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# ABSORPTION AND INTERMEDIARY METABOLISM OF PURINES AND PYRIMIDINES IN LACTATING DAIRY COWS

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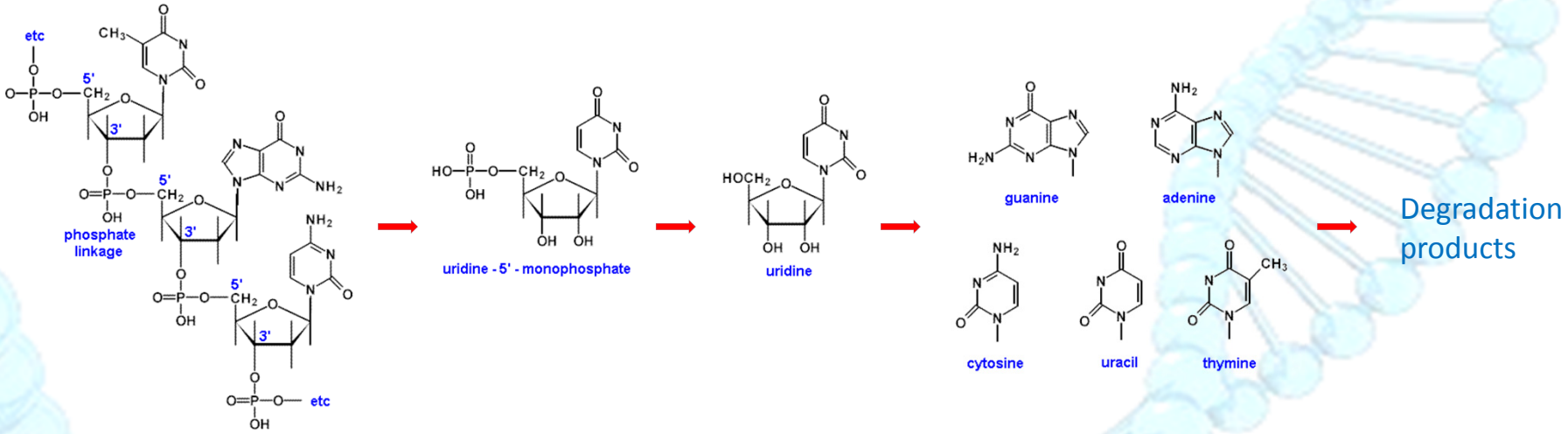
# LOW NITROGEN EFFICIENCY IN DAIRY CATTLE

- Nitrogen efficiency is low - 15-40%
- Consequences: economy and environment
- Purin and pyrimidine metabolism (nucleic acids)
- Microbial nucleic acids (DNA/RNA) amount to more than 20% of the total microbial nitrogen in ruminants
- We know: Uric acid and allantoin is excreted in urine
  Indirect marker of rumen microbial biosynthesis

# NUCLEIC ACID DEGRADATION




Nucleic acids → Nucleotide → Nucleoside → Bases

**Rumen:** Dietary nitrogen → microbial protein (75%) / microbial nucleic acids (25%)

**Small intestine:** microbial nucleic acids are digested and absorbed (NS, BS, DP)

# OBJECTIVE

- Improve utilisation of nitrogen
- Increase basic understanding of the intermediary turnover
- Purine and pyrimidine metabolites
  - ➢ Absorption from intestine
  - ➢ Hepatic metabolism
  - ➢ Overall splanchnic metabolism

# HYPOTHESIS

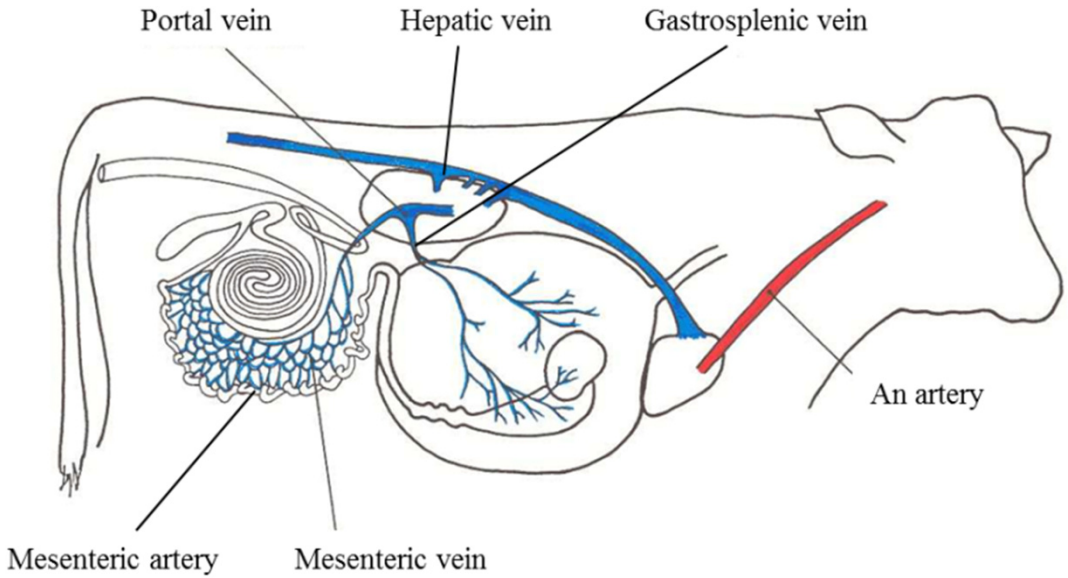
1. Purine and pyrimidine: absorbed from small intestine and undergo degradation across the intestinal wall and the hepatic tissue as nucleoside, base or/and degradation product
2. Purine and pyrimidine nitrogen is largely lost following degradation and product excretion

