AARHUS
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# A TECHNIQUE ABLE TO QUANTIFY METABOLITES OF THE PURINE AND ALSO OF THE PYRIMIDINE METABOLISM

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# LOW NITROGEN EFFICIENCY IN DAIRY CATTLE

- Discover new ways to improve nitrogen utilization in cattle
- Is there a potential to improve nitrogen efficiency from learning more about the purine and pyrimidine metabolism?
- Microbial nucleic acids correspond to more than 20% of the total microbial nitrogen compounds in ruminants
- Large quantities of allantoin, originating from the turnover of purines in the liver, is excreted in urine
- Presumable, also pyrimidines

# NUCLEIC ACIDS IN CATTLE RESEARCH

- The significance of microbial nucleic acids in nutritional physiology of ruminants have not been of interest
- The microbial supply in ruminants can be estimated by measuring purine derivatives in the urine
- A direct relationship between microbial nucleic acids entering the small intestine, and purine derivatives found in the urine

# AIM OF PROJECT

› Examine the effects of nutrition on the nucleic acid turnover in dairy cows to improve their utilization of nitrogen and/or to find new ways to monitor their nutritional status

› Improve basic understanding of the intermediary turnover of purines and pyrimidines in cattle

› Tools:

1. A quantitative multi-catheterized cow model
2. A method able to quantify components of the purine and pyrimidine metabolism from blood samples

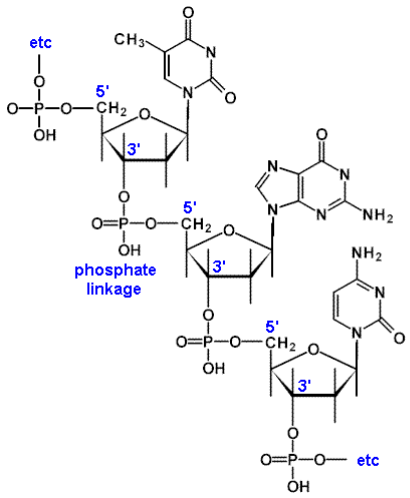
# OBJECTIVES

› Develop a method for determination of the quantitative absorption, turnover and excretion of metabolites of the purine/pyrimidine metabolism

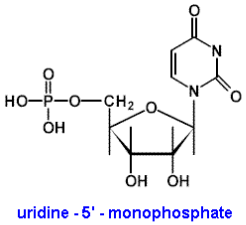
› Rapid, sensitive, specific and reliable

# WHICH COMPONENTS

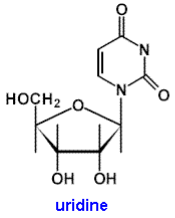
Target: Nucleotides, Nucelosides, bases and degradation products



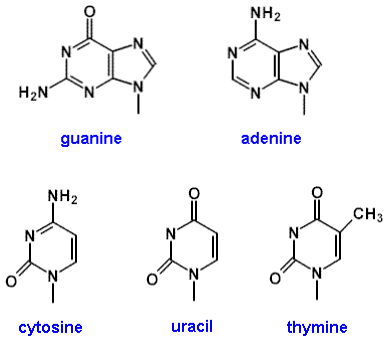

Nucleic acids
DNA/RNA




Nucleotide
(unstable)




Nucleoside




Base

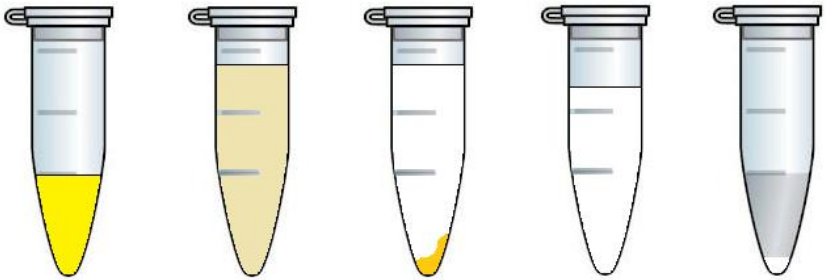
Degradation
products

# WHICH COMPONENTS

- **Bases**: Adenine, Guanine, Cytosine, Thymine, Uracil, Xanthine, Hypoxanthine
- **Nucleosides**: Adenosine, Guanosine, Cytidine, Uridine, Inosine, 2-Deoxyadenosine, 2-Deoxyguanosine, 2-Deoxycytidine, Thymidine, 2-Deoxyuridine, 2-Deoxyinosine
- **Nucleotides**: Adenylate (AMP), Guanylate (GMP), Cytidylate (CMP), Uridylate (UMP), 2-Deoxyadenylate (dAMP), 2-Deoxyguanylate (dGMP), 2-Deoxycytidylate (dCMP), Thymidylate (TMP or dTMP)
- **Purine degradation products**: Uric acid, Allantoin
- **Pyrimidine degradation products**: β-alanine, β-ureidopropionic acid, β-aminoisobutyric acid, β-ureidoisobutyric acid (not analysed)

