Session 13.

Sensing Physiology: Tools towards optimising livestock husbandry

Room: Modicana – Palazzo Affari 3rd Floor
Chair: Ákos / Garcia-Roche

Development of an HPLC method for the quantification of allantoin, creatinine and uric acid in Holstein cows' urine

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75th Annual Meeting of the European Federation of Animal Science, Florence, Italy (1st – 5th September 2024)

OUTLINE

1. INTRODUCTION
2. AIM
3. MATERIALS & METHODS
4. RESULTS & DISCUSSION
5. CONCLUSIONS

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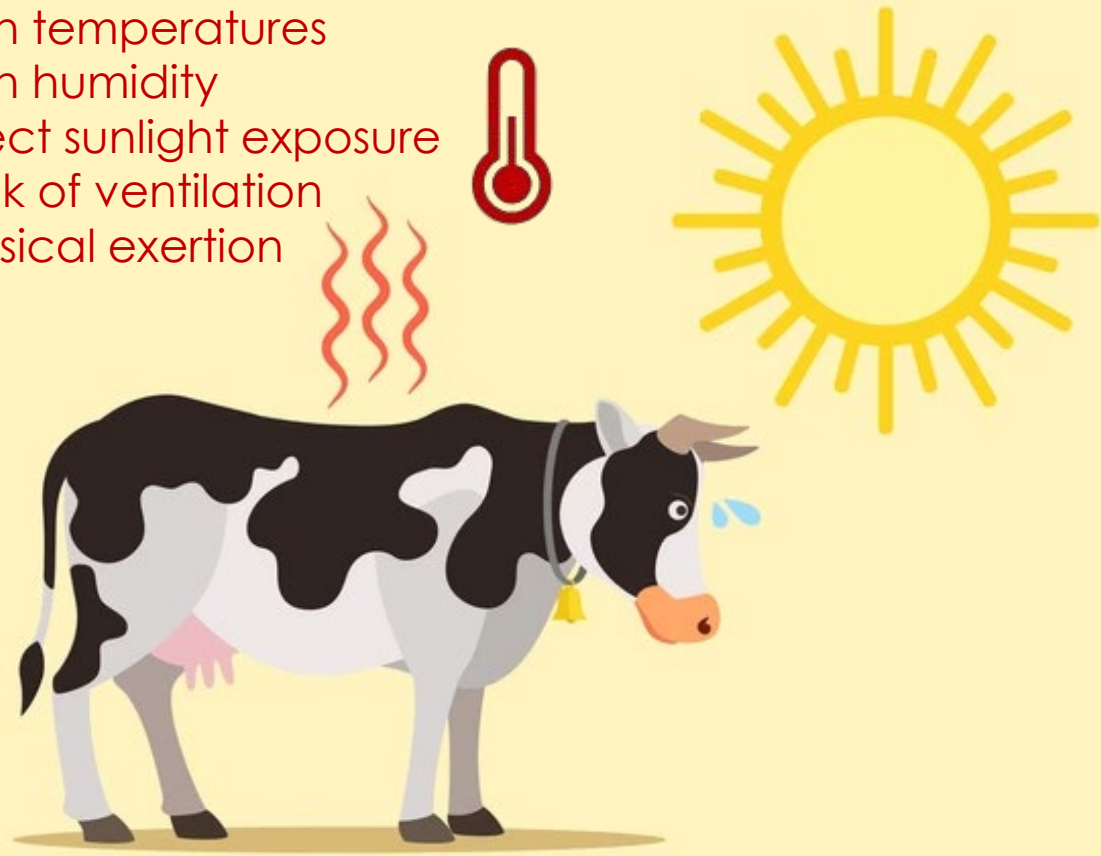
INTRODUCTION

HEAT STRESS

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

High temperatures
High humidity
Direct sunlight exposure
Lack of ventilation
Physical exertion

