Introduction onto a metabonomic study of peri-parturient dairy goats

Ana Elena Cismileanu[1], Celine Domange[2]

[1]National Research Institute for Animal Biology and Nutrition Balotesti (IBNA), Balotesti, Romania.

[2]UMR Modélisation Systémique Appliquée aux Ruminants, INRA, AgroParisTech, Université Paris-Saclay, 75005, Paris, France

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CONTEXT

- early lactation for dairy ruminants – require enormous energy and nutrient demand
- risk of pregnancy toxemia in late gestation

PURPOSE

2 experiments on goats

exp. 1: on 20 lactating goats (metabolic equilibrium)

exp. 2: on 20 peri-parturient goats (metabolic disorder)

saponin-based additive (4 weeks) for 10 animals in each exp.

analysis of metabolism (rumen + plasma + milk) by biochemical/zootechnical data

analysis of metabolism (plasma, rumen, milk/colostrum) by 1H-NMR technique = metabonome = identification of metabolites involved in individual metabolic signatures/trajectories by multivariate statistical analyses of NMR spectra

correlation of physiological/metabolic status with nature and presence of specific metabolites

Experimental design: Experiment 1 (during lactation)

Saponine ← Group 1 (10 goats in lactation)

Controls ← Group 2 (10 goats in lactation)

Analysis

RUMINAL LIQUID: → **ruminal metabolism**

pH, ammonia, VFA, protozoa number, ^{1}H-RMN

PLASMA: → **systemic metabolism**

glucose, urea, BHB, NEFA, cholesterol, bilirubin, HDL, magnesium, calcium, GGT, ALP, GOT, haptoglobin, ^{1}H-RMN

MILK: → **mammary metabolism**

protein, fat, ^{1}H-RMN

Measures and Analyses

Zootechnical	Exp1	Exp2
Body weigth	X	X
Milk yield & composition	X	
Ingestion dynamics	X	
Feed analysis	X	X
dry mater	X	X
van Soest	X	X
starch	X	X

Biochemical		
Ruminal fluids	Exp1	Exp2
Ph	X	X
NH_3	X	X
AGV	X	X
Protozoa	X	X
^{1}H RMN	X	X
Plasma goat		
GUBA	X	X
BIOCH	X	X
Haptoglobulin		X
^{1}H RMN	X	X
Plasma kids		
GUBA		X
IgG		X
Milk		
^{1}H RMN	X	
Collostrum		
IgG		X
^{1}H RMN		X