Tools:

- A quantitative multicatheterised cow model
- Developed and validated a LC-ESI-MS/MS analysis

# THE MULTICATHETERISED COW MODEL

- To investigate the inter-organ net fluxes

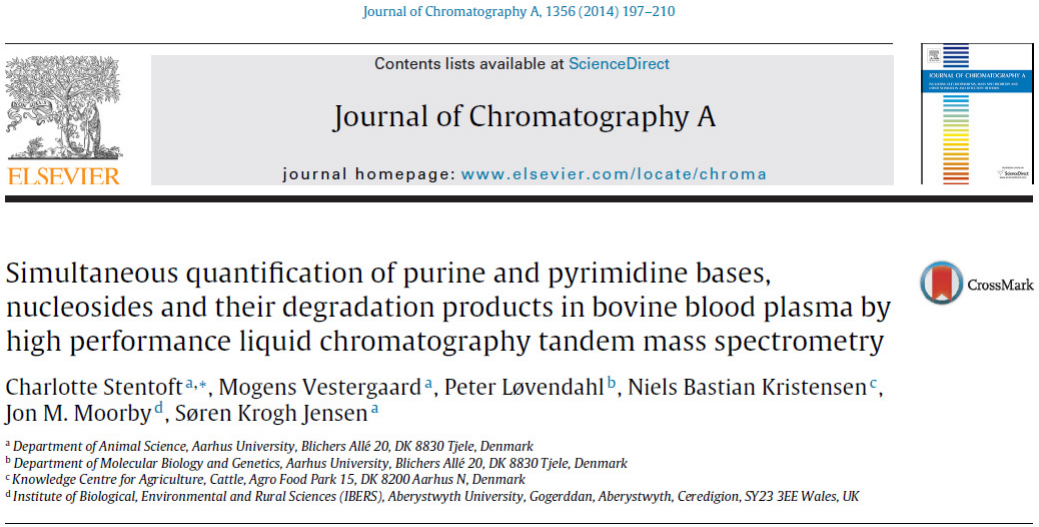



- Fitted with ruminal cannulas and indwelling catheters in splanchnic blood vessels
- Blood samples were obtained to calculate net fluxes (net uptake or net release)
- Positive net flux = release; negative net flux = up-take

# ANALYSIS

- Simultaneous quantification of 20 purine and pyrimidine bases, nucleosides and their degradation products in bovine blood plasma

Stentoft, C., M. Vestergaard, P. Lovendahl, N. B. Kristensen, J. M. Moorby, and S. K. Jensen. 2014. J Chromatogr A 1356:197-210.



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Simultaneous quantification of purine and pyrimidine bases, nucleosides and their degradation products in bovine blood plasma by high performance liquid chromatography tandem mass spectrometry

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# EXPERIMENT

## Animals:

- Four multicatheterized Holstein cows
- Basal TMR(CP: 126 g/kg DM) with a total protein content of 150 g/kg DM

