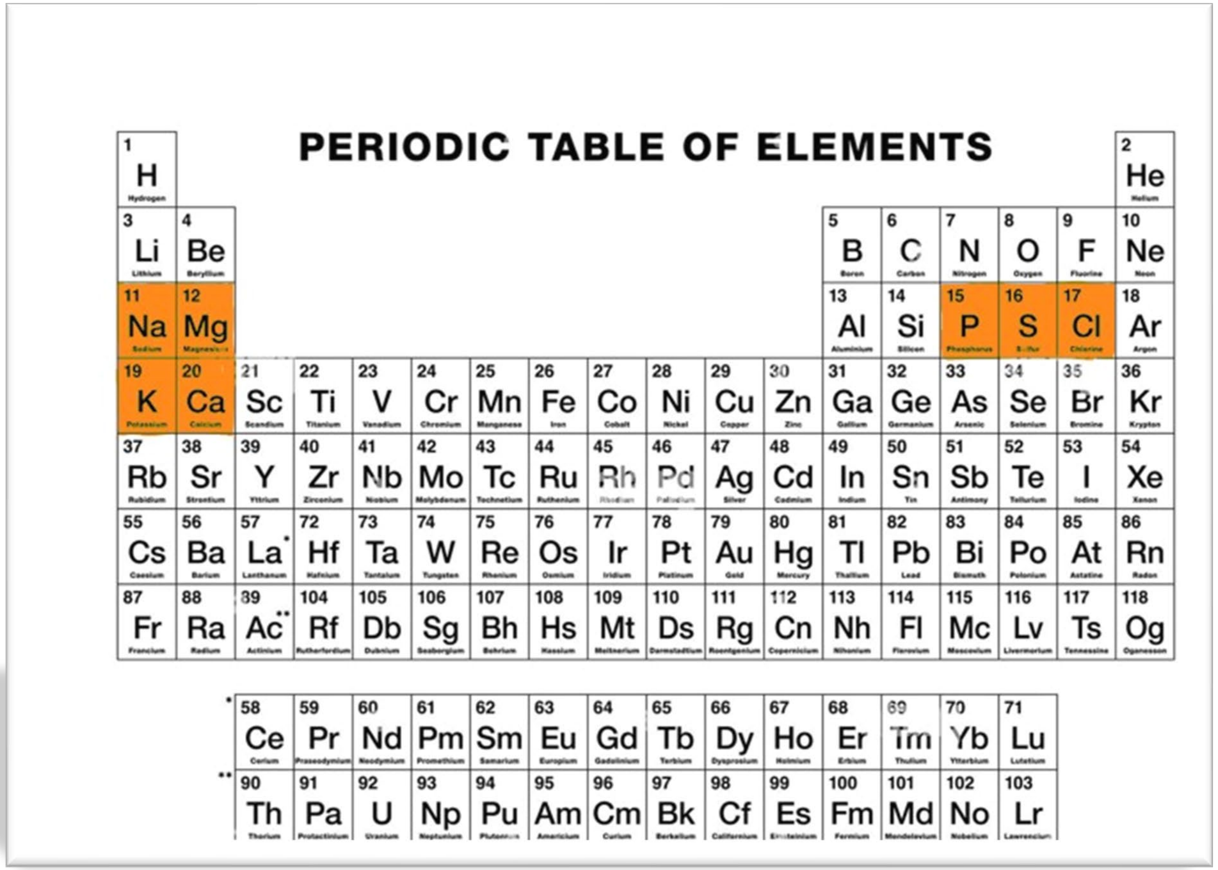
Assess the **effectiveness of energy dispersive X-ray fluorescence** for the prediction of:



- → **SODIUM**
- → **MAGNESIUM**
- → **POTASSIUM**
- → **CALCIUM**
- → **PHOSPHORUS**
- → **SULFUR**
- → **CHLORINE**

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Location

MATERIALS & METHODS

Location

Sample collection

9 commercial dairy farms

45 individual urine samples from Holstein Friesian

A.R.A. VENETO

MATERIALS & METHODS

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Mineral analysis

ICP-OES

MATERIALS & METHODS

Location

Sample collection

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Mineral analysis

ICP-OES

ED-XRF

MATERIALS & METHODS

ICP-OES reference analysis

1. <u>Mineralization step</u>

Urine samples were added with $\mathbf{H_2O_2}$ and $\mathbf{HNO_3}$ + heated in microwave at 200°C.

