Methodologies Developped in the Dep. of Animal Production and Food Science Technology

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Introduction

Microbiology: - DGGE

- 16S rDNA sequencing

Caecotrophy: - Urinary PD

- ^{15}N: AA (Lys and Thr)

Protein Turnover: - Synthesis : Phenylalanine

- Degradation : 3-Methylhistidine

Objective: Manipulation of caecal ecosystem

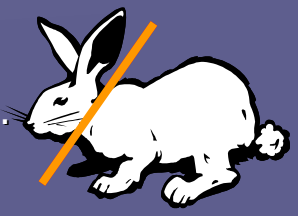
1) Type of food (structural vs non structural carbohydrates)

2) Voluntary Food intake (litter size)

3) Antibiotics

Caecotrophy

1) Wooden neck collar: Disturbs normal feeding behaviour.

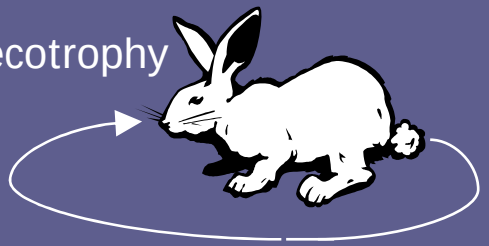


2) Purine derivatives: Dietary PB, Microbial PB → PB/N → MICROBIAL N

Duodenal PB → Absorption → Urinary PD

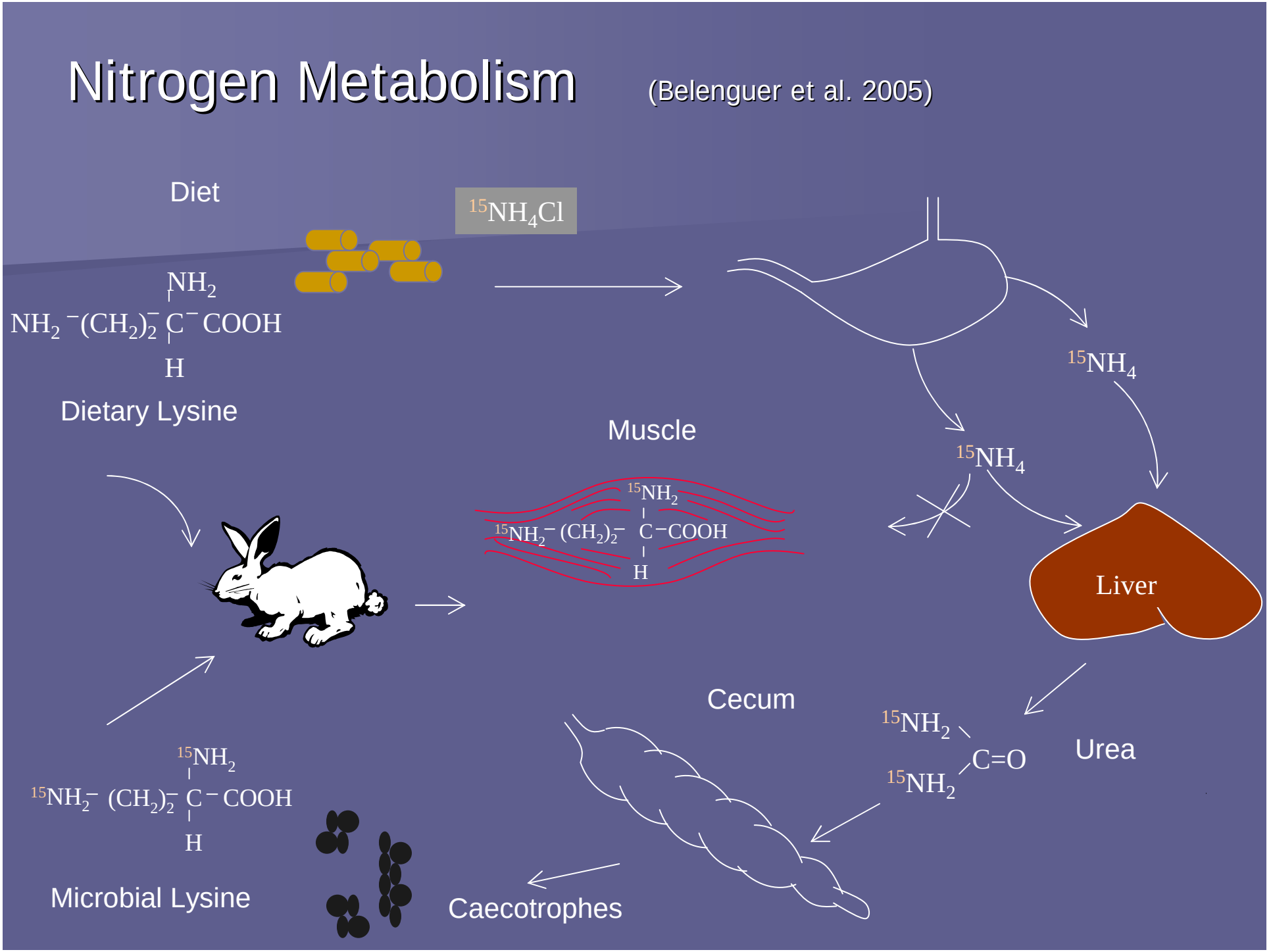
3) Isotopic technique:

$^{15}NH_4Cl$ (diet) → Caecal bacteria → Caecotrophy



^{15}N enrichment in tissues (muscle)

