



HAL
open science

Metabolomics analysis of liver to reveal profiles disruption in bovines upon steroid treatment

Giancarlo Biancotto, Roberto Stella, E. Mastrorilli, Roberto Angeletti, D. Bovo, M.C. Bellesso, Gaud Dervilly, Anne-Lise Royer, Bruno Le Bizec

► **To cite this version:**

Giancarlo Biancotto, Roberto Stella, E. Mastrorilli, Roberto Angeletti, D. Bovo, et al.. Metabolomics analysis of liver to reveal profiles disruption in bovines upon steroid treatment. *Metabolomics* 2016, Jun 2016, Dublin, Ireland. hal-04259799

HAL Id: hal-04259799

<https://hal.science/hal-04259799v1>

Submitted on 26 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

METABOLOMICS ANALYSIS OF LIVER TO REVEAL PROFILES DISRUPTION IN BOVINES UPON STEROID TREATMENT

Biancotto G.¹, Stella R.¹, Mastroilli E.², Angeletti R.¹, Bovo D.¹, Belleso M.C.¹, Dervilly-Pinel G.³, Royer A.L.³, Le Bizec B.³

1. Istituto Zooprofilattico Sperimentale delle Venezie, Department of Chemistry, Legnaro (PD) - Italy
 2. Istituto Zooprofilattico Sperimentale delle Venezie, Department of Food Safety, Legnaro (PD) - Italy
 3. Laboratoire d'Étude des Résidus et Contaminants dans les Aliments (LABERCA), ONIRIS, LUNAM Université, Nantes - France

INTRODUCTION

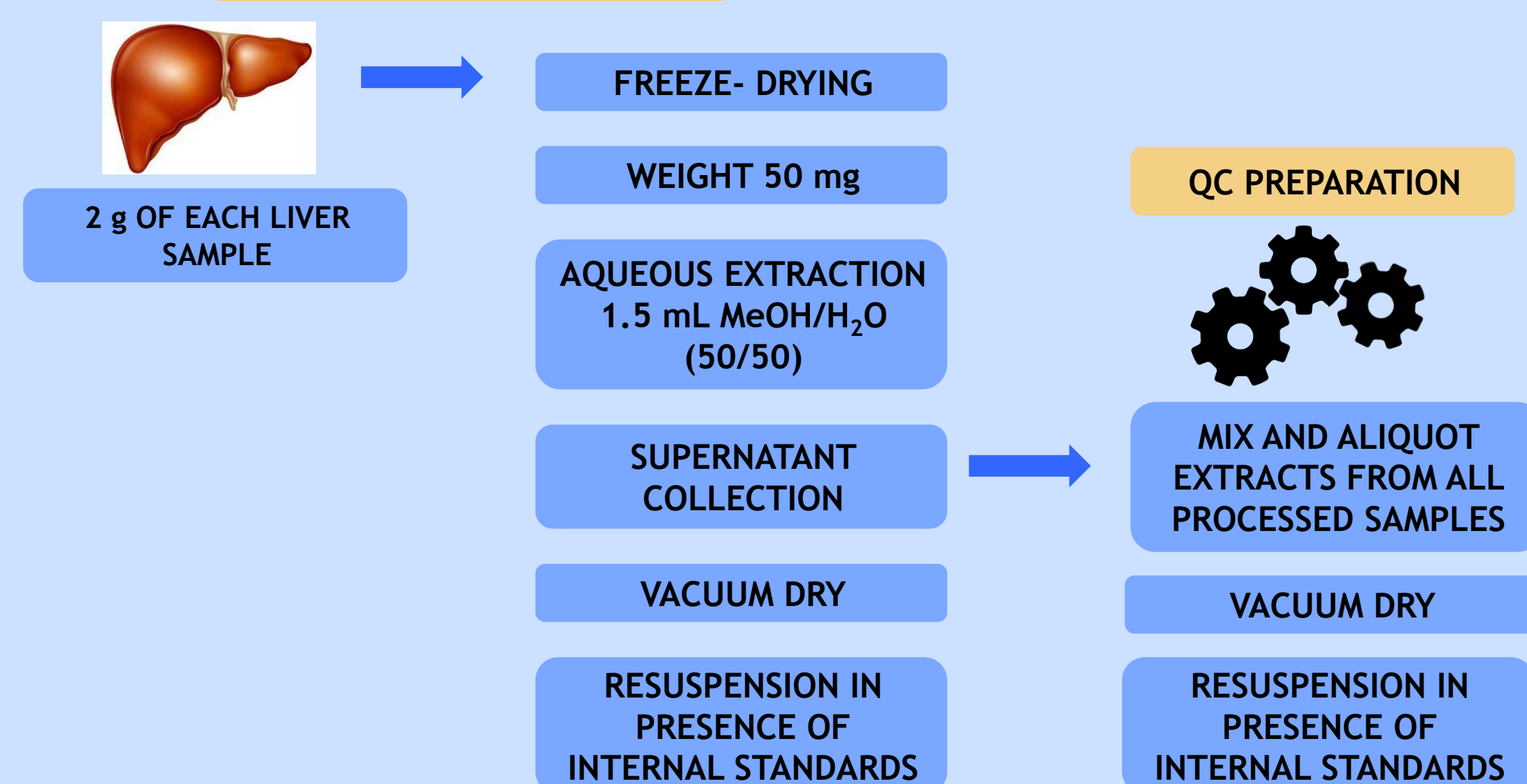
The surveillance of illegal anabolic practices in bovine meat production is necessary to guarantee consumers' health. Screening strategies based on the recognition of indirect biological effects are considered by the community as promising tools to overcome some limitations of classical analytical methods and might therefore concur to ensure safer food for the consumer.