2. <u>Dilution and filtration steps</u>

Mineralized samples were added with ultrapure water and filtered into plastic tubes.

MATERIALS & METHODS

ICP-OES reference analysis

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Sample preparation is necessary

3. <u>ICP-OES analysis</u>
Instrument settings, chemical parameters and physical conditions were selected before starting the analysis of mineral elements.

ICP-OES SPECTRO ARCOS (Ametek)

ED-XRF predictive analysis

No preparatory steps are necessary.

MATERIALS & METHODS

ED-XRF predictive analysis

No preparatory steps are necessary.

Urine samples were weighed (5 mL) and placed in **ED-XRF plastic cups.**

MATERIALS & METHODS

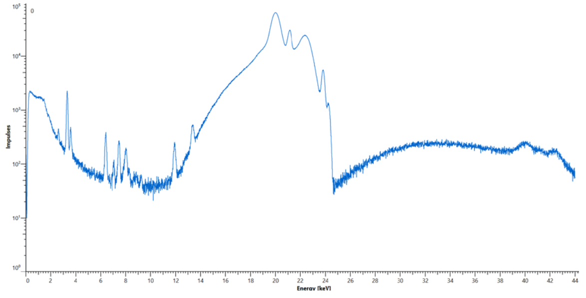
ED-XRF predictive analysis

No preparatory steps are necessary.

Urine samples were weighed (5 mL) and placed in **ED-XRF plastic cups.**

Information on **type of sample** and **exact weight** was provided to the instrument as the accuracy of the ED-XRF method.

ED-XRF Spectro Xepos 5P (Ametek)



ED-XRF spectrum of an urine sample

MATERIALS & METHODS

Statistical analysis

✓ The accuracy of ED-XRF technique was assessed using 2 data sets:

TRAINING SET

70% of the samples

n = 30

↓

ICP-OES analysis *vs* ED-XRF technique

↓

R^2_{TR}

MATERIALS & METHODS

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ICP-OES analysis *vs* ED-XRF technique

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$\mathbf{R^2_{TR}}$

TESTING SET

30% of the samples

n = 15

↓

ICP-OES analysis *vs* mineral concentrations predicted through the training equations

↓

$\mathbf{R^2_{TE}}$ **and** $\mathbf{RPD_{TE}}$

MATERIALS & METHODS

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ICP-OES analysis *vs* mineral concentrations predicted through the training equations
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R^2_{TE} **and** RPD_{TE}

✓ For each mineral, training and testing set were independently prepared.

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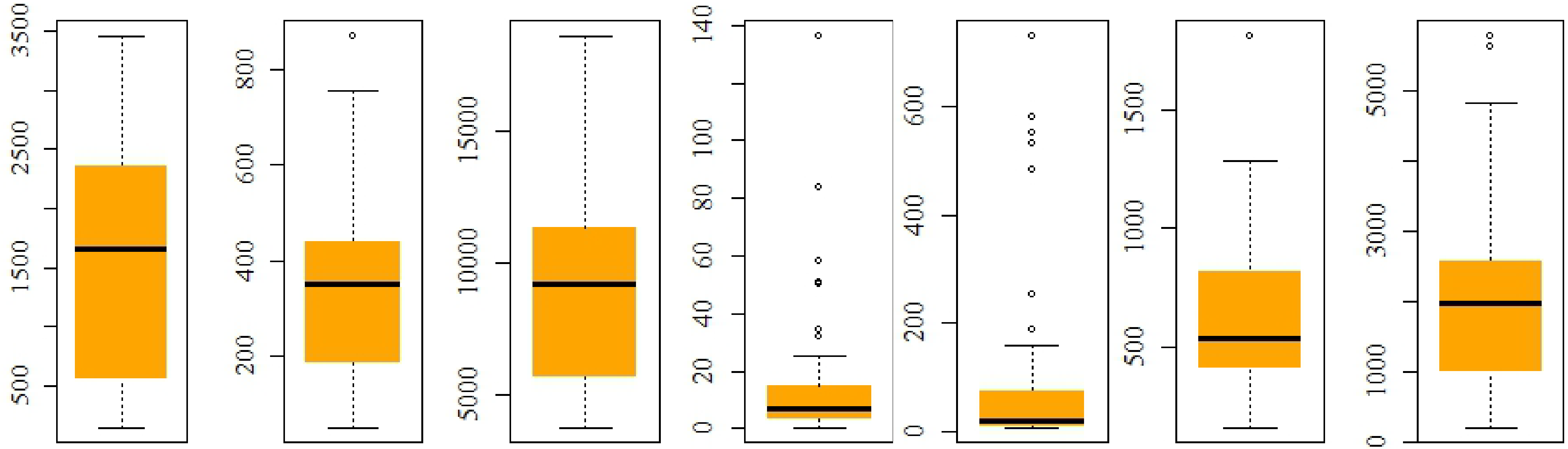
Descriptive statistics of urine mineral elements concentrations measured through ICP-OES