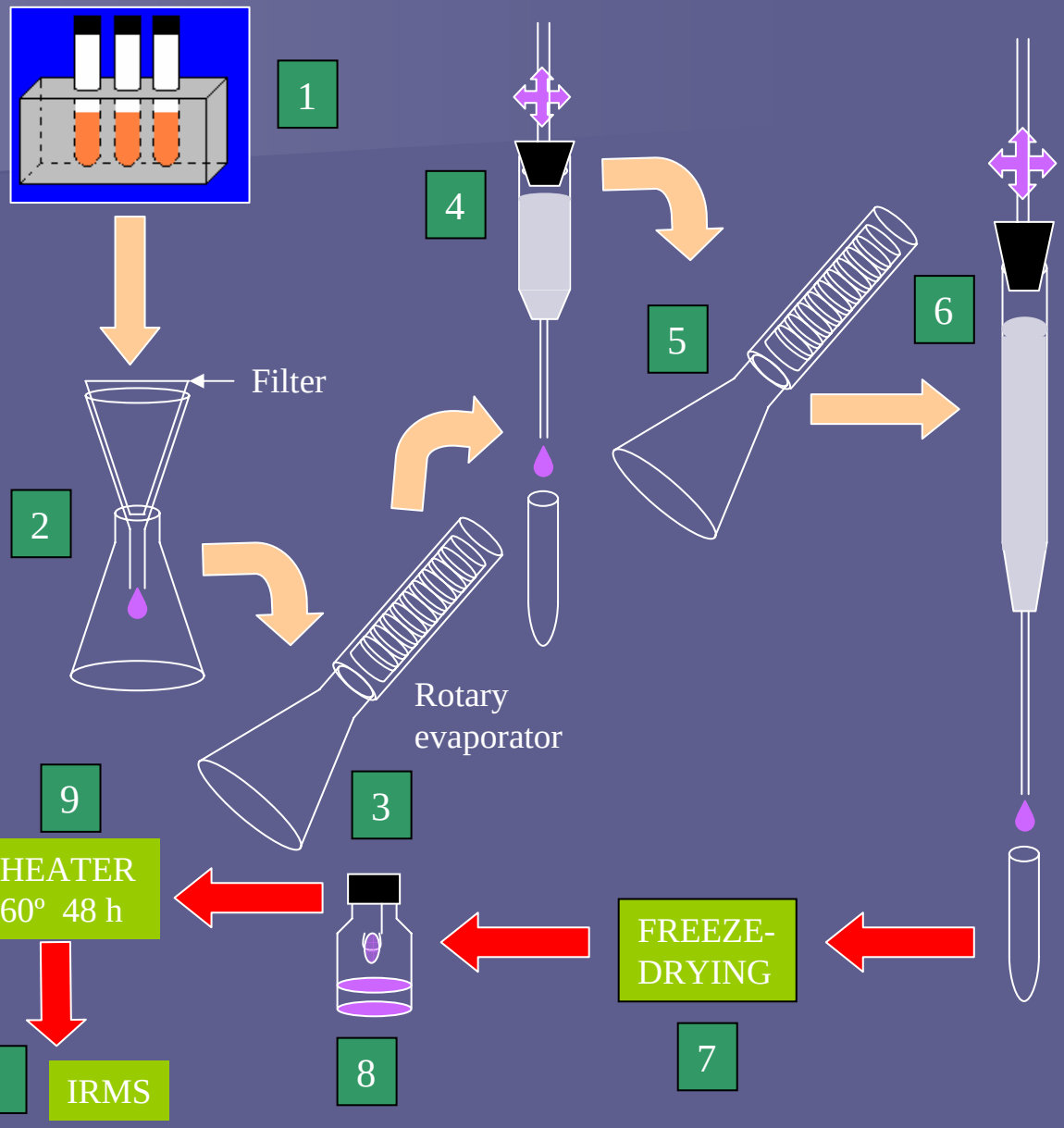
Nitrogen Metabolism (Belenguer et al. 2005)



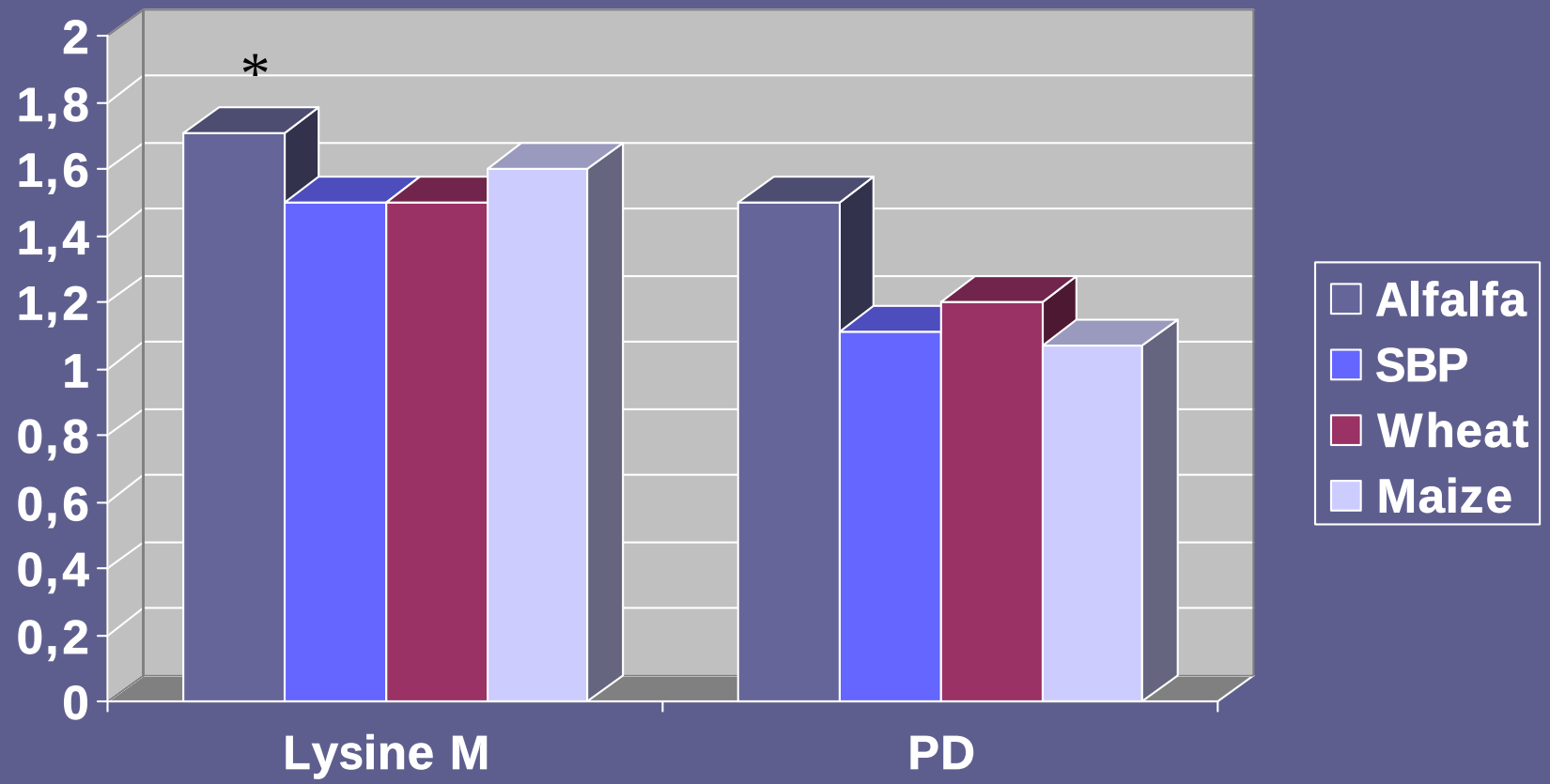
Chemical Analysis

ION-EXCHANGE CHROMATOGRAPHY

1) Hydrolisis
2) Filter and dryness by rotary evaporator
3) Add NaOH to increase pH and eliminate NH3 while drying again
4) Precolumn: small column to wash the sample
5) Dry in rotary evaporator and adjust pH with buffer
6) Column: ion-exchange chromatography. Change pH. Isolation of lysine in some tubes.
7) Freeze and freeze-dry the sample.
8) Isolation of N-lysine: digestion with H2SO4, addition of NaOH, hermetically closed bottle for three days.
9) Dry the paper.
10) IRMS