# METHOD (PRE-TREATMENT)

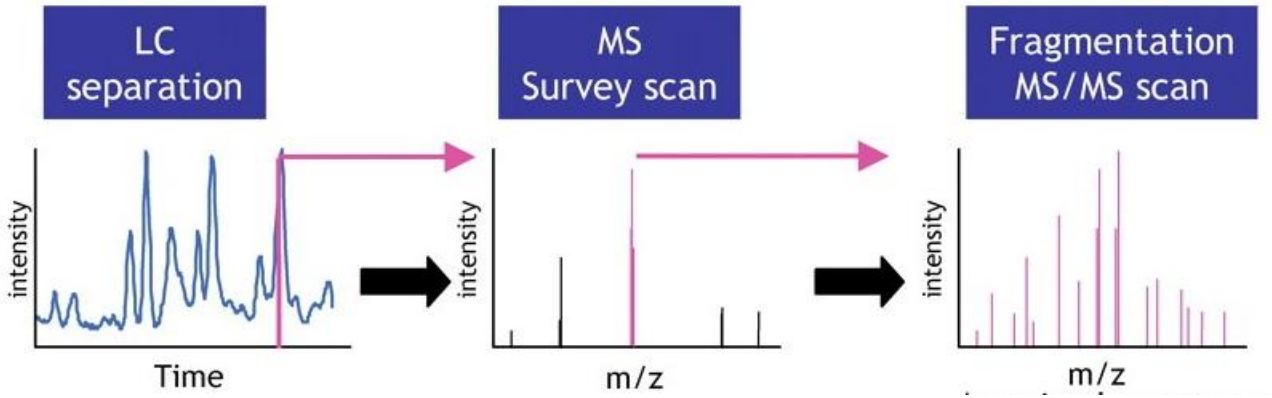
› Component specific pre-treatment - simple and repeatable
› Ethanol precipitation, filtration and evaporation/re-solution



› Stability


# METHOD (LC-MS/MS)

› A HPLC electrospray ionization tandem mass spectrometry-based technique (LC-MS/MS)



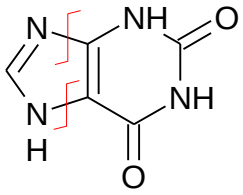

› LC-MS/MS – quantitative analysis of compounds in biological matrices (specificity and sensitivity)

› Water, plasma (blood) and urine

# METHOD (STANDARDS AND MATRIX EFFECTS)

› Calibration standards and isotope-labeled internal standards

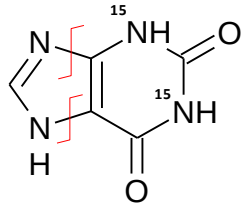
› Distinguish between the components and quantify these individually - easy and reliable



**Xanthine (NL 43)**
**Parent: 151 *m/z***
**Daughter: 108 *m/z***

Structur
the same

Weight
different



**Xanthine* (NL 43)**
**Parent: 153 *m/z***
**Daughter: 110 *m/z***

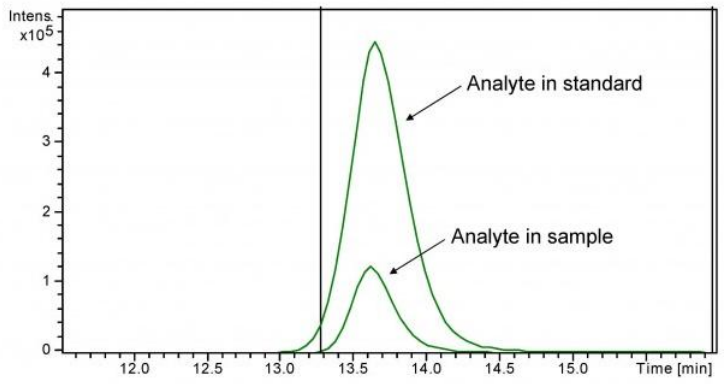
› Correct for day to day variations in pre-treatment as well as instrument variations

# METHOD (STANDARDS AND MATRIX EFFECTS)

› Matrix effects – molecules from the sample can interfere with the ionization process - cause ion suppresion or enhancement

› Matrices: water, plasma and urine

› Ex. Signal in water from 2 µmol/L Xanthine
›  Signal in plasma from 2 µmol/L Xanthine