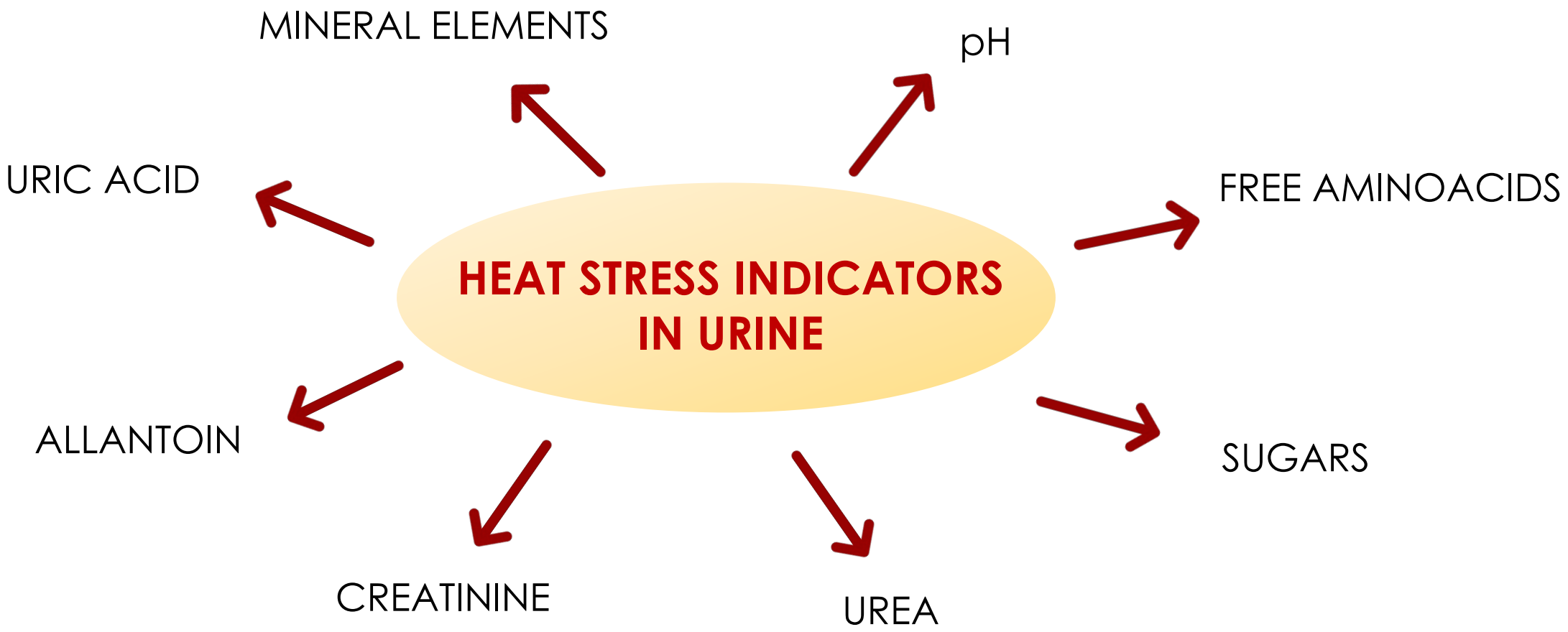


Respiratory rate and body temperature
Incidence of mastitis and ketosis

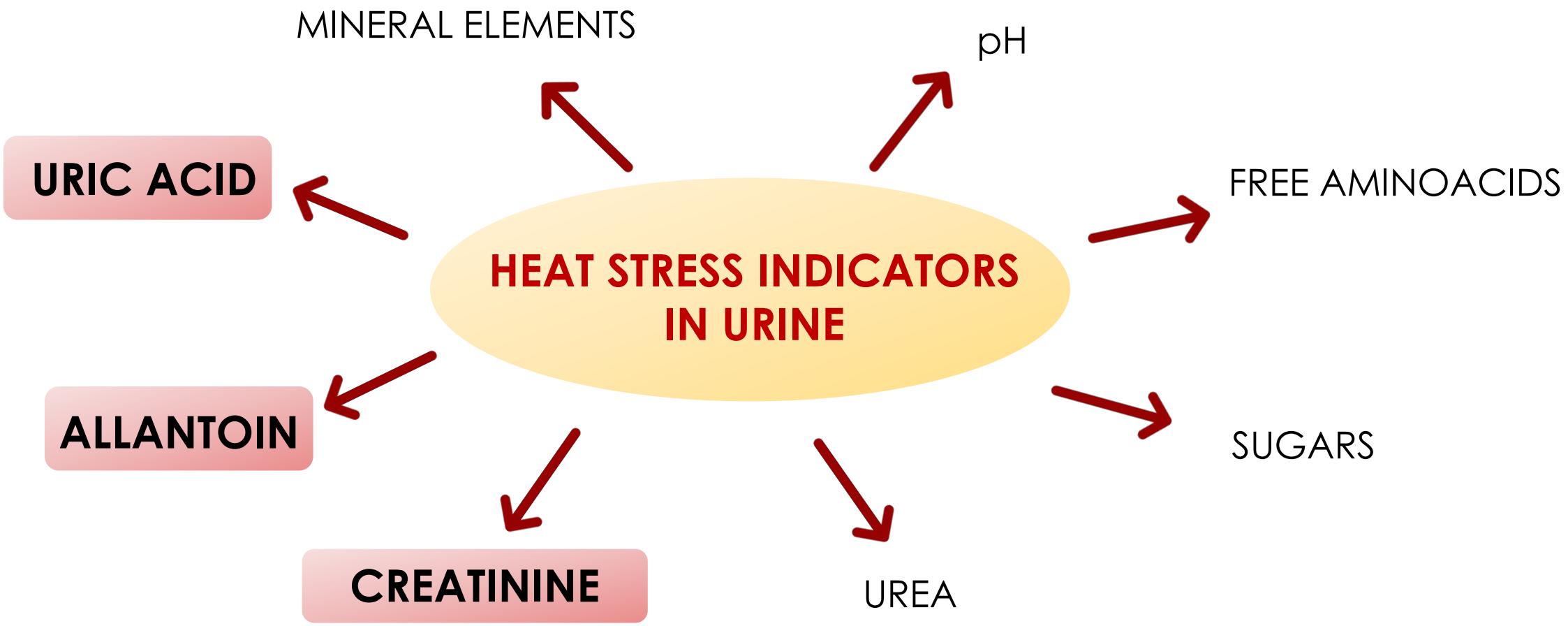
Milk production

Dehydration and electrolyte imbalances
Alteration in milk quality
Reproductive problems

INTRODUCTION



INTRODUCTION



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AIM

The aims of the present study were to:

1) Determine allantoin, creatinine and uric acid in urine samples through HPLC method.

AIM

The aims of the present study were to:

1) Determine allantoin, creatinine and uric acid in urine samples through HPLC method.

2) Assess repeatability, reproducibility and recovery of the HPLC method used to determine urine compounds related to heat stress.

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MATERIALS & METHODS

North of Italy

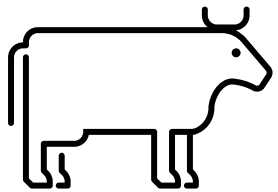
1,275 Holstein Friesian cows

21 farms → Temperature Humidity Index (THI)

From April to November 2023

Each cow was sampled once
- milk
- urine

MATERIALS & METHODS



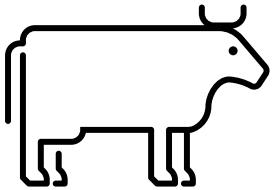
CombiFoss™ 7 (FOSS)

MILK COMPOSITION:

- ✓ Fat, protein, casein and lactose (%)
- ✓ Urea (mg/dL)
- ✓ SCC (cells/mL)

- ✓ Milk yield (kg/d)
- ✓ Days in milk
- ✓ Parity

MATERIALS & METHODS



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Reversed-phase HPLC
(Agilent Technologies)

URINE COMPOUNDS:

- ✓ Allantoin
- ✓ Creatinine
- ✓ Uric acid

} µg/mL

MATERIALS & METHODS

Urines preparation before HPLC analysis

Starting sample

1 mL of each urine sample

Centrifugation step

at room temperature for 15 min at 13,000 g

Dilution step

in the proportion 1:10 with ultrapure water

Filtration step

using 0.22 µm syringe filter

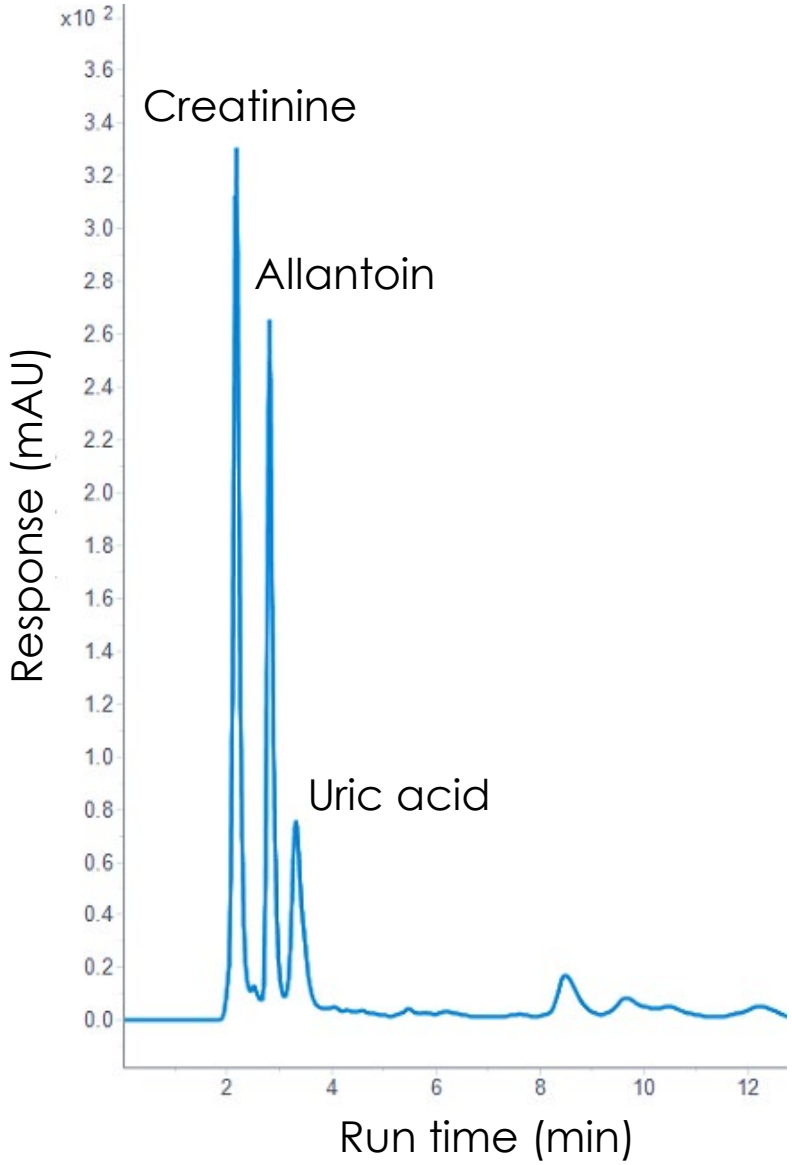
Sample in vial can be analyzed through HPLC analysis

MATERIALS & METHODS

HPLC analysis

- Instrument: Agilent 1260 Infinity II LC system
- Reverse-phase analytical column C18 preceded by a pre-column
- Linear gradient elution
- Mobile phase: 10 mM potassium dihydrogen phosphate solution
- Flow rate: 1 mL/min
- Absorbance detection at 220 nm
- Injection volume: 20 µL

Identification and **quantification** were carried out using **external standards** at the highest available purity level.