Effect of different cereal (*wheat vs maize*) and fibre (*alfalfa vs sbp*) source on N recycling in growing rabbits



Caecotrophy: Contribution of Lys

Diet: 18.89% PB

Intake LL:230g DM/d – 41.4 g PB/d

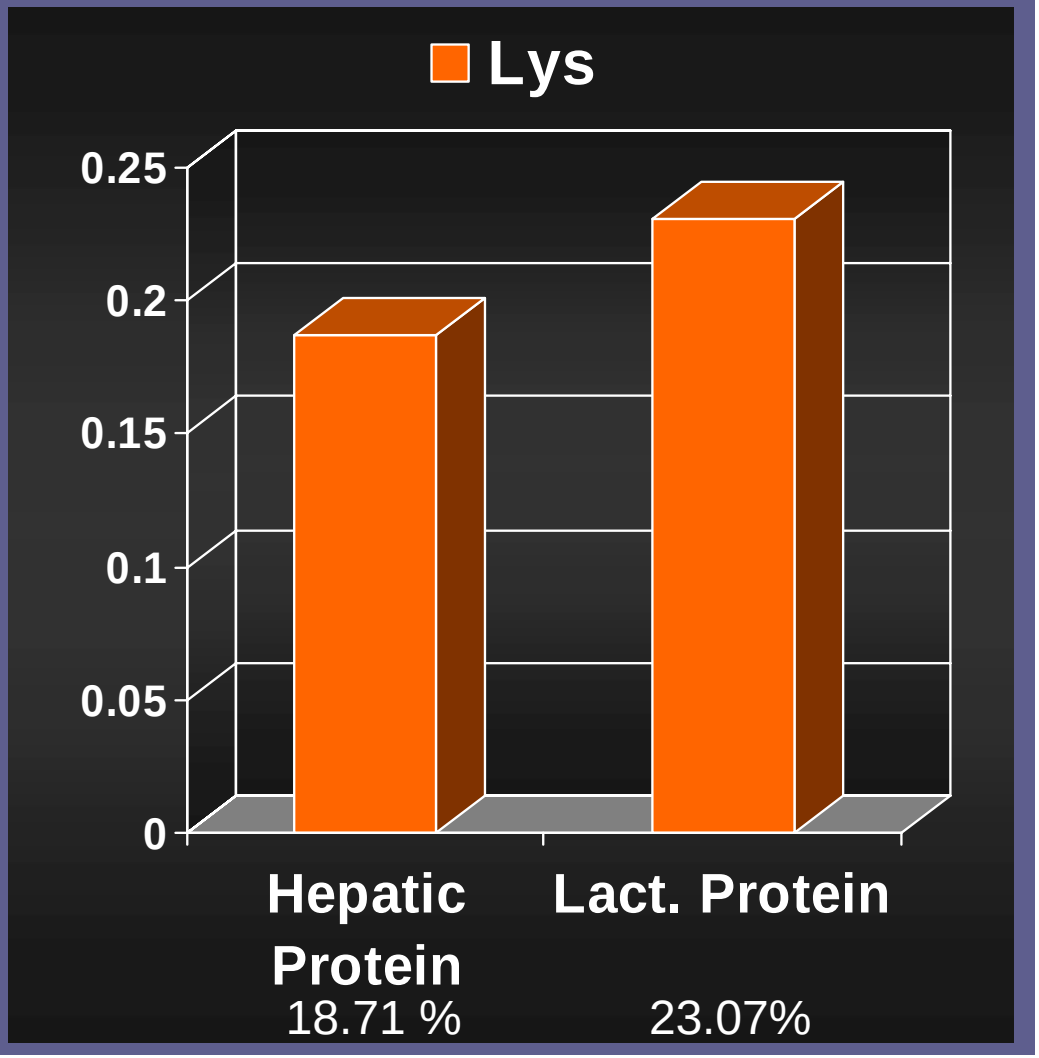
HL:260 gDM/d – 49.1 g PB/d

Caecotrophes: 28.4 % PB

LL: 9.52 g /.28= 34 g caecotrophes

HL: 11.29 g/ .28= 40.32 g caecotrophes

Lactating does