Minerals, mg/kg	Na	Mg	K	Ca	P	S	Cl
Mean (± SD)	1625.96 (± 1003.84)	333.57 (± 198.62)	8961.91 (± 3405.98)	16.00 (± 24.74)	97.14 (± 176.32)	639.60 (± 331.02)	2042.38 (± 1371.29)



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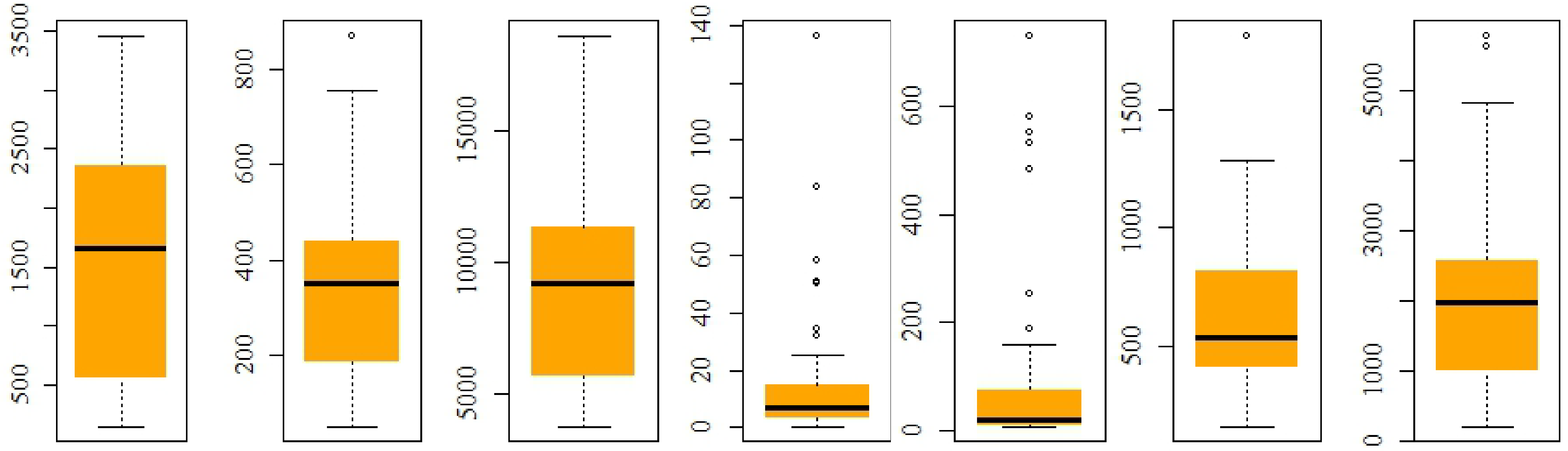
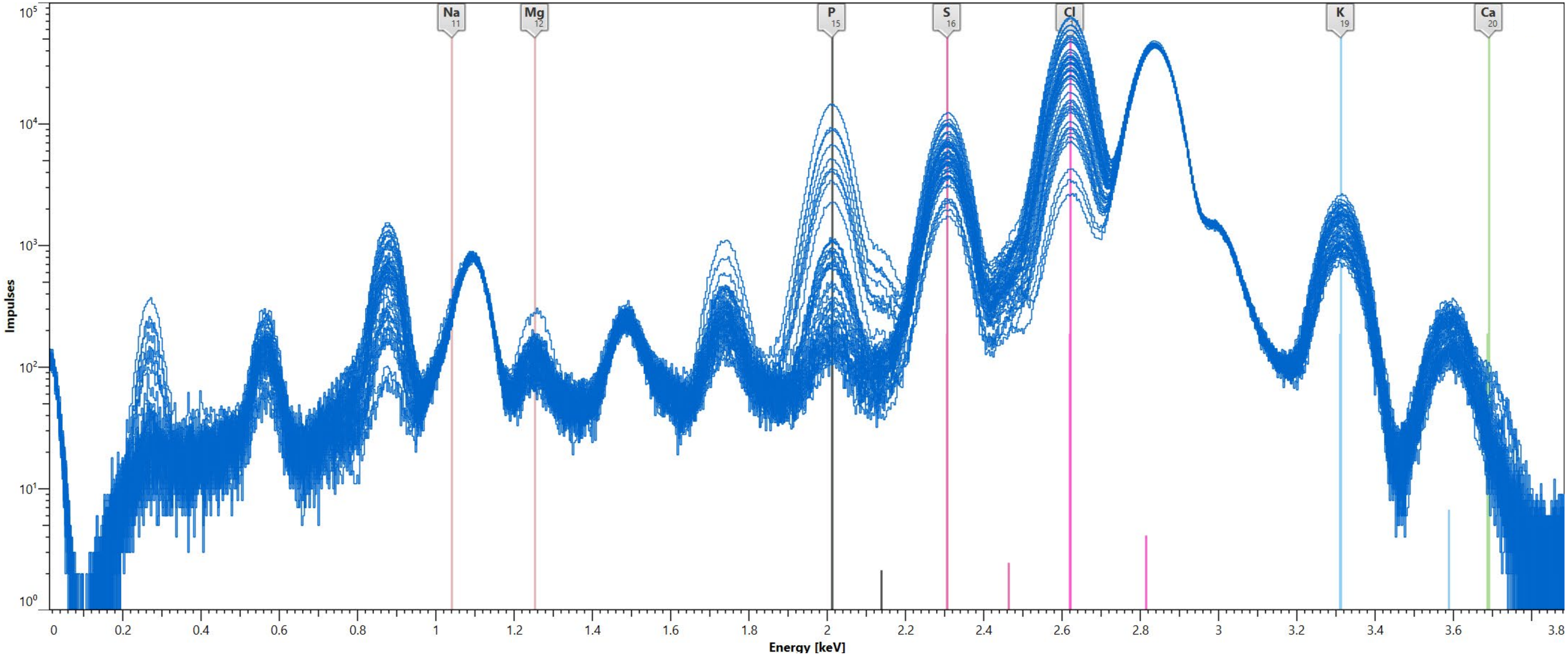