Environment	Exp 1	Exp 2
Temperature	X	X
Hygrometry	X	X

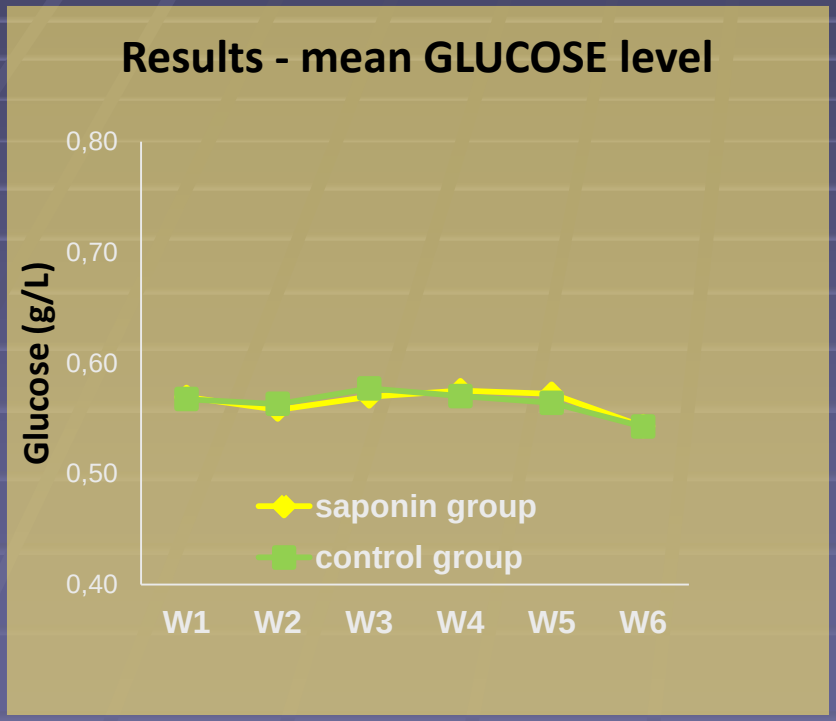
Glucose
Urea
NEFA
βHB

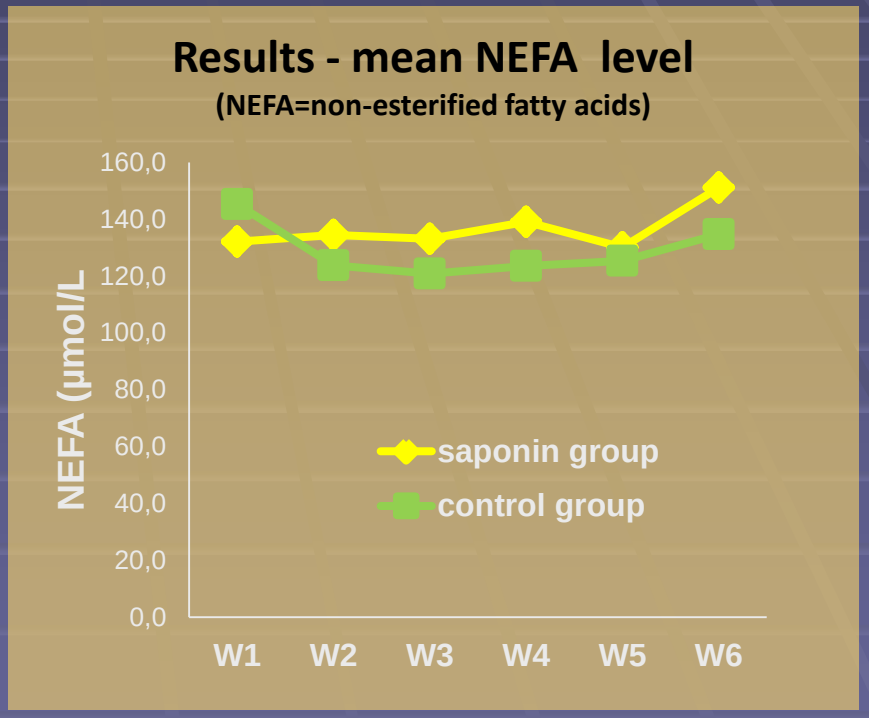
Glucose
Urea
NEFA
βHB
Cholestérol
Bilirubin
Albumine
Cho HDL
Mg
Ca
γGT
ALP
SGOT

Results – mean values of the ruminal liquid parameters

	Saponin group	Control group
pH	6.75	6.78
ammonia (mg/L)	67.65	70.78
protozoa number ($x10^5$ cells)	12.48	12.88
VFA – C2%	65.9	67.6
VFA – C3%	18.0	17.0
VFA – C4%	12.1	12.0