MATERIALS & METHODS

Validation of the HPLC method

- ✓ Individual urine samples from **10 cows** analyzed for **5 days**
- ✓ Single breed → Holstein Friesian
- ✓ Single experimental farm
- ✓ Average parity = 2.3
- ✓ Average days in milk = 140.3

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Allantoin
Creatinine
Uric acid

$$y_{ij} = \mu + Day_i + Cow_j + (Day \times Cow)_{ij} + e_{ij}$$

R software v. 4.3.1

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Overall intercept | Random effects | Random residual term

R software v. 4.3.1

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RESULTS & DISCUSSION

Descriptive statistics[1] of urine traits

Urine trait	Mean	SD	CV, %	Minimun	Maximum
Allantoin, µg/mL	3,071.10	1,273.87	41.48	238.20	8,385,30
Creatinine, µg/mL	505.91	209.80	41.47	42.54	1,431.84
Uric acid, µg/mL	225.05	105.27	46.78	23.59	1,071.71

[1]SD: standard deviation; CV: coefficient of variation.

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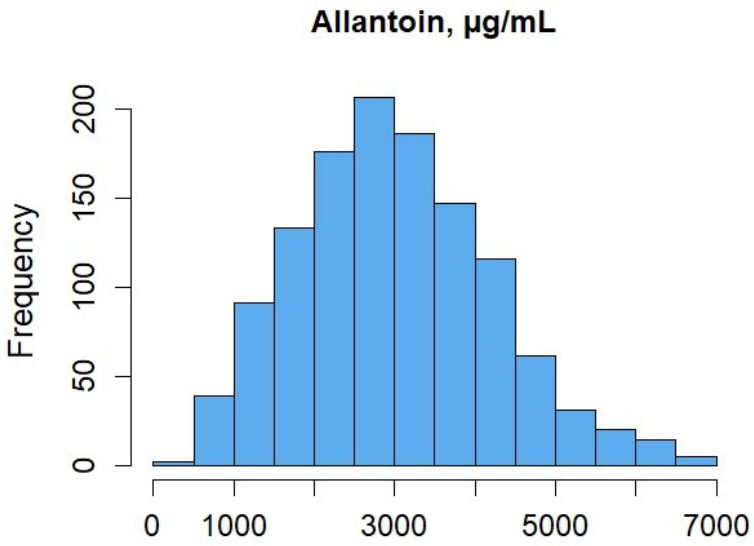
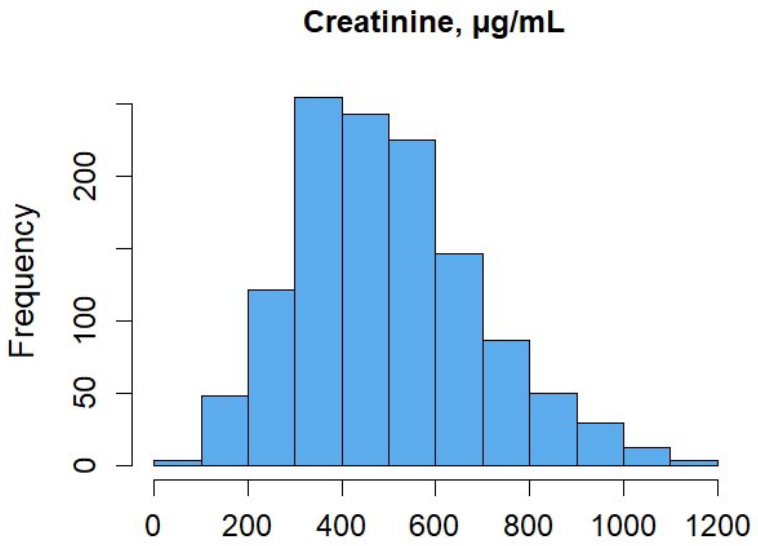
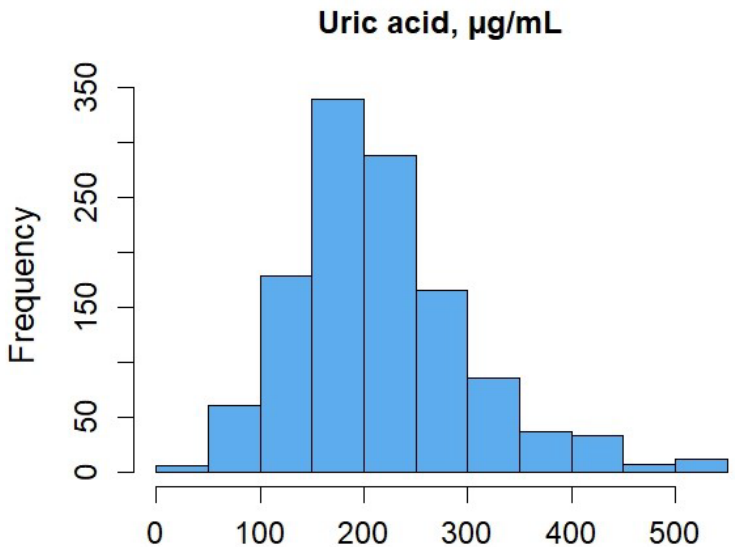
RESULTS & DISCUSSION