RESULTS & DISCUSSION

X-ray fluorescence spectrum of urine samples (*n* = 45) and excitation energies associated to each mineral elements



RESULTS & DISCUSSION

Fitting statistics[1] of the prediction models calculated within training set and testing set

Minerals, mg/kg	Training set		Testing set		
	R^2_{TR}	SE_{TR}	R^2_{TE}	SE_{TE}	RPD_{TE}
Na	0.71	366.87	0.76	495.55	2.10
Mg	0.79	108.48	0.64	172.35	1.10
K	0.83	1,481.42	0.51	2,449.22	1.38
Ca	0.92	8.15	0.63	14.51	1.86
P	0.89	46.04	0.76	144.19	0.96
S	0.89	85.99	0.80	161.56	1.69
Cl	0.95	269.06	0.89	490.87	2.64

[1]R^2_{TR}: coefficient of determination in training set; SE_{TR}: standard error in training set; R^2_{TE}: coefficient of determination in testing set; SE_{TE}: standard error in testing set; RPD_{TE}: ratio performance to deviation in testing set.

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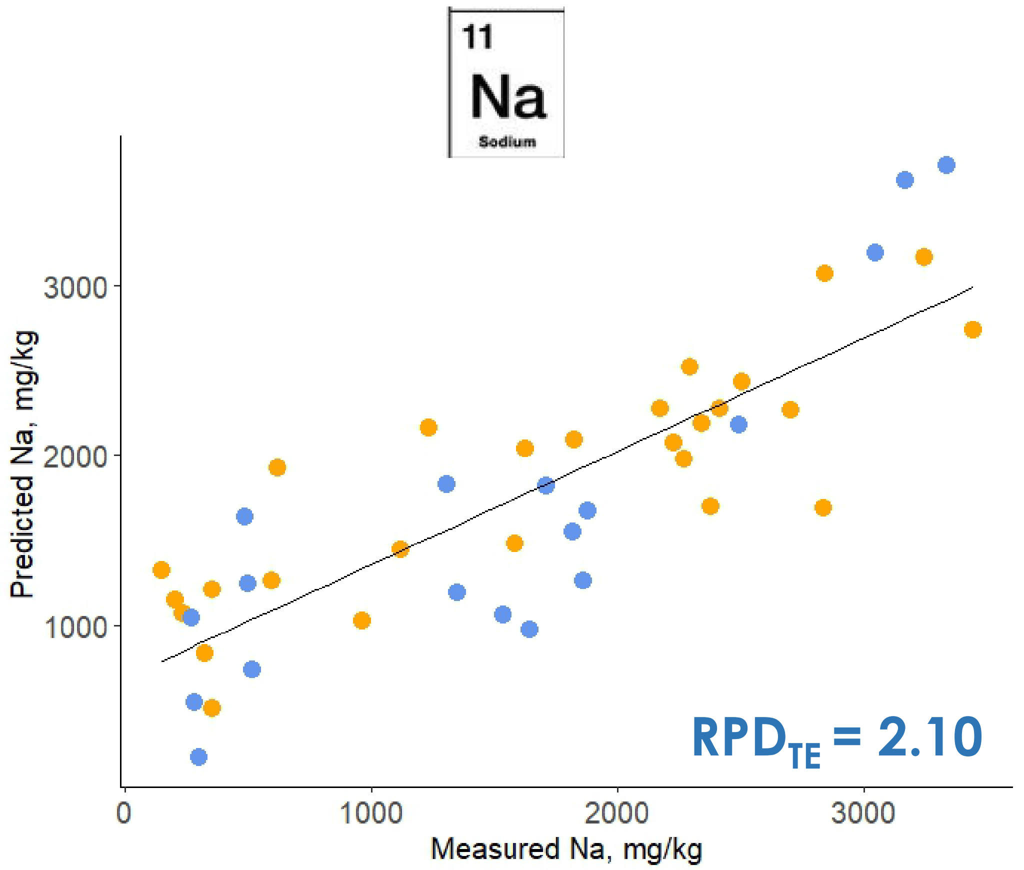
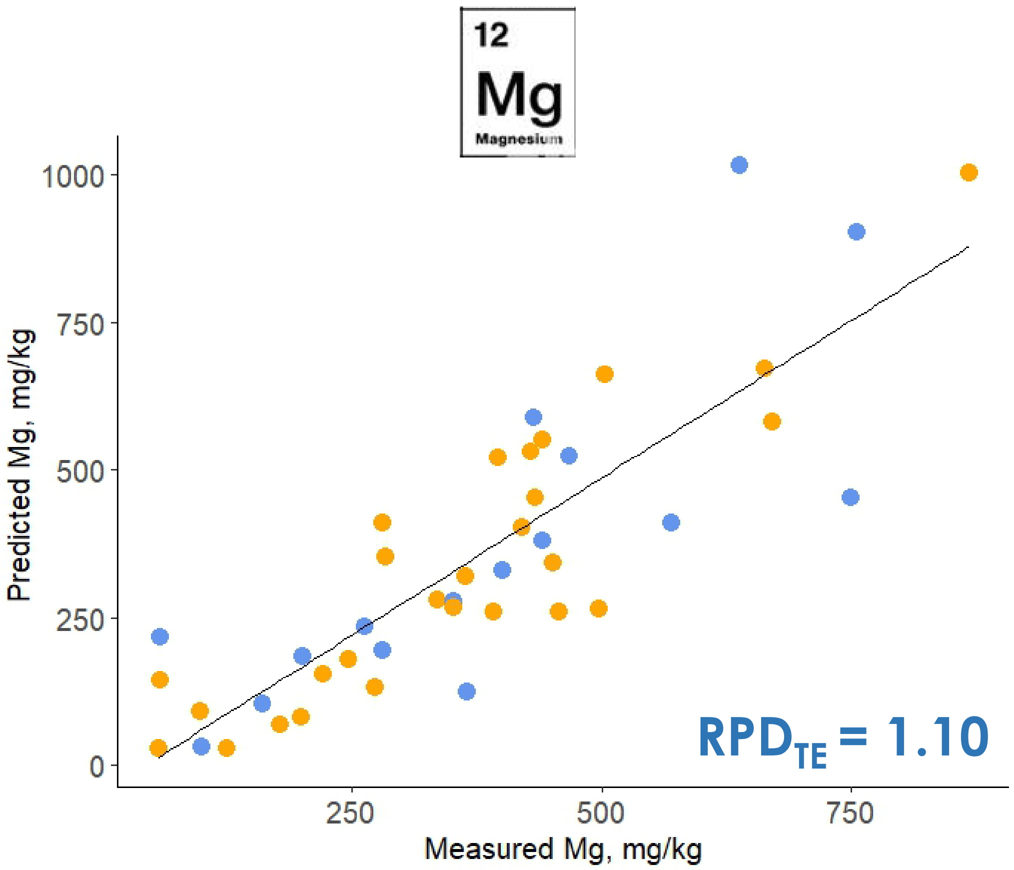
RESULTS & DISCUSSION