Given that hormonal therapies influence the physiology of an organism, strategies based on the detection of metabolic changes that occur following anabolic practices are promising approaches to identify their misuse [1-3]. The present work is aimed at characterizing the metabolic profile induced in liver by administration of anabolic steroids, and to identify potential disturbances in the hepatic metabolism. A total of 32 liver samples, 16 from untreated bulls and 16 from bulls treated with an ear implant (Revalor-XS[®]) containing trenbolone acetate (200 mg) and estradiol (40 mg), were analyzed following a LC-HRMS-based metabolomics analysis using RP and HILIC chromatographic separations.

SAMPLE COLLECTION AND PREPARATION



EXTRACTION OF METABOLITES



DATA ACQUISITION AND PROCESSING



HRMS

Full Scan 70-1000 m/z
 70,000 resolution (at 200 m/z)
 Positive ESI
 Negative ESI

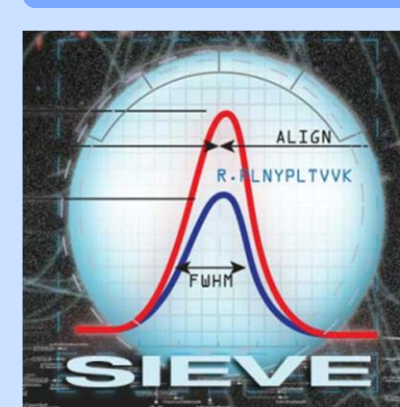
HILIC

Phase A: H₂O + 10 mM ammonium acetate + 10 mM acetic acid
 Phase B: ACN
 Flow: 0.3 mL min⁻¹
 Run time: 30 minutes