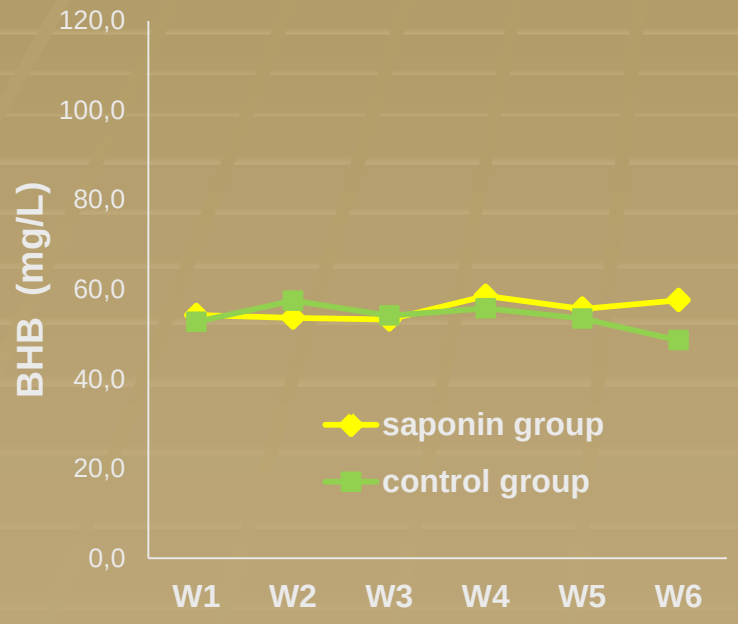
Results – mean values of the plasma biochemical parameters



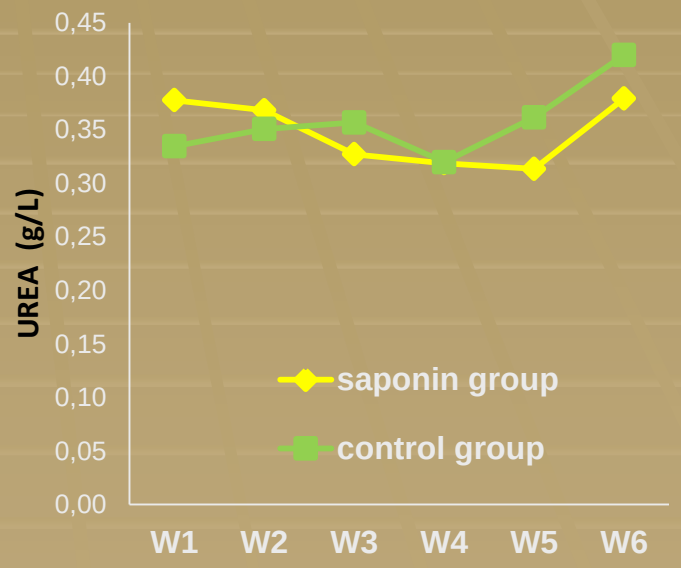


Results - mean BHB level

(BHB=beta-hydroxybutirate)