TRAINING SET
TESTING SET

Measured through ICP-OES *vs* predicted through ED-XRF





RESULTS & DISCUSSION

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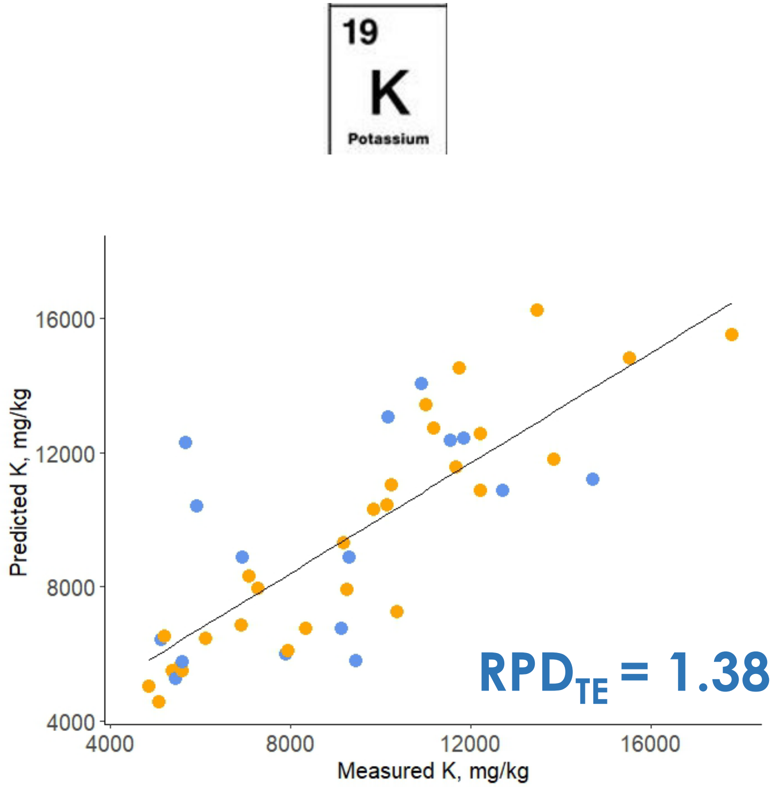