Distribution density plot of urine traits





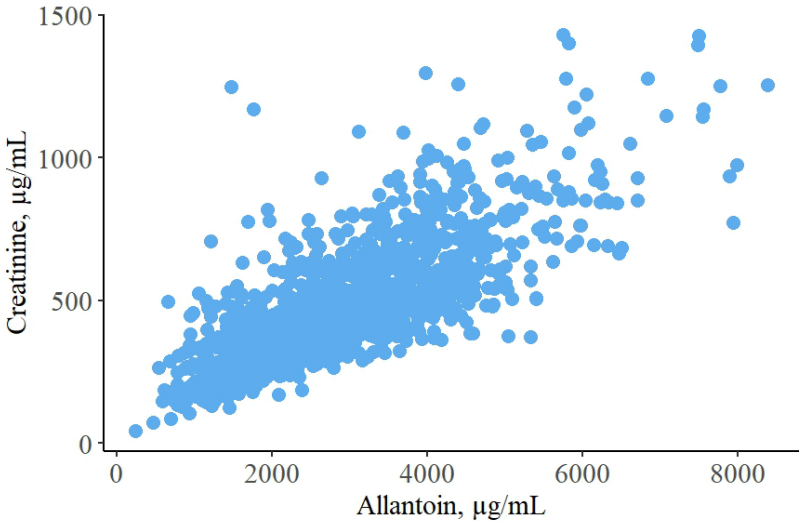


All traits are normally distributed

RESULTS & DISCUSSION

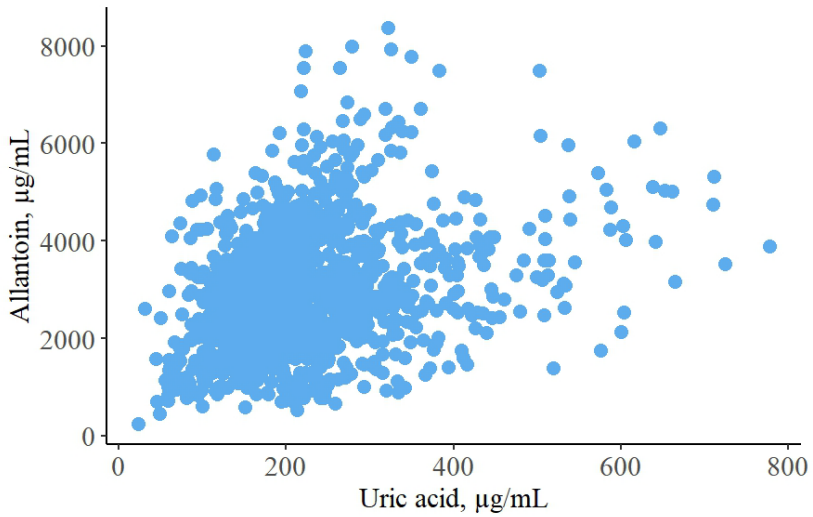
Pearson correlation coefficients between urine traits