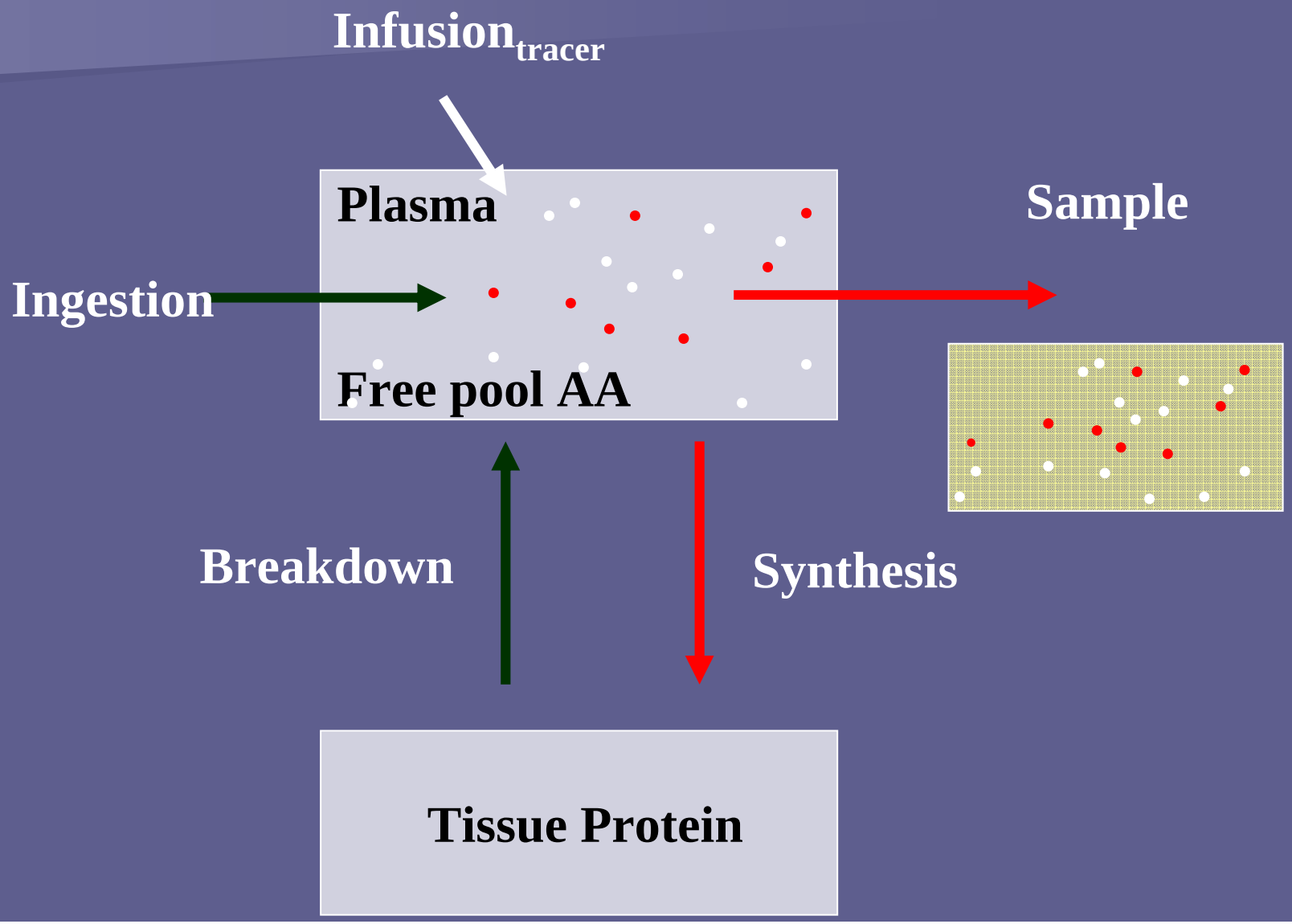
Protein turnover

- Synthesis : Flooding dose Method

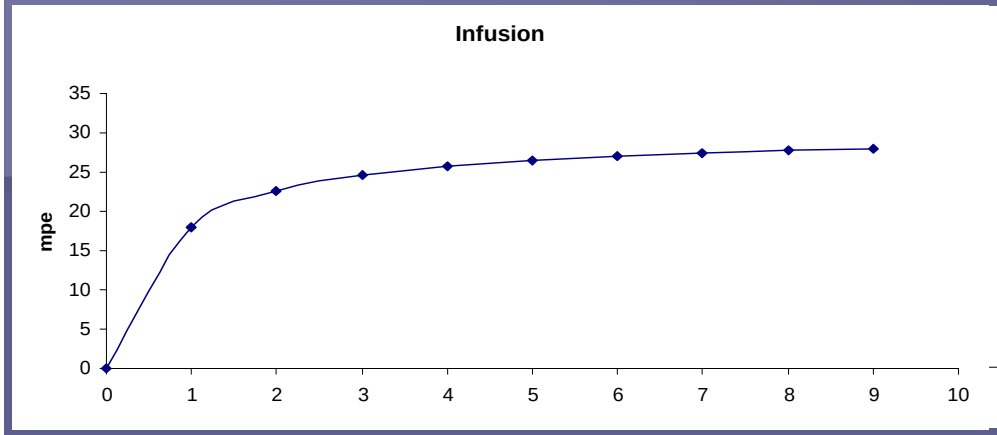
  L-Phenylalanine-d_5

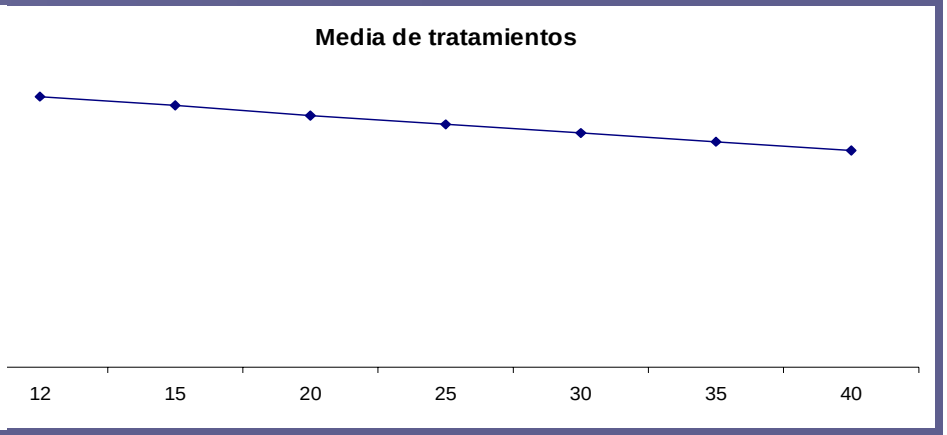
- Degradation: 3-Methylhistidine

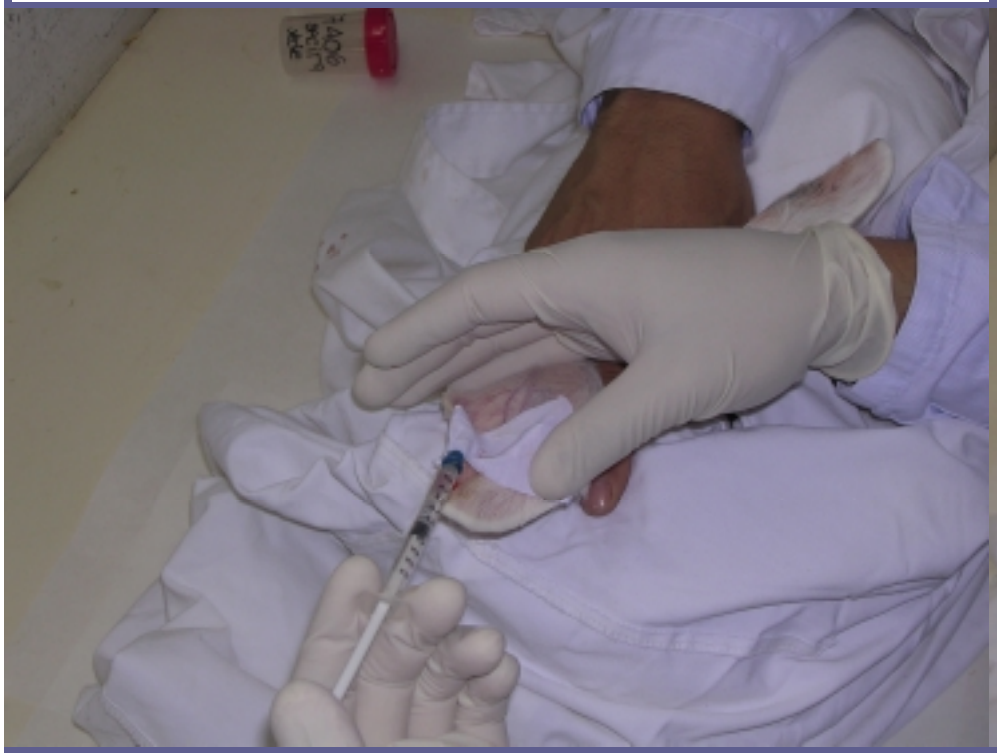
Principle: "flood" the free AA pools, minimizing the differences between extracellular and intracellular free AA isotopic enrichment