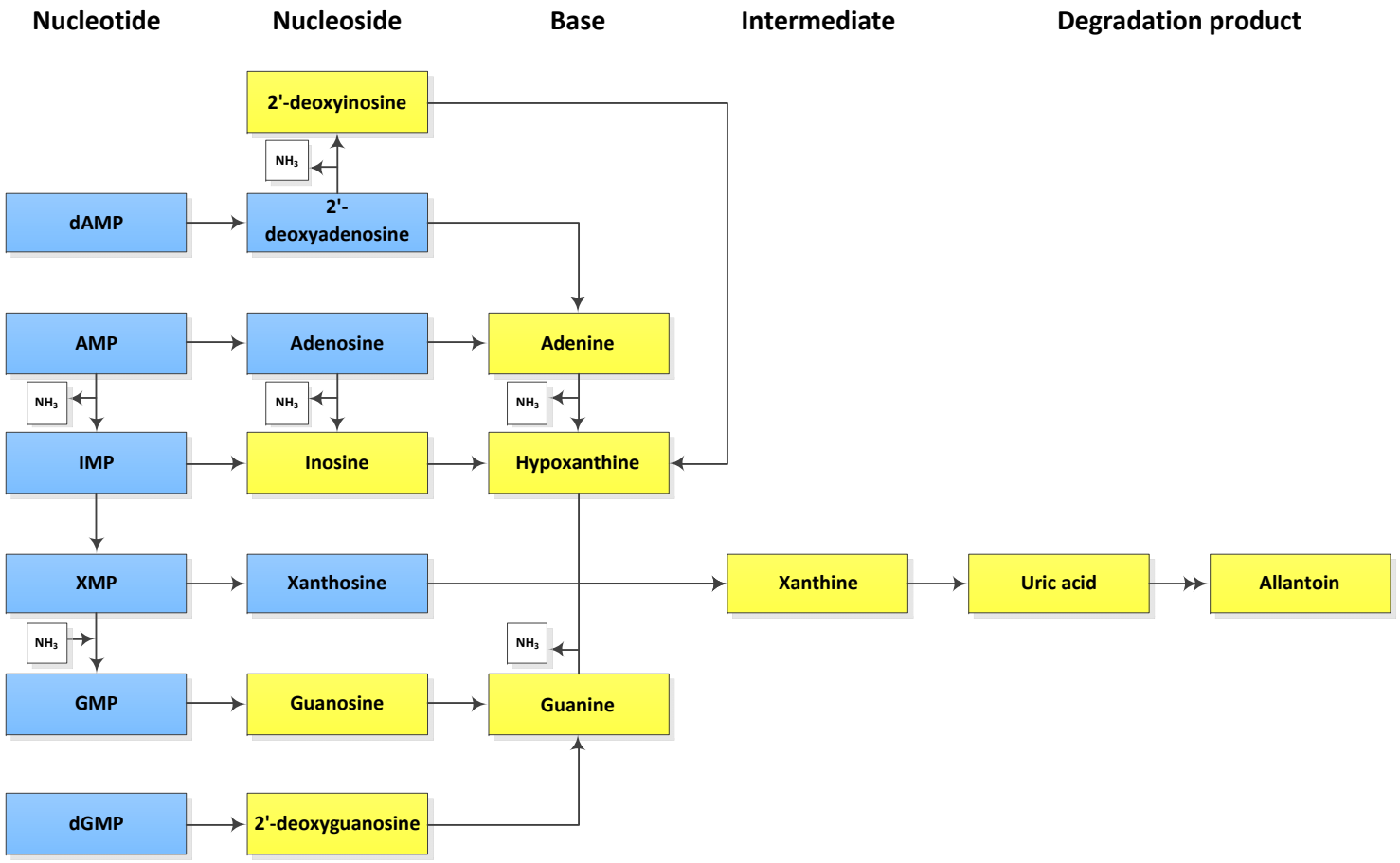
## Samples:

- Arterial, hepatic portal, hepatic and gastrosplenic vein blood (plasma)
- Eight hourly samplings, beginning ½ h before feeding

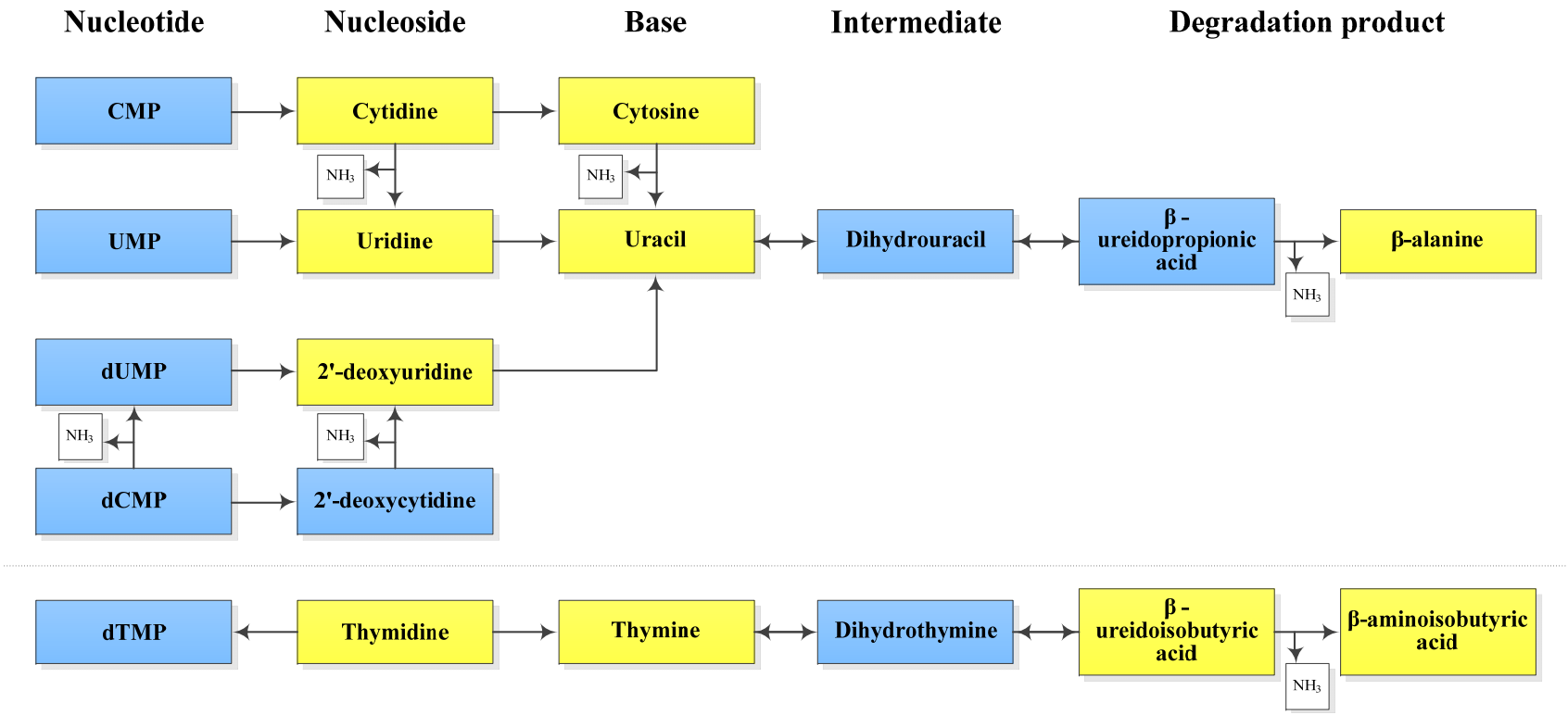
## Examine:

- Plasma concentrations
- Net portal-drained viscera flux (absorption), metabolite types and levels
- Net hepatic flux (liver metabolism)
- Net total splanchnic flux (overall)

# PURINE METABOLISM

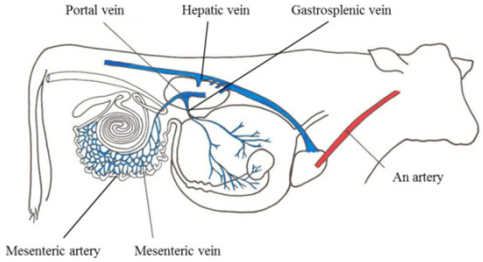


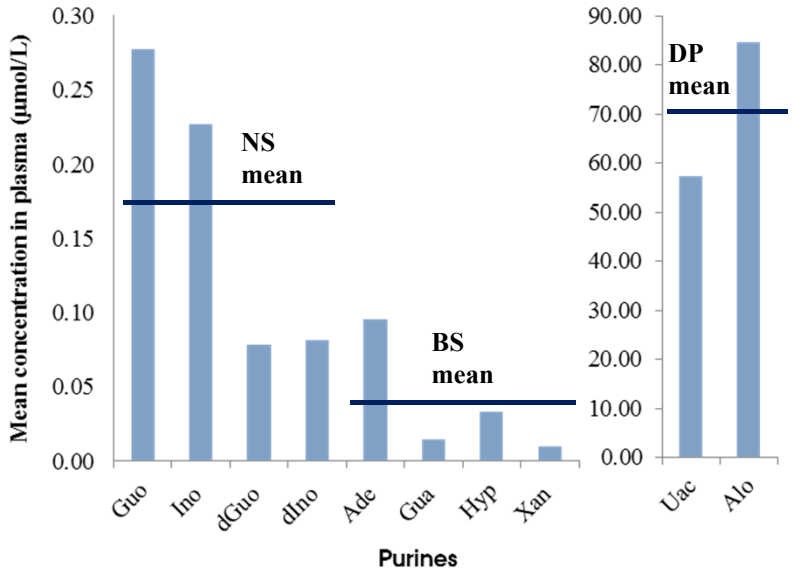
# PYRIMIDINE METABOLISM

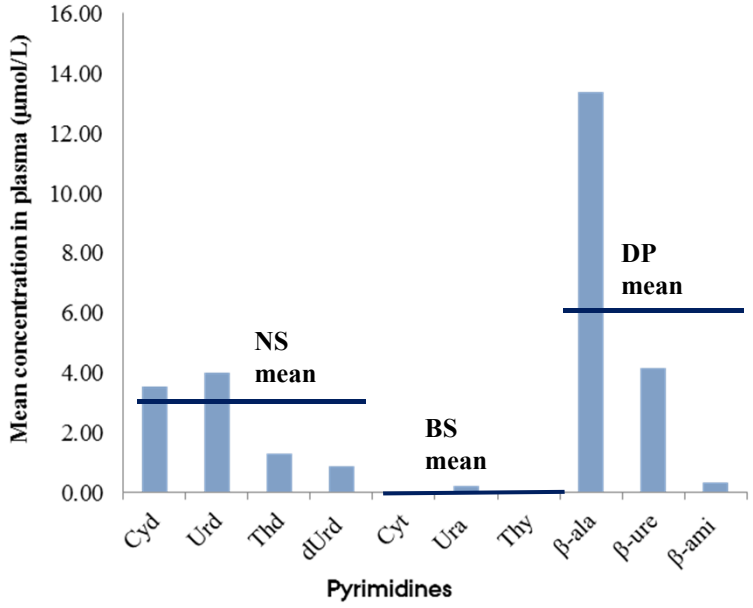


# RESULTS: PLASMA CONCENTRATIONS

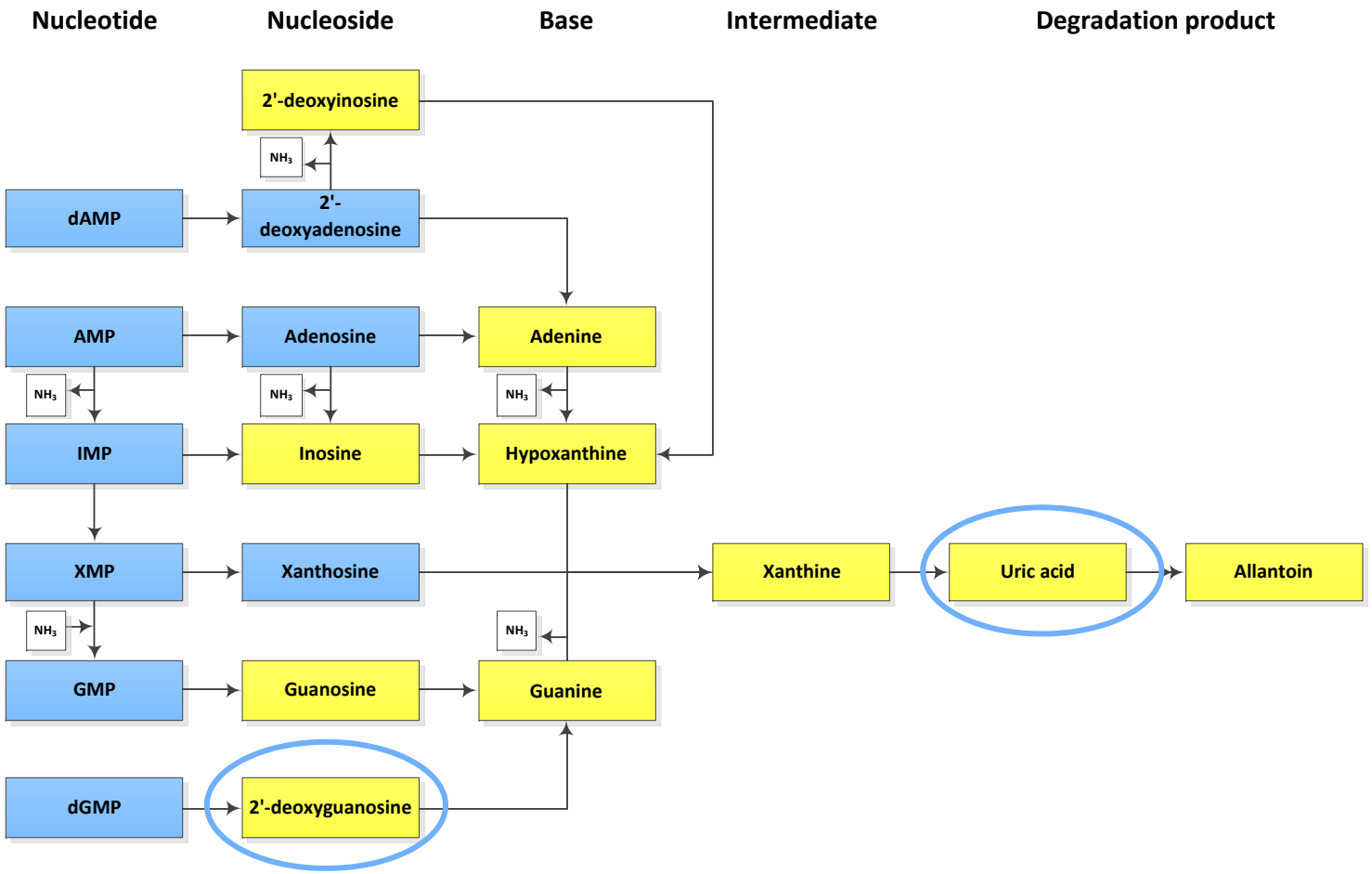
- All 20 metabolites indentified in all four types of plasma