Allantoin vs creatinine



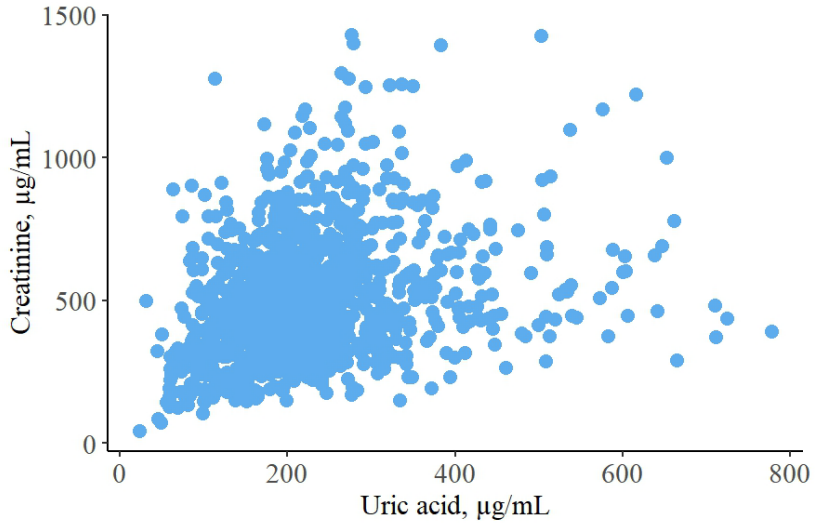
r = 0.76
***p* < 0.001**

Uric acid vs allantoin



r = 0.31
***p* < 0.001**

Uric acid vs creatinine

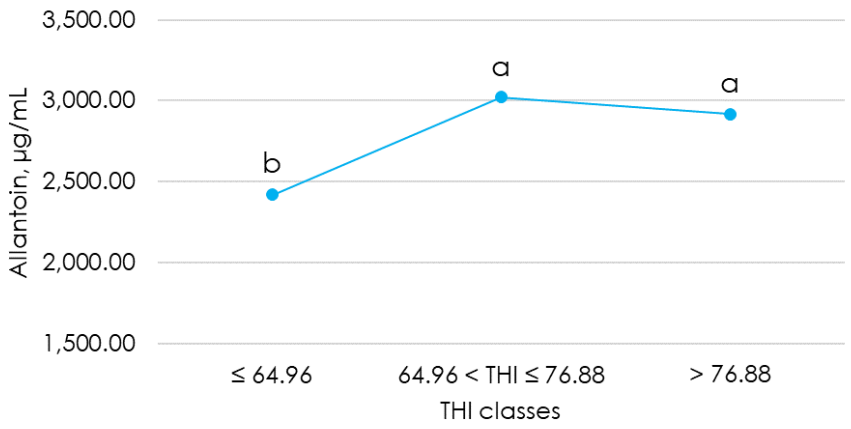


r = 0.28
***p* < 0.001**

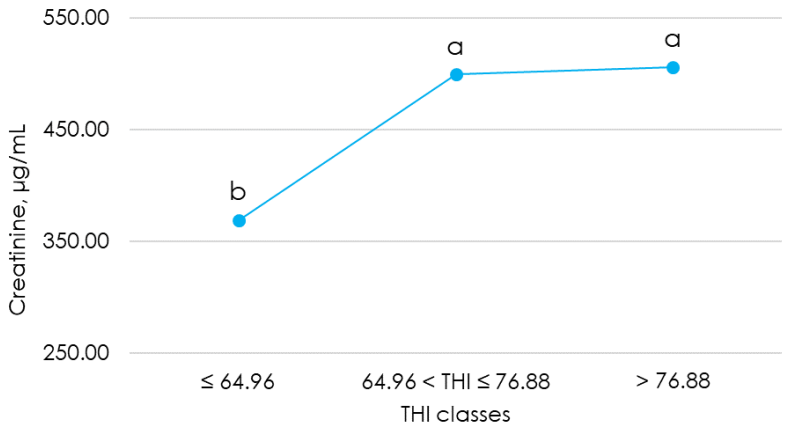
RESULTS & DISCUSSION

Effect of THI on urine traits (µg/mL)