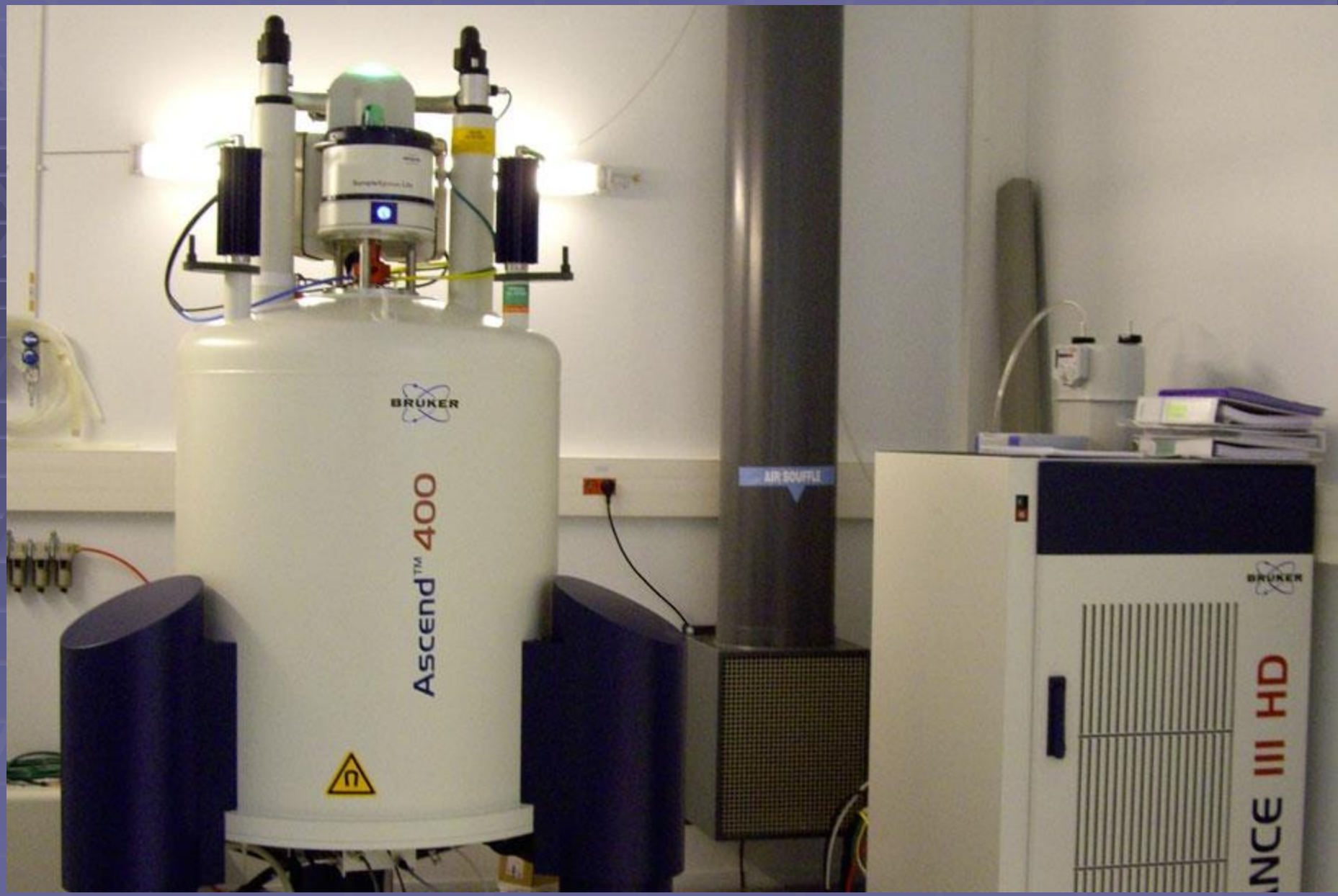


Results - mean UREA level



1H-NMR spectroscopy measurement

- Bruker Avance III HD 400 NMR Spectrometer (Bruker BioSpin, Germany), operating at a 1H frequency of 600.19 MHz, and equipped with a standard 5-mm 1H TCI CryoProbe.
- tilt angle = 90^{O}
- number of data points (TD) = 32768
- number of scans (NS) = 256
- spectral width (SW) = 11 ppm
- acquisition time (AQ) = 2.45 s
- relaxation delay (D1) = 2.5 s
- receiver gain (RG) = 362
- centre of window (OSP) = 4.709 ppm
- sample – combined with deuterated water in special narrow tubes


BRUKER
Ascend™ 400
AIR SOUFFLE
BRUKER
NCE III HD

1H NMR METABOLITES IDENTIFICATION

- by querying metabolomic databases for their chemical shift
- metabolite is identified by its peak(s) located along the chemical shift scale; the peaks reflect the unique interactions of protons in the chemical structure

DATA PROCESSING

- creating specific matrices (buckets) able to be processed statistically: data conversion, validation by 1-4 D methods. The result is the metabolite profiling for the samples in concordance with metabolic pathways of aminoacids, carbohydrates, lipids, energy, cofactors and vitamins, nucleotides, and for tricarboxylic acids cycle.