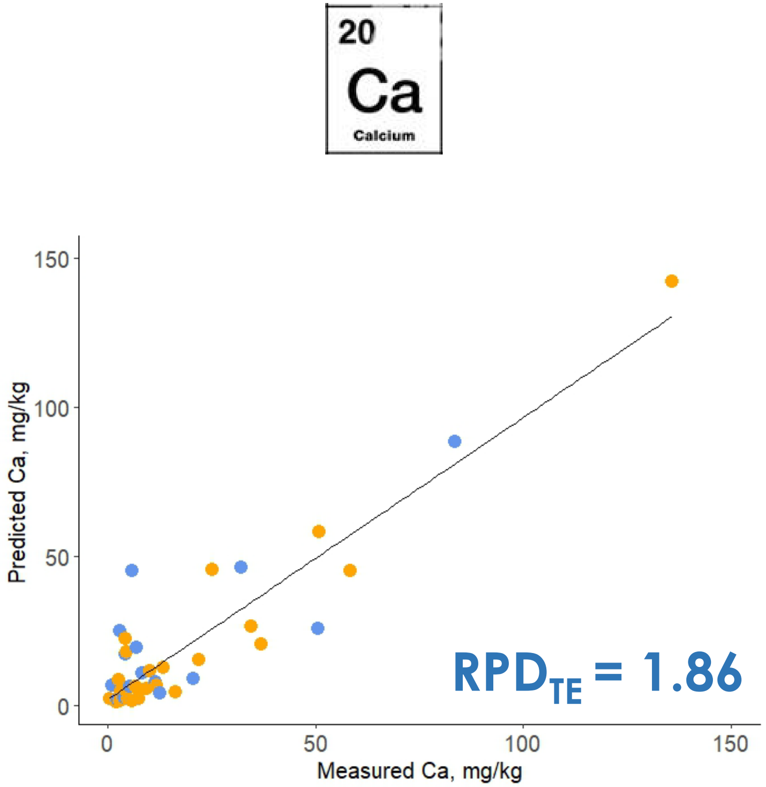

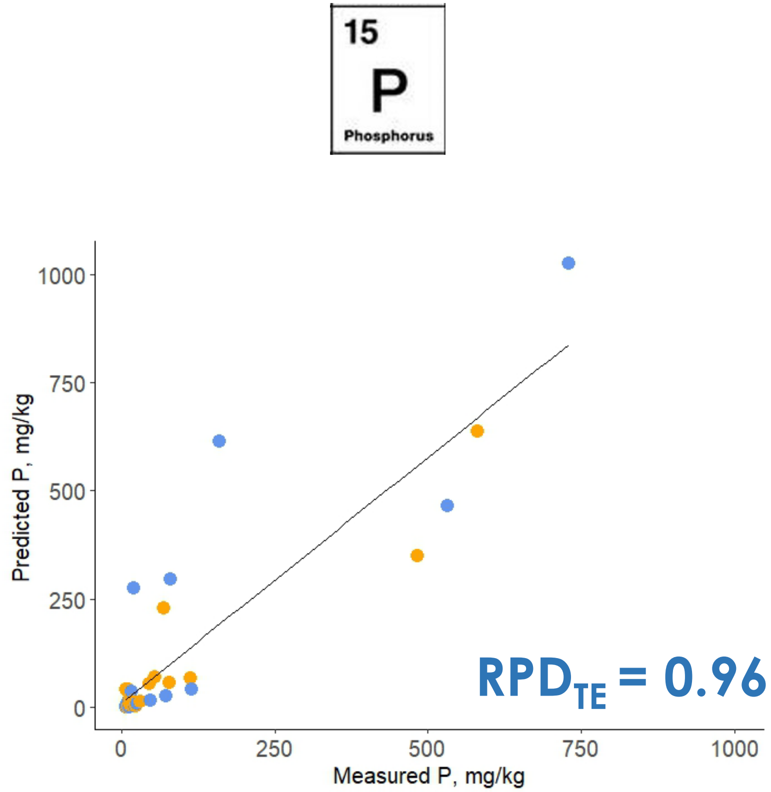

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