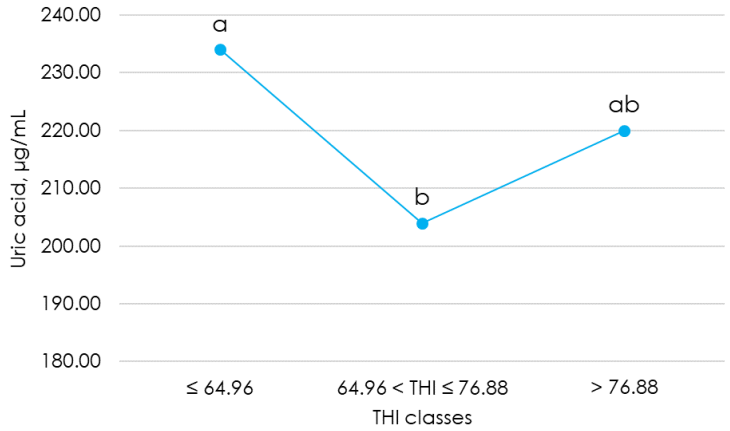
Allantoin



Creatinine



Uric acid



$$THI = (1.8 * MT) - (1.0 - MU/100) * (MT - 14.39) + 32$$

where:
MU = mean humidity
MT = mean temperature

Preliminary analysis

RESULTS & DISCUSSION

Repeatability (RT), coefficient of repeatability (RT%), day of analysis reproducibility (RD_{Day}), and day of analysis coefficient of reproducibility (RD_{Day}%) for urine traits

Urine trait	RT	RT%	RD_{Day}	RD_{Day}%
Allantoin, µg/mL	70.53	99.96	1055.04	90.86
Creatinine, µg/mL	14.91	99.94	44.80	99.39
Uric acid, µg/mL	11.73	95.86	24.34	78.03

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RESULTS & DISCUSSION

Recovery (%) and relative standard deviation of repeatability (RSD_r) for urine traits

Urine trait	Spike level 1		Spike level 2		Spike level 3	
	Recovery	RSD_r	Recovery	RSDr	Recovery	RSDr
→ Allantoin, µg/mL	104.36	0.12	106.05	1.51	102.97	1.07
Creatinine, µg/mL	97.71	0.04	98.70	0.21	96.66	0.41
→ Uric acid, µg/mL	95.61	1.67	94.18	0.09	91.12	2.85

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CONCLUSIONS

- ✓ A **simple and fast protocol** followed by a cheap HPLC method was validated for the simultaneous determination of **allantoin**, **creatinine** and **uric acid**.

- ✓ This method can be usefully adopted for large-scale studies aiming to investigate **phenotypes related to heat stress** in dairy cows.

- ✓ Results represent the first step for the development of **reliable method** capable of correlating **THI data with indicators of heat stress**.

Thank you for your attention!

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RESULTS & DISCUSSION

Repeatability (RT) and reproducibility (RD) formulas from the International Organization for Standardization (ISO)

$$RT = 1.96\sqrt{2\sigma^2{}_e}$$

$$RD_S = 1.96\sqrt{2(\sigma^2{}_{Day} + \sigma^2{}_{DayxCow})}$$

$$RT\% = \frac{\sigma^2{}_{Cow} + \sigma^2{}_{Day} + \sigma^2{}_{DayxCow}}{\sigma^2{}_{Cow} + \sigma^2{}_{Day} + \sigma^2{}_{DayxCow} + \sigma^2{}_e} \times 100$$

$$RD_S\% = \frac{\sigma^2{}_{Cow}}{\sigma^2{}_{Cow} + \sigma^2{}_{Day} + \sigma^2{}_{DayxCow} + \sigma^2{}_e} \times 100$$

ISO.1994,b