> Standard curves made in the same matrix as the sample

Matrix effects: Concentration is the same but peak areas are very different

# RESULTS

- Quantification & Validation - 23 purine and pyrimidine bases, nucleosides and degradation products - a single run

- **Bases**: **Adenine**, **Guanine**, **Cytosine**, **Thymine**, **Uracil**, Xanthine, Hypoxanthine

- **Nucleosides**: Adenosine, Guanosine, Cytidine, Uridine, Inosine, 2-Deoxyadenosine, 2-Deoxyguanosine, 2-Deoxycytidine, Thymidine, 2-Deoxyuridine, 2-Deoxyinosine

- **Nucleotides**: Adenylate (AMP), Guanylate (GMP), Cytidylate (CMP), Uridylate (UMP), 2-Deoxyadenylate (dAMP), 2-Deoxyguanylate (dGMP), 2-Deoxycytidylate (dCMP), Thymidylate (TMP or dTMP)

- **Purine degradation products**: Uric acid, Allantoin

- **Pyrimidine degradation products**: β-alanine, β-ureidopropionic acid, β-aminoisobutyric acid, β-ureidoisobutyric acid (not analysed)

# PRESENCE IN PLASMA AND URINE

- QUALITATIVE

## Blood plasma

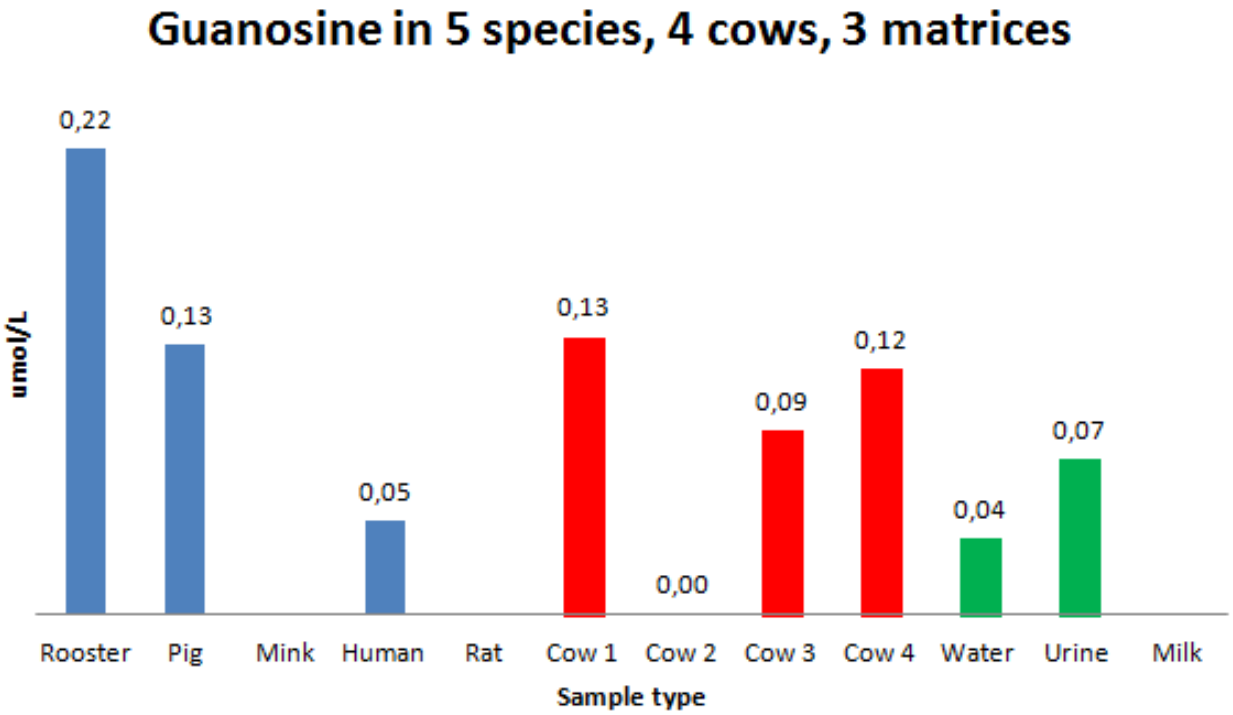
Uric acid
+
Nucleosides