DATA ANALYSIS by MULTIVARIATE STATISTICAL EVALUATION

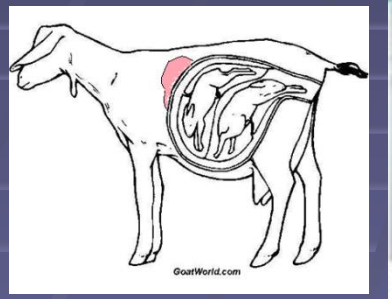
- **uses the preliminary biological knowledge to analyse metabolite patterns from an integrative point of view**
- **by statistics as PCA, PLS-DA, OPLS-DA on integrals of the peaks are identified metabolite components which vary in a significant systematically manner; example: in glucose metabolism not only glucose is involved, but also specific aminoacids**
- **Identification of peri-parturient metabolic markers**

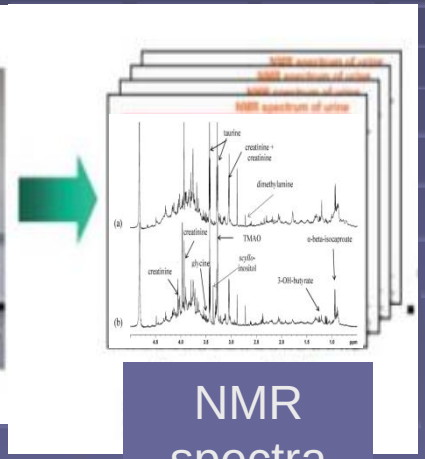
A novel statistical model of data processing for 1D NMR spectra is the Bayesian automated metabolite analyzer for NMR (BATMAN) R package: a regression model using a vector of spectral intensities and a matrix, each column of which is a spectral template corresponding to a metabolite. It can construct a template spectrum for each metabolite.

The BATMAN model includes more: a vector of relative concentrations for the metabolites, a matrix of wavelets, a vector of wavelet coefficients, a vector of Gaussian errors.
It is widely used because it enhances the robustness of the biomarker/metabolite selection process.

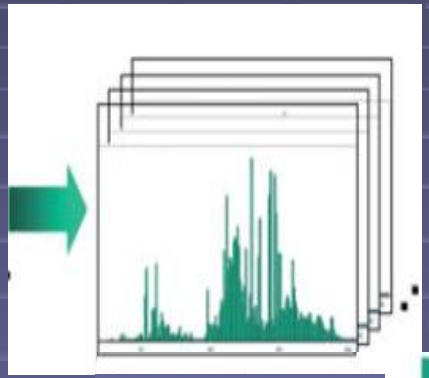
NMR analyses

Biological samples





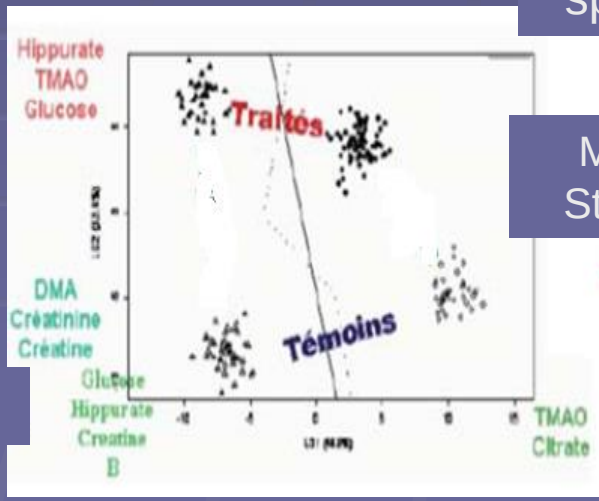
NMR spectra



Bucketing

Database

Multidimensional Statistical analysis



CONCLUSION

- In the end of analysis will be obtained metabolic fingerprints for lactating and peri-parturient goats.
- Metabolic differences between lactating and peri-parturient goats can be detected by NMR-based metabonomic study before clinical manifestation appears.

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