# RESULTS: SPLANCHNIC FLUXES OF PURINES



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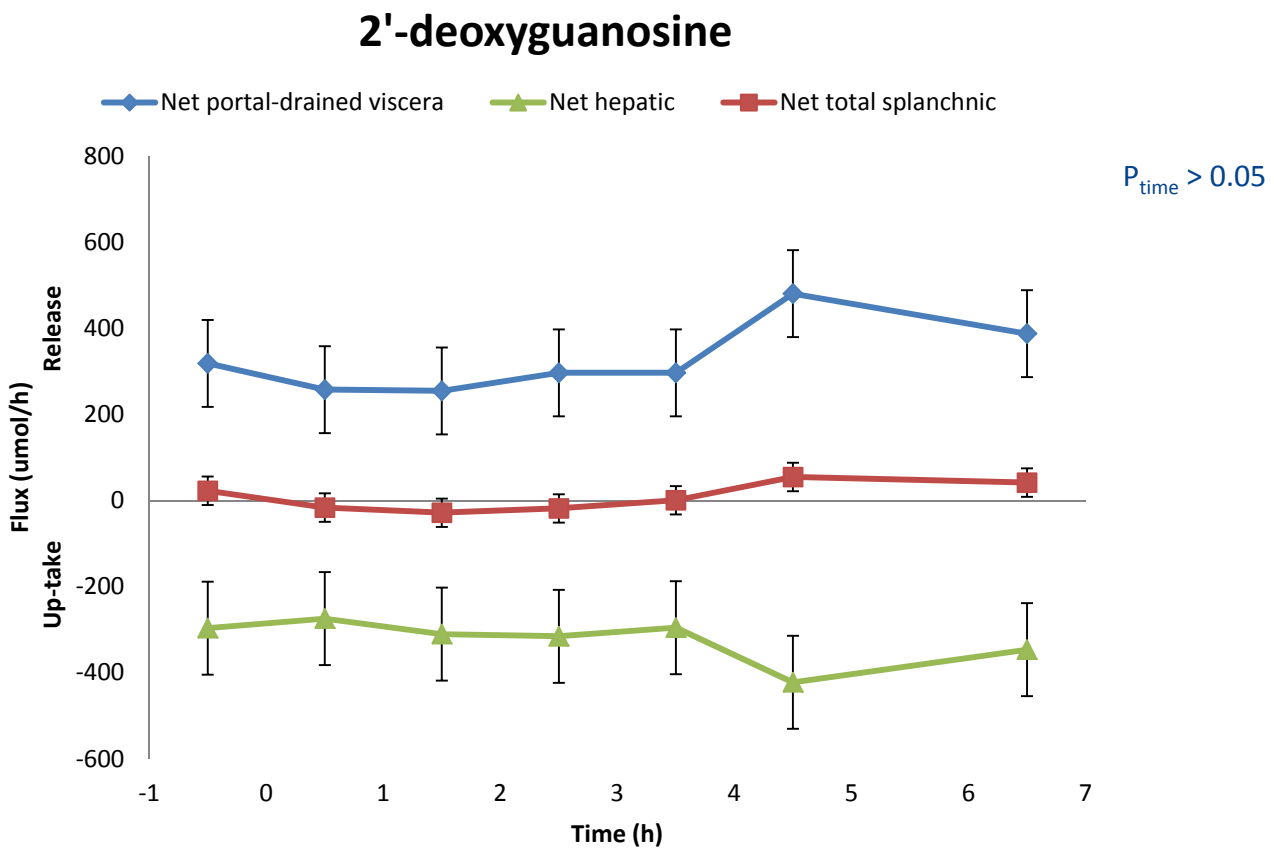


$P_{time} > 0.05$

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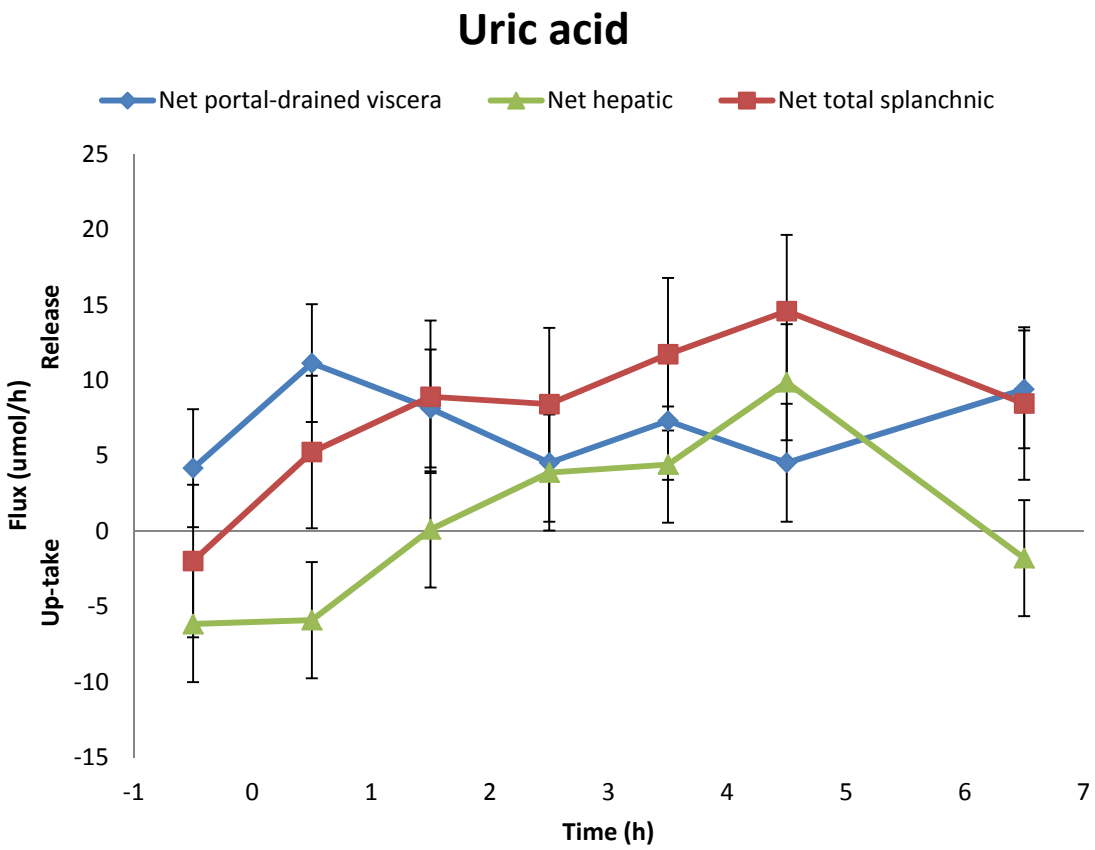