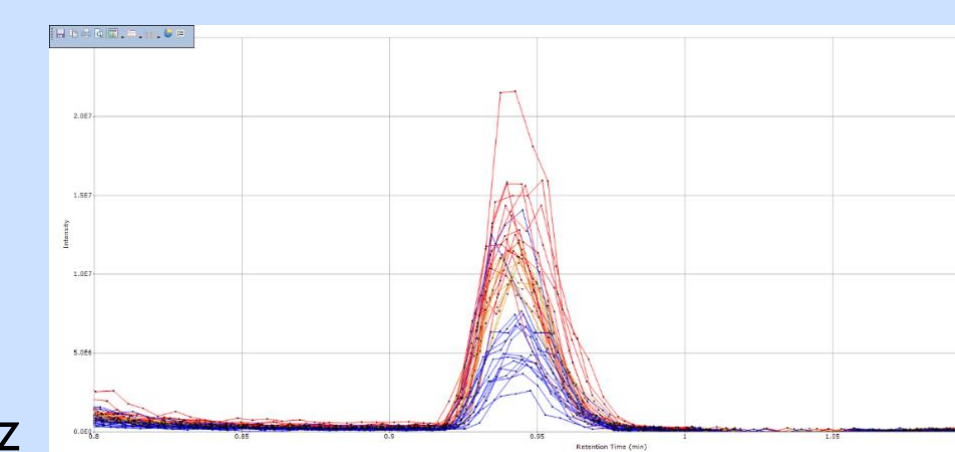
REVERSED PHASE

Phase A: H₂O + 0.1% acetic acid
 Phase B: ACN + 0.1% acetic acid
 Flow: 0.4 mL min⁻¹
 Run time: 30 minutes

PROCESSING



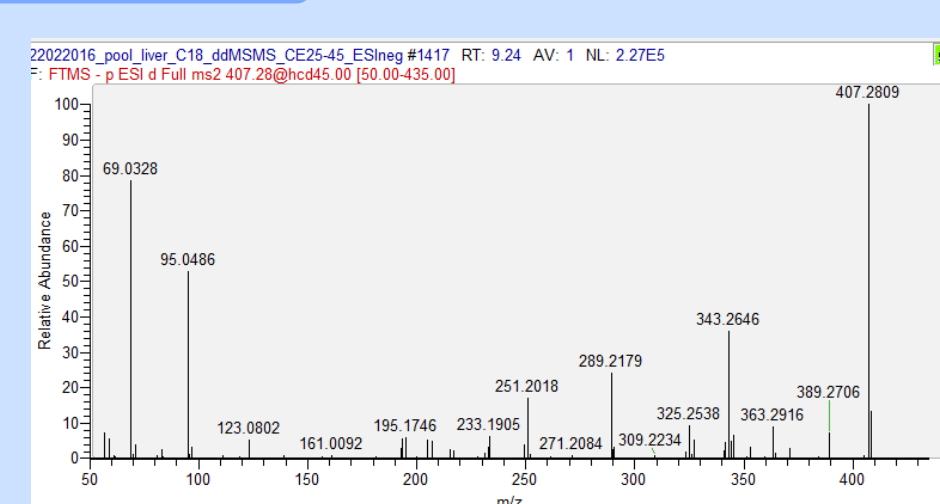
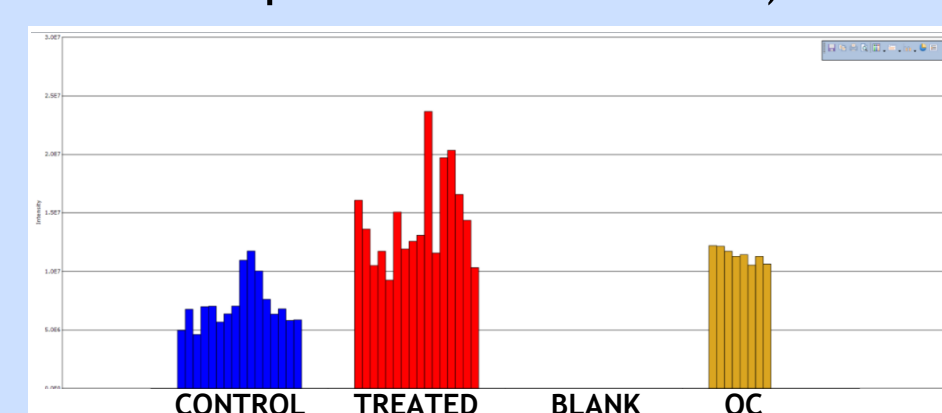
Chromatographic peaks alignment
 Frames extraction
 Peaks integration
 Selection of interesting features
 Putative identification based on m/z