TRAINING SET
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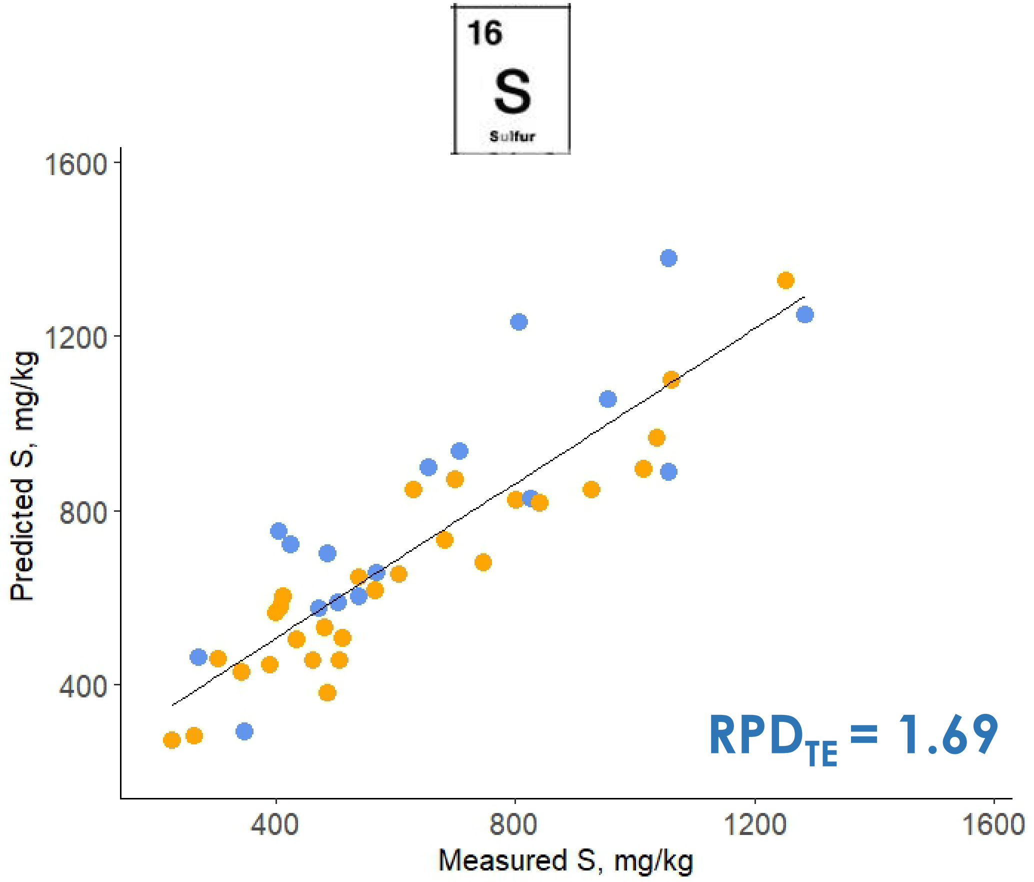