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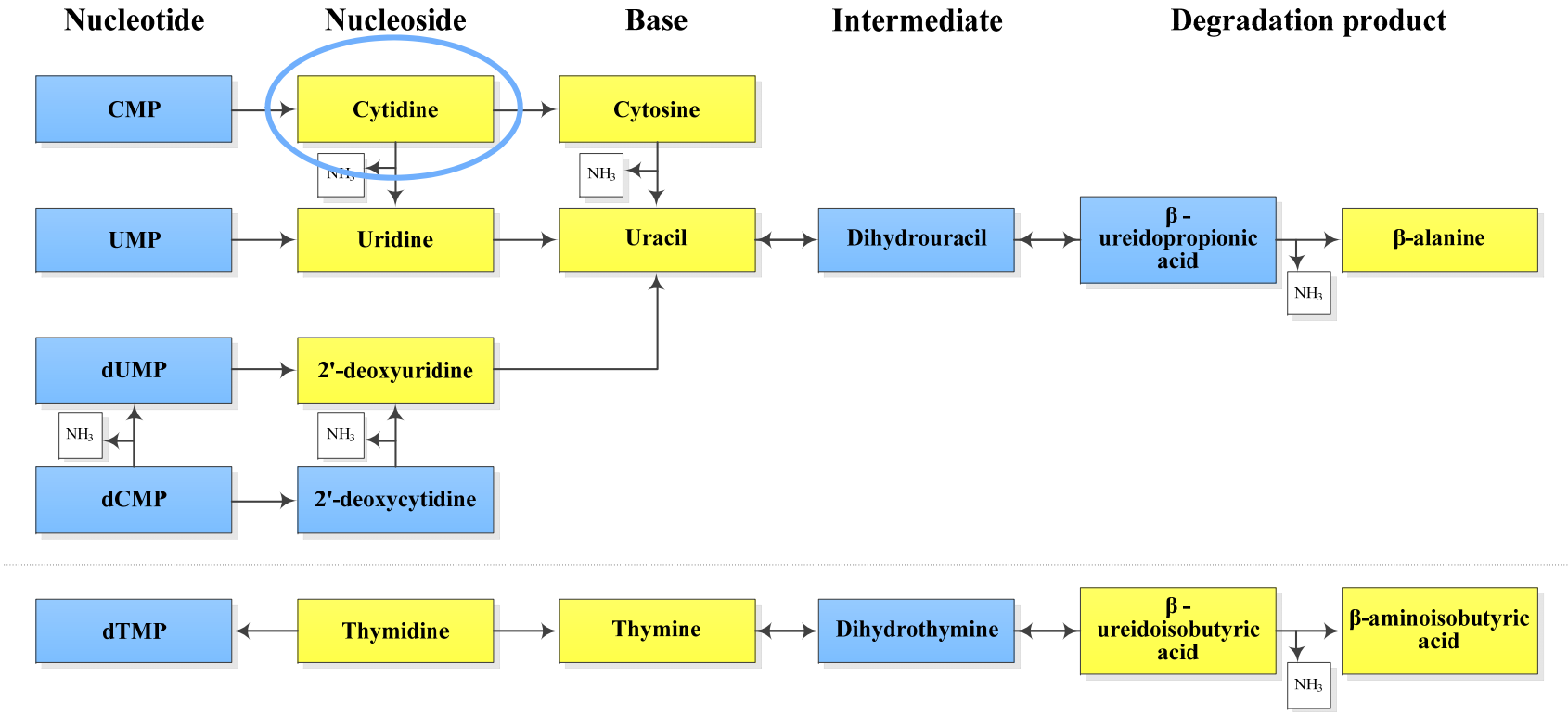
# RESULTS: SPLANCHNIC FLUXES OF PYRIMIDINES