IDENTIFICATION OF BIOMARKERS

MS/MS STRUCTURAL ELUCIDATION

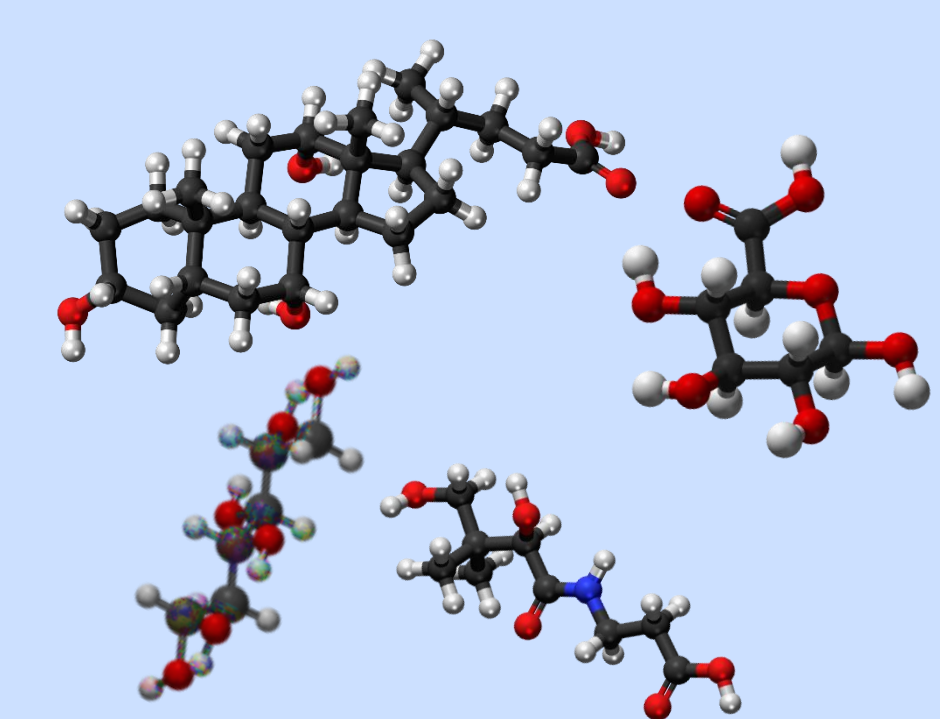
QC samples were analysed using FullScan + DDA (140,000 resolution + targeted MS/MS of potential biomarkers)



RT and MS/MS were compared to those of reference material

DATA COMPARISON

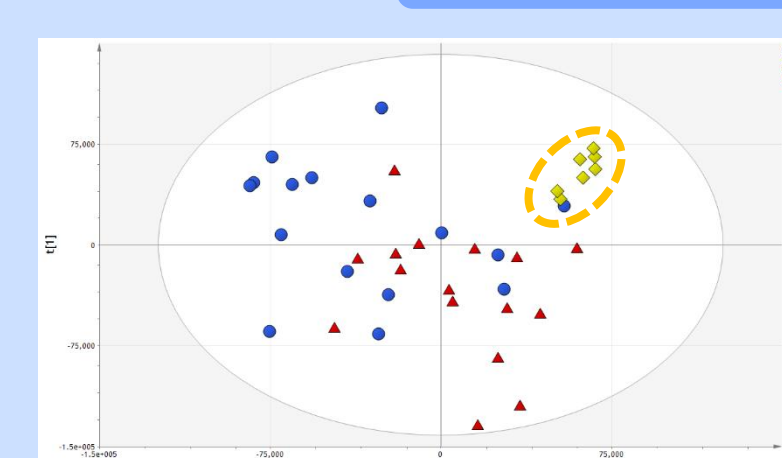
6 out of 8 potential markers of anabolic treatment have been identified by another lab analysing the same liver tissues with a different acquisition and processing platform