Flooding dose Method: L-Phenylalanine-d5





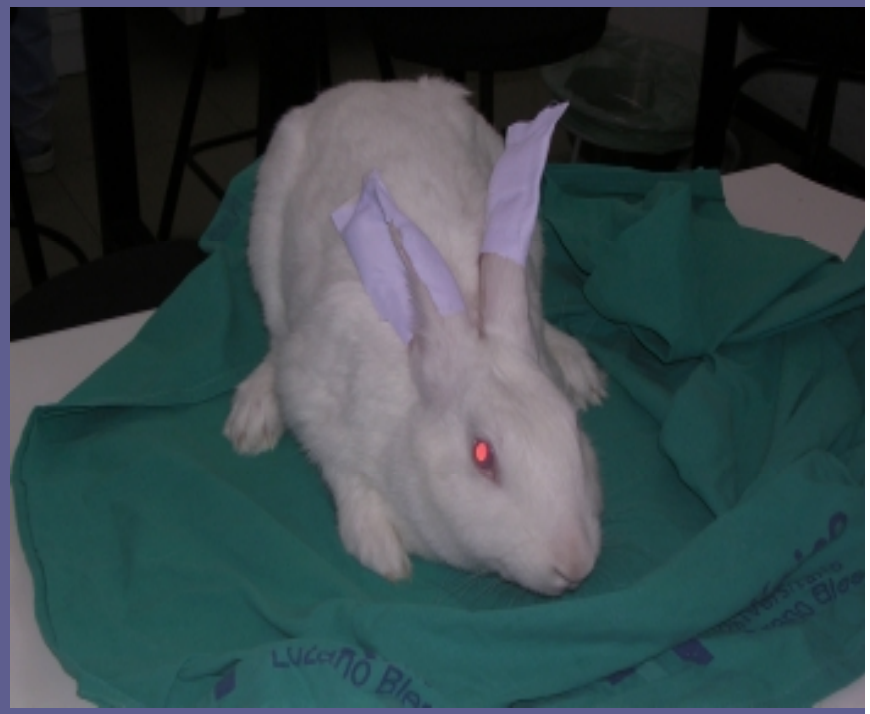


Fractional Synthesis Rate

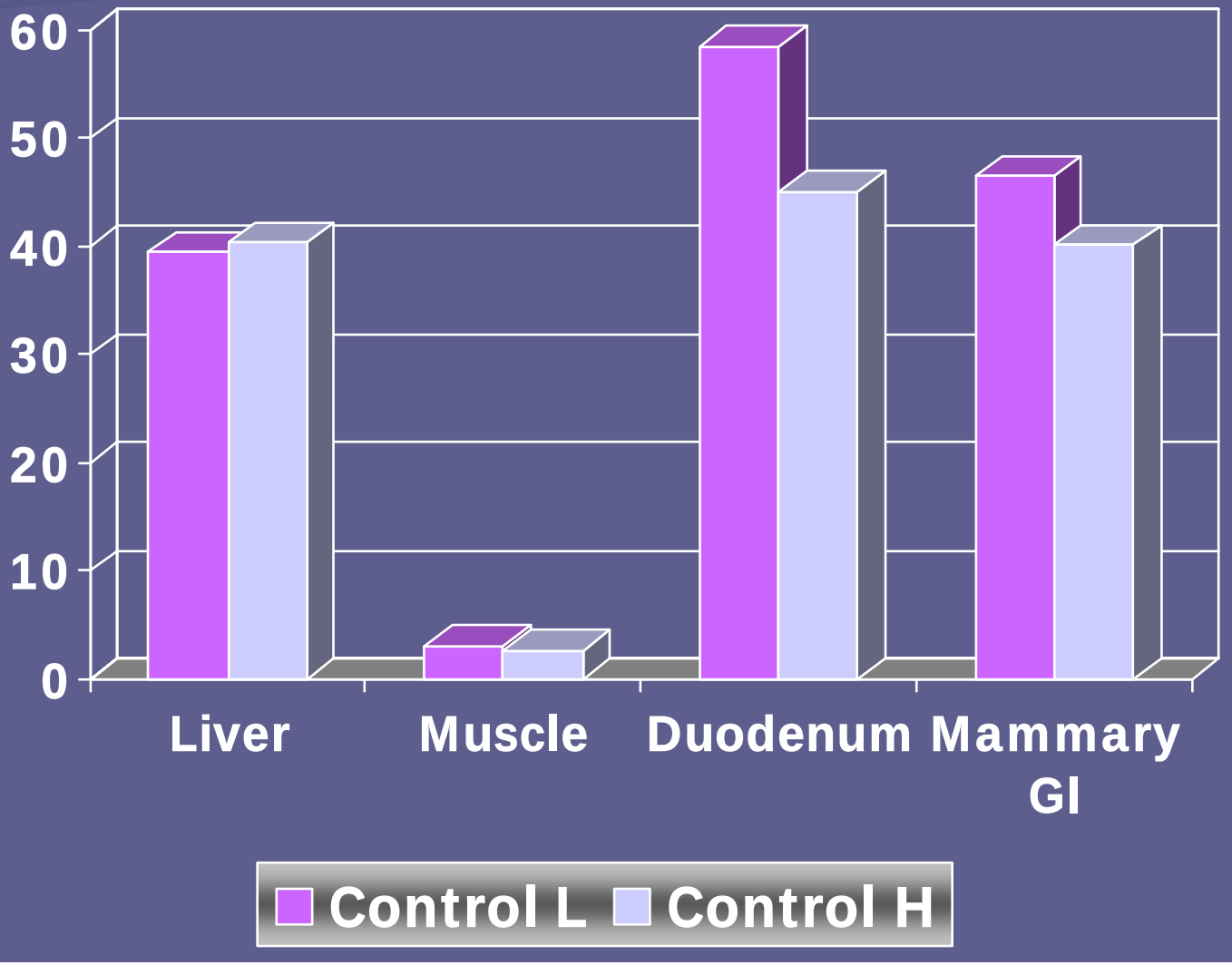
The fractional rate of protein synthesis can be calculated as

$$\text{FSR (\%/ d)} = \frac{Sb}{Sa} \times \frac{100}{t}$$

where *Sb* is the isotopic enrichment of the protein-bound labeled AA and *Sa*, is the enrichment of the precursor pool in the duration of labeling in minutes



