Utilisation of doubly stable isotope labelled *Lactobacillus johnsonii* in humans

Klaus D. Wutzke, I. Oetjens

University of Rostock Children’s Hospital, Research Laboratory, Germany

[www.kinderklinik-rostock.de](http://www.kinderklinik-rostock.de), Forschungslabor
Introduction

*Lactobacillus johnsonii* (La1), a probiotic *lactobacillus* strain of human origin, is able to adhere to the intestinal mucosa.

One of the important properties of probiotics is the ability to survive in the intestine.

For evaluation of this phenomenon, La1 was used for doubly labelling with $^{15}$N and $^{13}$C to investigate the digestion of orally administrated dILa1 in humans.
Aim of the Study

Investigation of the metabolic fate of doubly $^{13}$C-, $^{15}$N-labelled *Lactobacillus johnsonii* (dlLa1)

- the $^{13}$CO$_2$-exhalation,
- the urinary and faecal $^{13}$C- and $^{15}$N-excretion, respectively,
- and the corresponding isotopic enrichment of specific blood plasma fractions.
- Furthermore, the data were correlated to the oroecaecal transit time (OCTT).
Material and Methods

Universal labelling of La1 with $^{13}\text{C}$ and $^{15}\text{N}$ by fermentation (Biostad, Braun, Melsungen, Germany) in a medium containing

- $[^{15}\text{N}]\text{yeast extract}$ (prior labelled by $[^{15}\text{N}]\text{H}_4\text{Cl}$) and
- $[^{13}\text{C}_6]\text{glucose}$ (Campro Scientific, Berlin)

dlLa1- and raffinose-administration

- 10 healthy subjects, mean age 25.9 years, mean BMI: 23.4
- 8.00 a.m. together with breakfast
- dosage: 90 mg wet vital dlLa1/ kg body weight + 10 g raffinose

Sample collection

- Breath (14 h), urine (48 h), faeces (48 h), blood (2 h)

$^{13}\text{C}$- and $^{15}\text{N}$-measurement by IRMS (Tracer-mass 20-20, SerCon, Crewe, U.K., FANci2, Fischer ANalysen Instrumente Leipzig)

Evaluation of OCTT by a raffinose $\text{H}_2$-breath test (Stimotron, Wendelstein, Germany)
Fermentor Biostad B, B. Braun Melsungen
Results
OCTT measurements by a raffinose-H₂-breath test

[Graph showing OCTT measurements over time (h) with [ppmOB] on the y-axis and time [h] on the x-axis. The graph displays a peak at the OCTT time point, indicating elevated [ppmOB].]
$^{13}$CO$_2$-Enrichment and cumulative percentage exhalation
$^{15}$N-enrichment of urinary total-N and urinary ammonia

![Graph showing enrichment over time]
Isotope enrichment in different fractions of the blood

[Graph showing isotope enrichment for supernatant, fibrinogen, and plasma protein precipitate]

[Legend: 15N (green), 13C (orange)]
Percentage total $^{15}$N-excretion and $^{15}$N-incorporation

- Renal: 12.4%
- Faecal: 50.0%
- Incorporation: 37.6%
Percentage total $^{13}$C-excretion and $^{13}$C-incorporation

- Expiratory: 8.6%
- Renal: 1.3%
- Faecal: 50.2%
- Incorporation: 39.9%
Conclusion

In comparison to the OCTT of 3.7 h both stable isotopes appear after 30 min in breath and urine indicating that dLlA1 is rapidly digested in the small bowel before reaching the caecum.

This is confirmed by $^{13}$C-and $^{15}$N-enrichments of blood plasma fractions.

The ingestion of dLlA1 led to an excretion of 50% of both stable isotopes.

Our combination of measuring the expiratory, urinary and faecal excretion of $^{13}$C- and $^{15}$N-enriched metabolic degradation products of doubly labelled *Lactobacillus johnsonii* in correlation to the orocaecal transit time is a novelty.