## Urine

Allantoin & uric acid
+
Bases
+
Pyrimidine Degradation products

# GUANOSINE IN DIFFERENT MATRICES

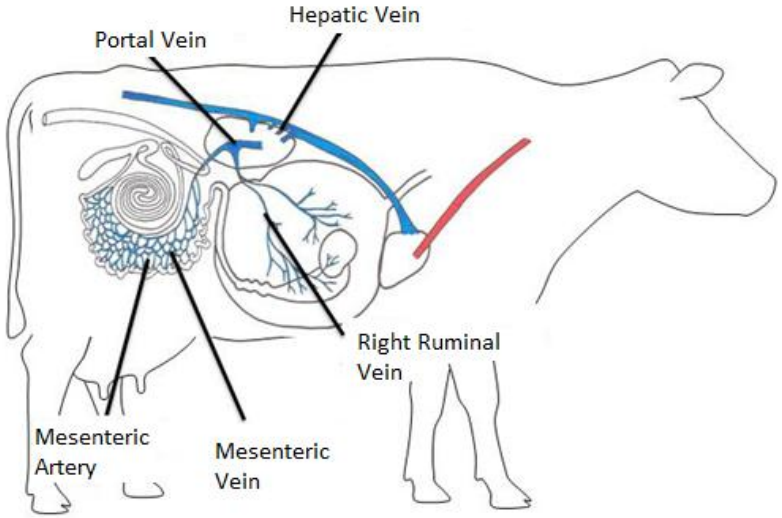
- AN EXAMPLE



# SPLANCHNIC CATHETERIZED COW MODEL

› Holstein cows at different lactation stages fitted with ruminal cannulas and permanent indwelling catheters in major splanchnic blood vessels

› Quantitative model – bloodflow

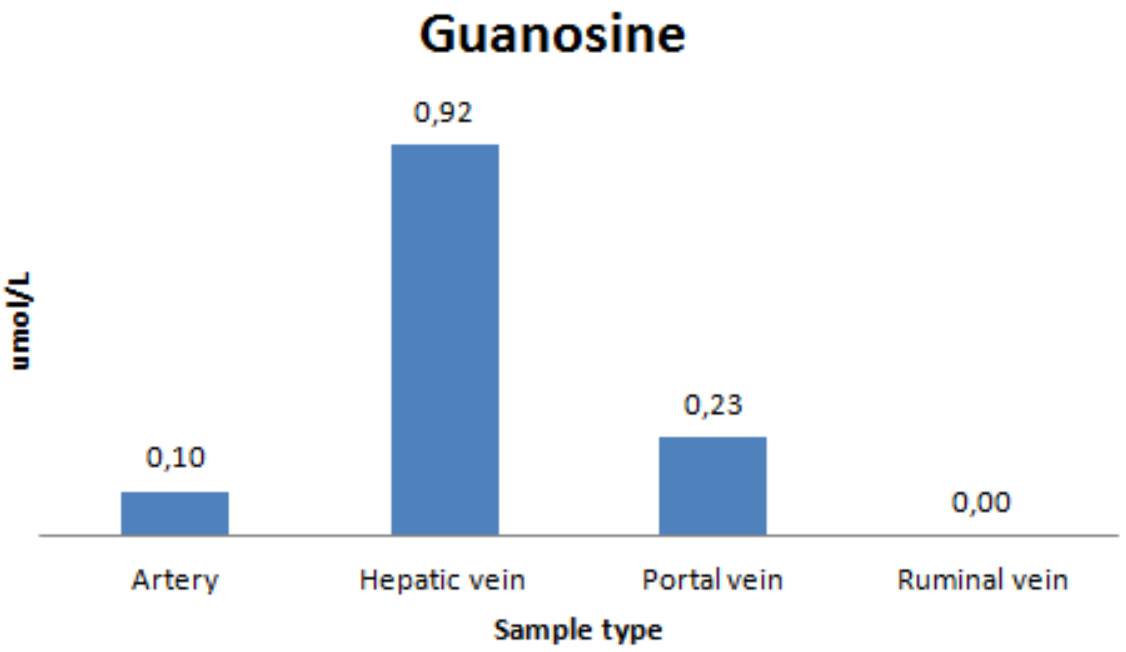



› Artery
› Portal vein (digestive tract)
› Hepatic vein (liver)
› Ruminal vein (rumen uptake)

> Milk
> Urine
> Rumen fluid

# TURNOVER IN A CATHETERIZED COW MODEL

- AN EXAMPLE



# CONCLUSION AND PERSPECTIVES

- Quantify components not only from the purine metabolism but also the pyrimidine metabolism in blood samples
- Validation (accesment of matrix effects, LOD, LLOQ, intra- og interday variations, ruggedness, recovery etc.)
- Start analysing samples and examine the intermediary turnover of purines and pyrimidines in dairy cattle
- Possible further develop the method to include other matrices – urine and milk