FSR


60
50
40
30
20
10
0
Liver
Muscle
Duodenum
Mammary Gl
Control L
Control H

3-Methylhistidine

- Urine of humans, rats, cattle and rabbit ⇦ skeletal muscle

⇧

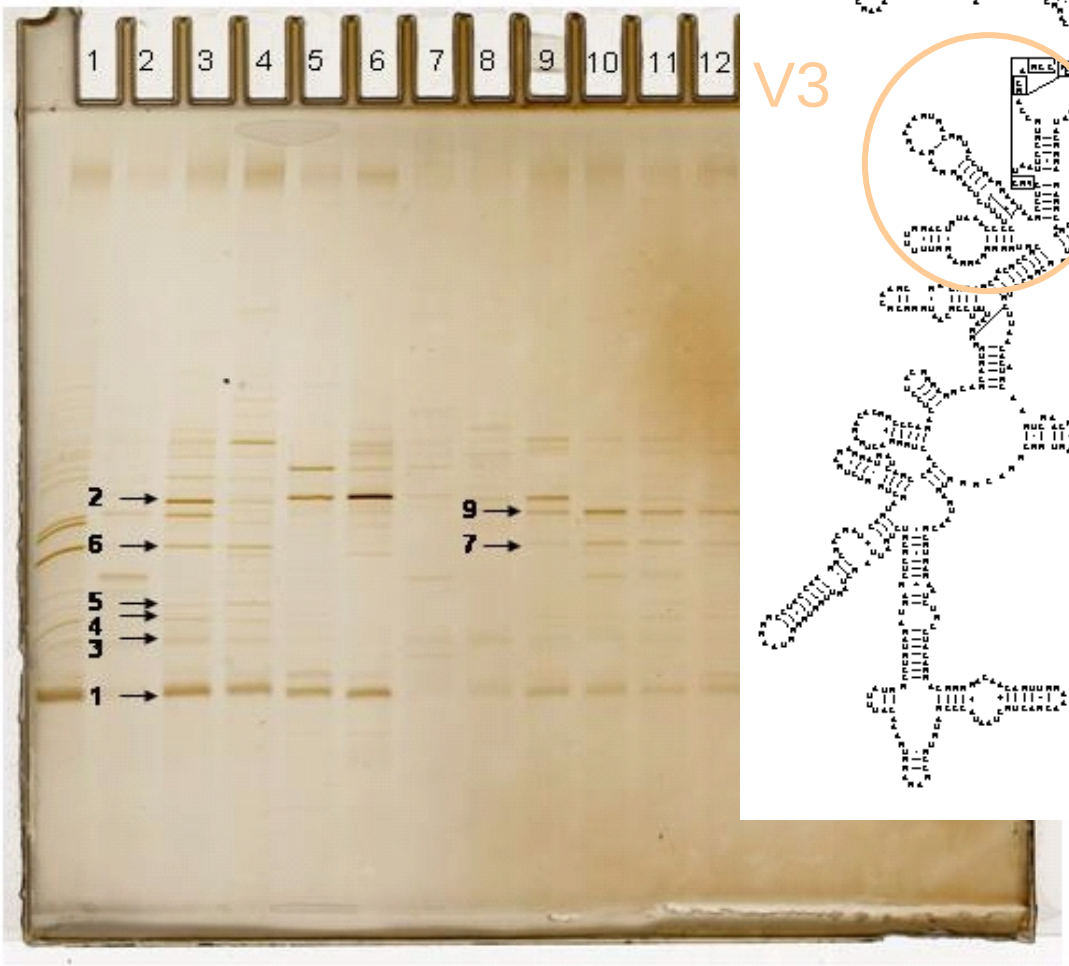
actin and myosin

- 3-methylhistidine is not used for protein synthesis
- Simple, no destructive to measure the muscle protein breakdown *in vivo.*
- Several measures in one animal
- No need of radioisotopes

Microbiology

- Dilution and Counting
- DGGE (Denaturing gradient gel electrophoresis)
- 16S rDNA Sequencing