8 potential markers have been identified

STATISTICAL HANDLING

4 DATA MATRICES WERE ANALYSED



Unsupervised PCA was run to verify the acceptability of the analytical session using QC samples

Frames were filtered for:
 - Treated/Control ratio (T/C < 0.7 or T/C > 1.5)
 - Student T-test p value (< 0.05)
 - CV % measured for QC samples in a given working session (< 30%)

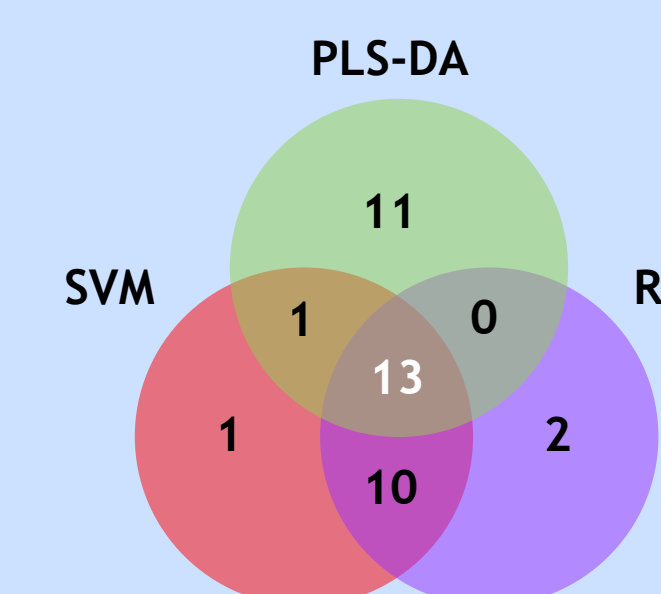
3 MULTIVARIATE ANALYSES WERE PERFORMED FOR SELECTION OF BIOMARKERS

SVM
Support Vector Machine

PLS-DA
(Partial Least Square - Discriminant Analysis)

RF
(Random Forest)

Top 25 significant frames common to all statistical tools were selected as potential markers to be identified



CONCLUSIONS

Different multivariate statistical tools were applied to the datasets to select common metabolites for classification of samples and to reveal potential biomarkers on the basis of their significant changes in concentrations after administration of sexual steroids.

The identity of 8 candidate biomarkers was confirmed using reference standard material and fragmentation spectra. Moreover, a subset of biomarkers was also validated by a different laboratory that performed the same analyses using an independent instrumental and elaboration platform, confirming the robustness of the results achieved.

The identified metabolites can be considered as candidate markers of the proposed anabolic treatment with sexual steroids and will be further tested using independent animal sets. Their biochemical interpretation is under evaluation.

References

- 1 - Kouassi Nzoughe J, Dervilly-Pinel G, Chéreau S, Biancotto G, Monteau F, Elliott CT, Le Bizec B.. Metabolomics 2015;11(5):1184-1196.
- 2 - Jacob C, Dervilly-Pinel G, Biancotto G, Monteau F, Le Bizec B.. Metabolomics 2015;11(1):184-197.
- 3 - Kouassi Nzoughe J, Dervilly-Pinel G, Gallart-Ayata H, Biancotto G, Le Bizec B.,. Metabolomics 2015;11(6):1884-1895.

Authors thanks Italian Ministry of Health for funding (RC IZS VE 01/2012, CUP B28C13000350001, and RF IZS VE 2008, 1157188), and Merck Animal Health for kindly providing the ear implants Revalor XS[®]