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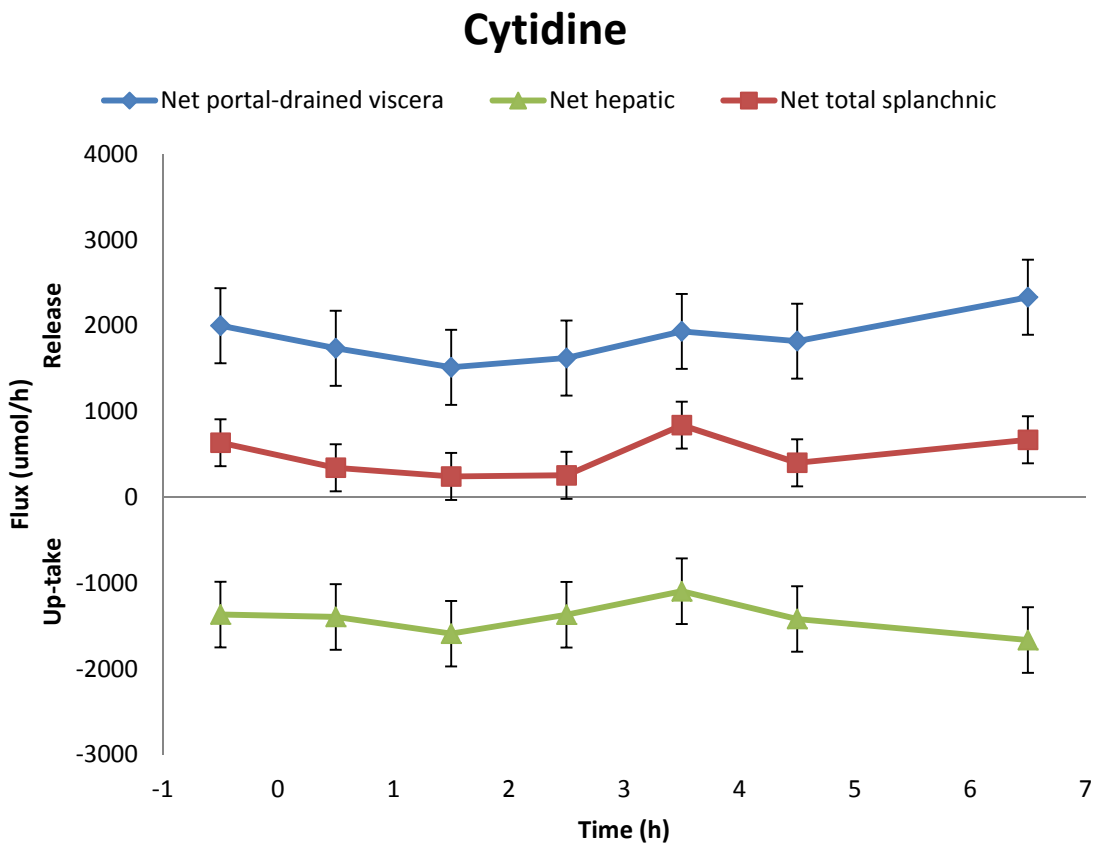
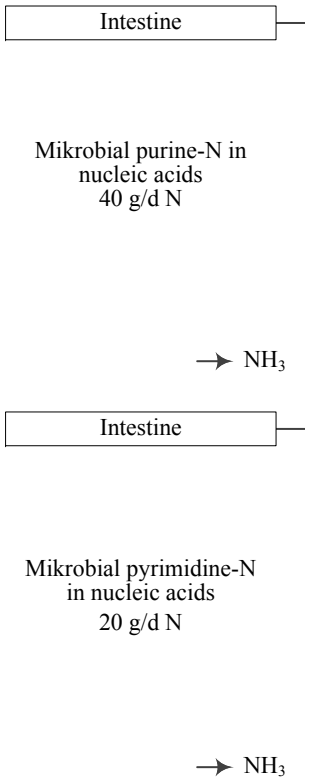


# RESULTS: PURINE AND PYRIMIDINE NITROGEN CONTRIBUTION

## - Splanchnic fluxes of nitrogen



# CONCLUSION

- The metabolic processes was different

## Absorption

- Purine: Mainly; DP and minor; NS and BS
- Pyrimidine: Mainly; NS and BS and minor; DP

## Degradation

- Purine: NS and BS fully degraded to DP
- Pyrimidine: Partly degraded, some release of NS and DP

## Excretion

- Purine: DP (lost)
- Pyrimidine: Unknown. However outlet into N-metabolism (recycled)

# SCIENTIFIC PERSPECTIVES

## Examine the effect of i.e. protein level and roughage type

- Varying rumen microbial biosynthesis
- Reveal if it is possible to manipulate or use this system
- Optimising and making more efficient the utilisation of purine and pyrimidine nitogen in ruminants