DGGE


1
2
3
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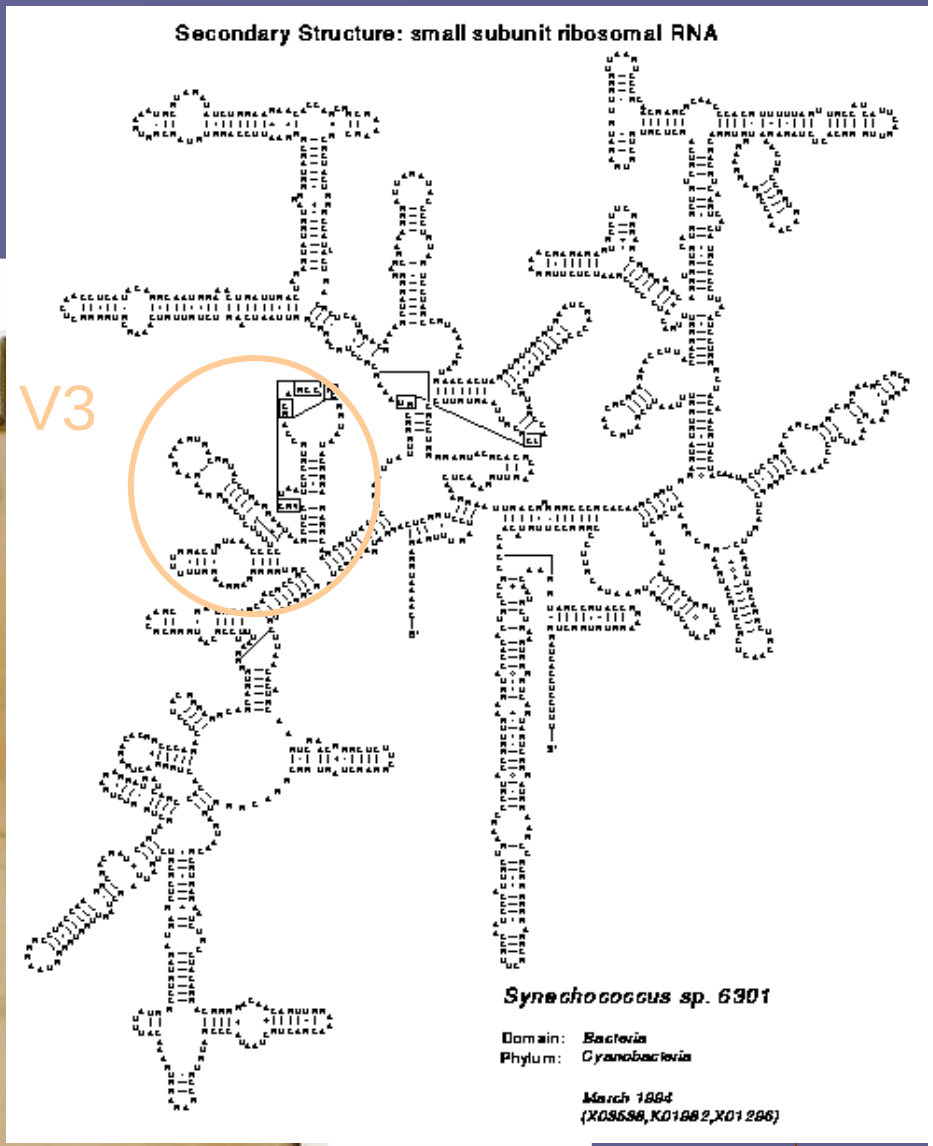
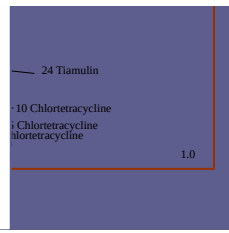
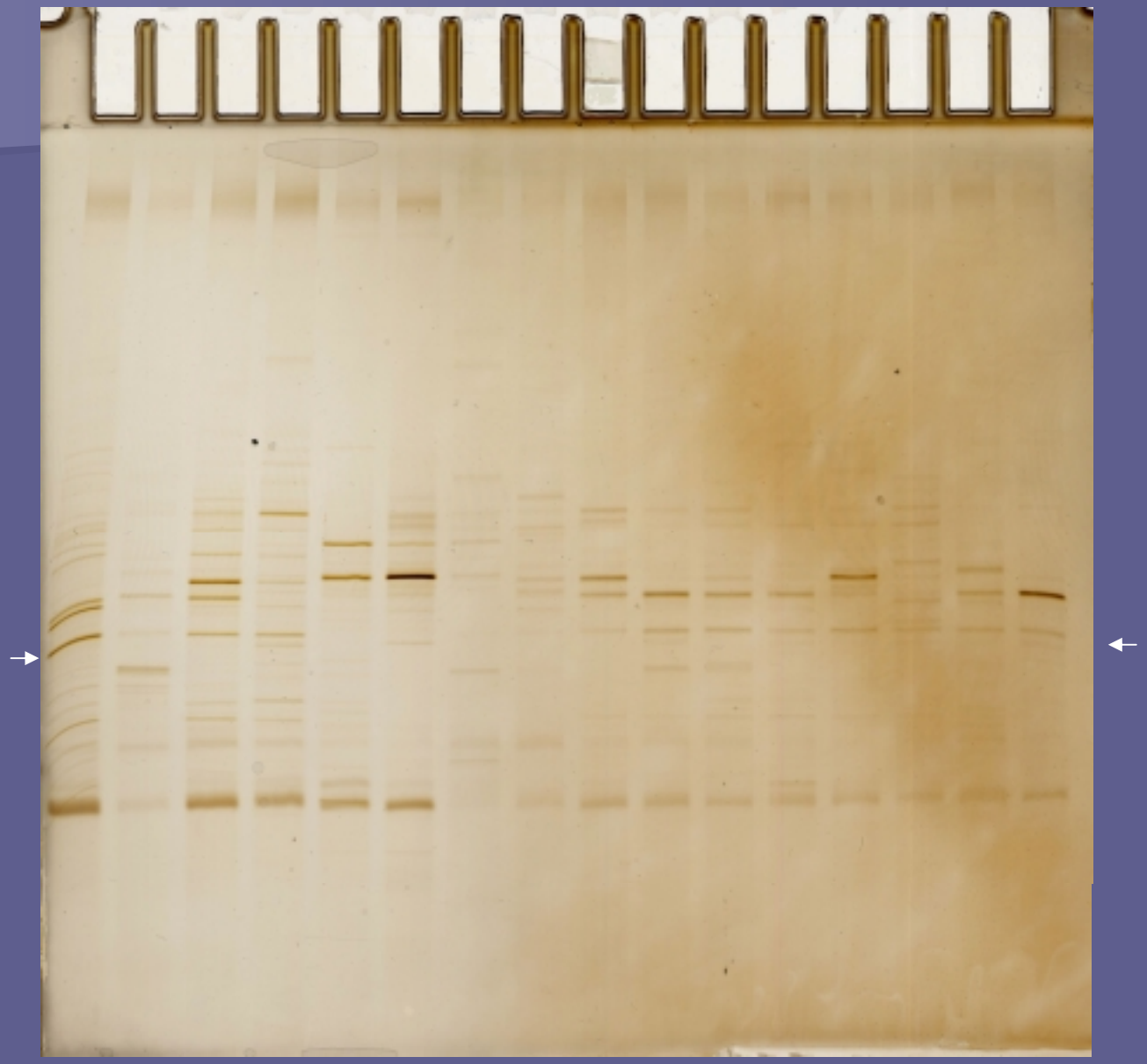
10
11
12
2
6
5
4
3
1
9
7


Secondary Structure: small subunit ribosomal RNA
V3
Synechococcus sp. 6301
Domain: Bacteria
Phylum: Cyanobacteria
March 1994
(X05536,K01882,X01296)