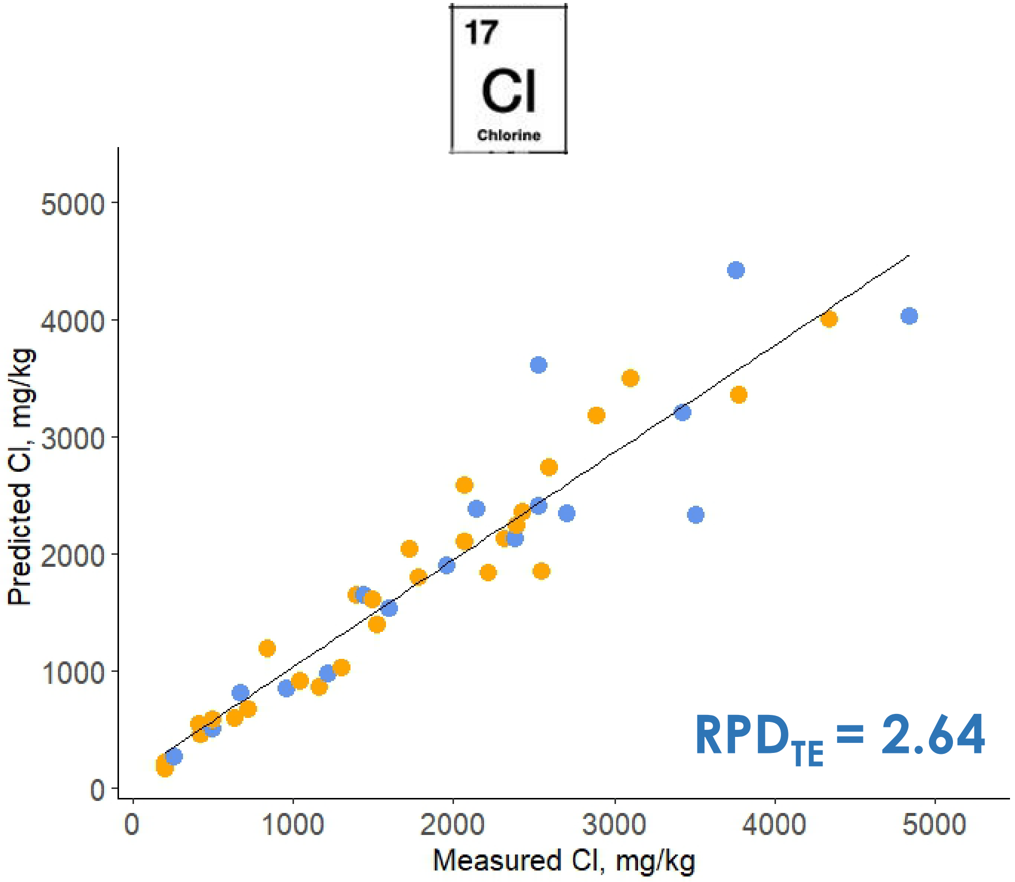

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CONCLUSIONS

- ✓ Results represent the first step for the development of **rapid technique to predict urine mineral elements** as indicators of heat stress in dairy cows.

- ✓ **Better results** were obtained for **S** and **Cl**.

- ✓ There is **alignment among the training and the testing sets** regarding the good prediction accuracies for **Na**, **Mg**, **P**, **S** and **Cl**.

- ✓ **ED-XRF prediction models** can be used to analyze **unprocessed urine samples** for monitoring heat stress and mineral status in dairy cows.

Thank you for your attention!

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