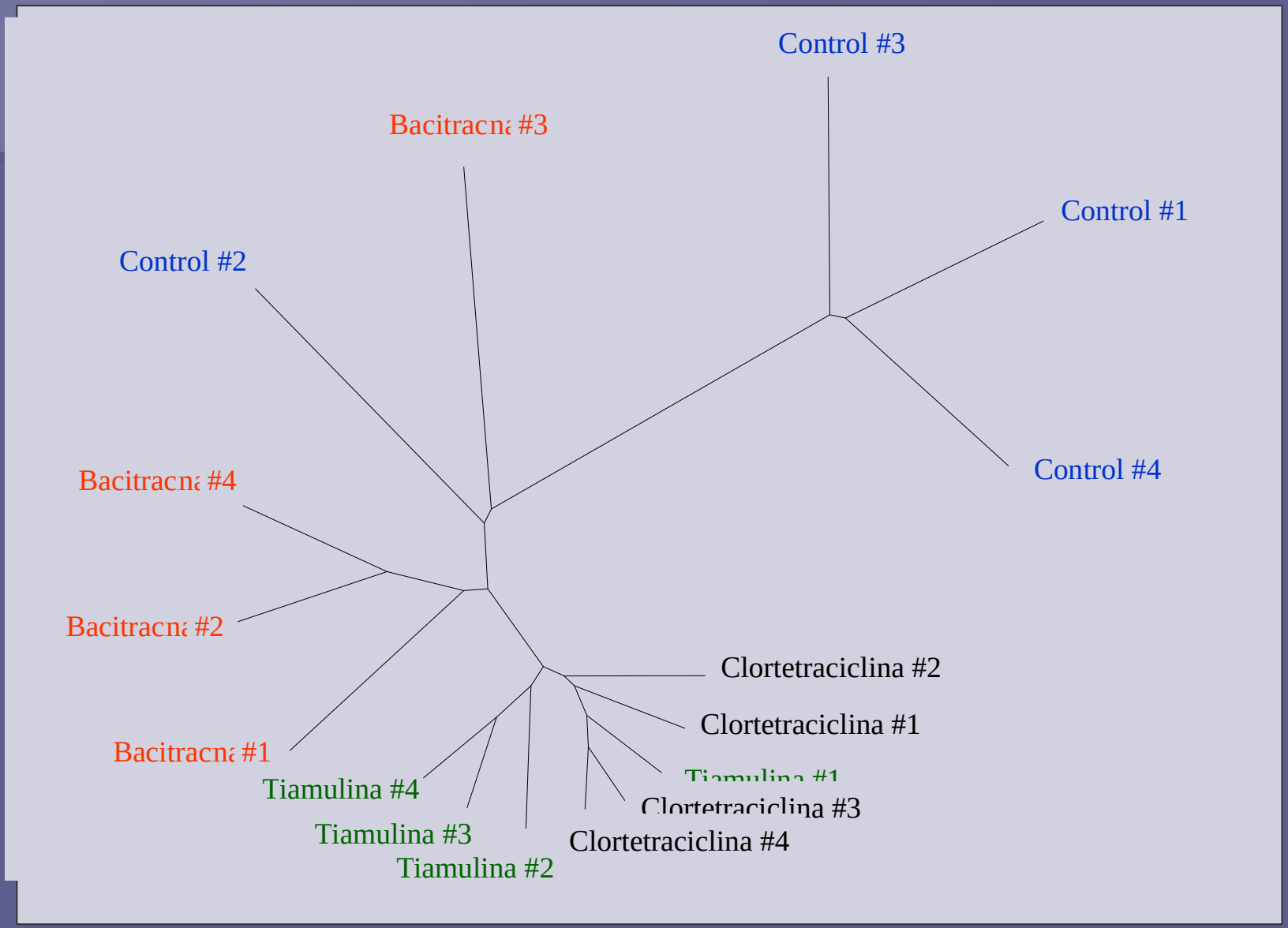

24 Tiamulin
10 Chlortetracycline
Chlortetracycline
hlortetracycline
1.0

DGGE

Control / Bacitracin / Chlortetra. / Tiamulin



Phylogenetic tree (DGGE)



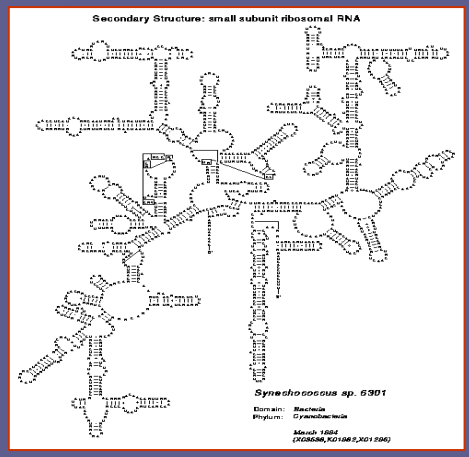
16S rDNA Sequencing

Samples

Extraction

DNA

PCR



Amplification
16S rRNA

Clonning
E. coli

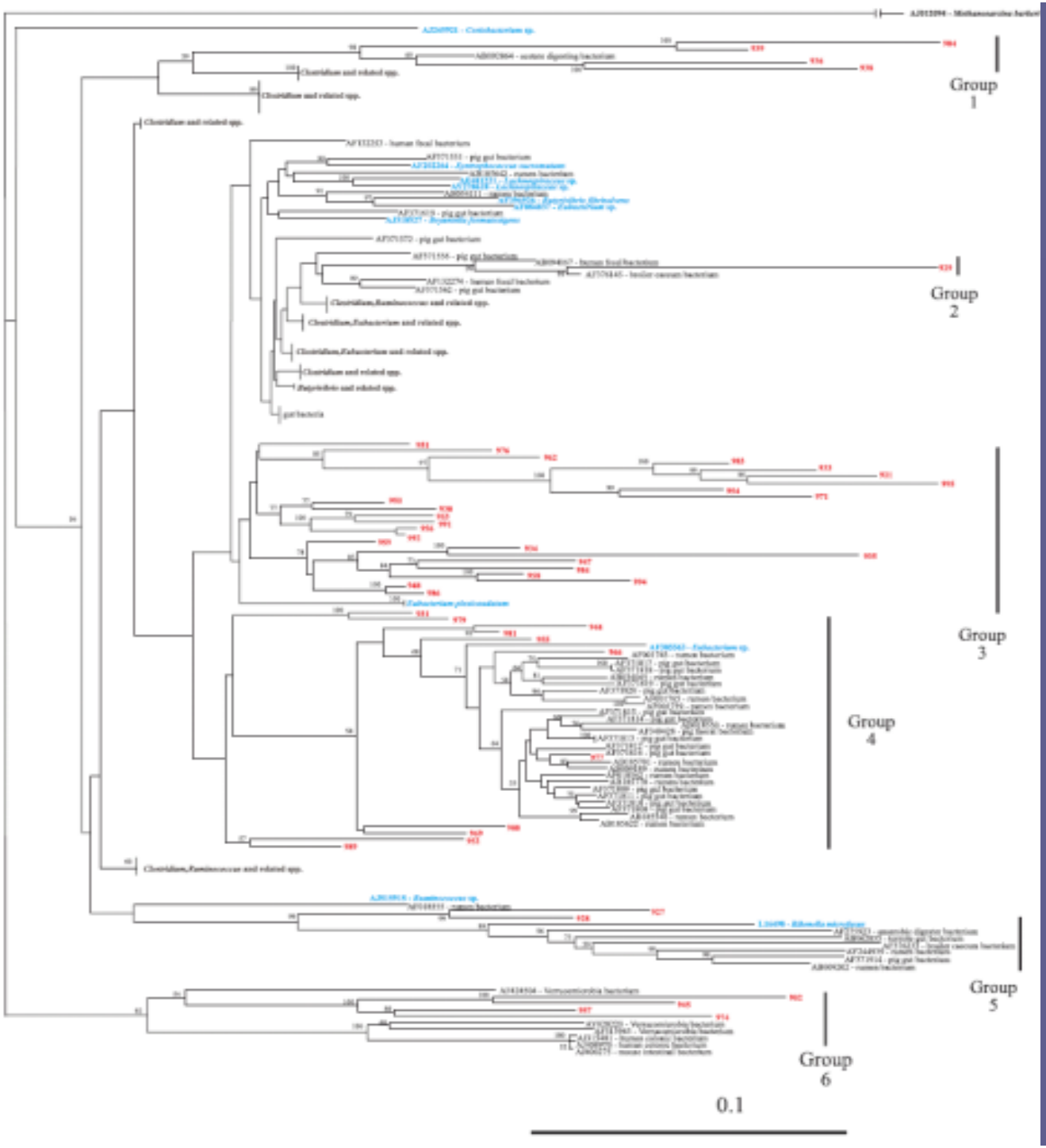
Clones

Sequencing

16S rRNA Sequences

Sequences analysis

Phylogenetic tree



- 44 new sequences (red)

-6 different groups

- Gr. 3: 24 of the new sequences, none of the registered

- Gr. 4: sequences belonging to ruminal content and gut and faeces of pigs

- Gr. 1 y 2: *Clostridium* spp.

FEMS Microbiology Letters 2005

Thank you !!

DGGE

- **Methodology**, denaturing gradient gel electrophoresis, is based upon the different melting properties of double-stranded DNA molecules in an increasing gradient of denaturant concentration (urea and formamide) at a fixed elevated temperature. Detection of sequence variation is facilitated by the addition of a GC-rich region (GC-clamp) to one end during fragment amplification, thus creating a high stability domain.