METABOLOMICS ANALYSIS OF LIVER REVEALS UNEXPECTED CONTAMINATION OF CLENBUTEROL IN BOVINES

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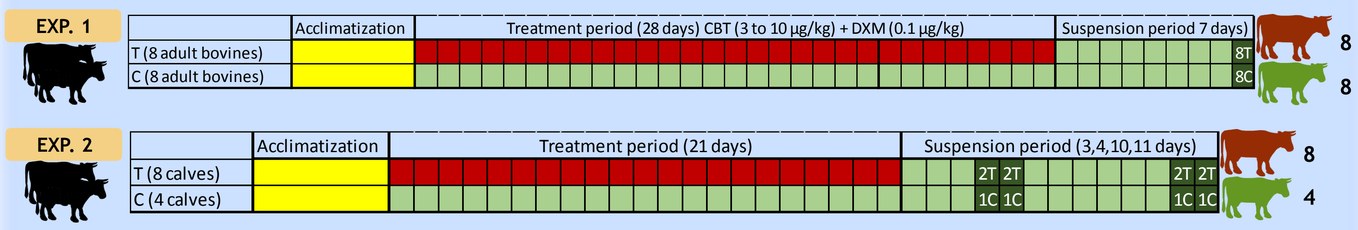
INTRODUCTION

Insight of metabolome of tissues or biological fluids can represent a new tool to highlight growth-promoting treatments in food producing animals, provided that an alteration of the metabolic patterns took place as a consequence of the administration of such active compounds.
In this work , we investigated the possible effects of clenbuterol administration on the metabolic profile of bovines liver.
The study was conducted on 2 separate groups of animals: in the first experiment, 8 adult bovines received (clenbuterol + dexamethasone) whereas other 8 animals were kept as control. In a second experiment, 8 veal calves received clenbuterol only, and 4 calves were kept as controls.
PCA and PLS from the first study reveal a set of features which correctly classify animals according to their corresponding group. When the predictive model was applied to the second group, 4 control samples were assigned to the treated group. However, low concentrations of CBT were found in the retina of these 4 control animals: the model highlighted a potential unexpected low contamination of control animals with clenbuterol.

MATERIALS and METHODS

ANIMAL TREATMENT AND SAMPLING SCHEME



SAMPLE PREPARATION

2 g OF EACH LIVER SAMPLE

EXTRACTION OF METABOLITES

- FREEZE- DRYING
- WEIGHT 50 mg
- AQUEOUS EXTRACTION 1.5 mL MeOH/H_2O (50/50)
- SUPERNATANT COLLECTION
- VACUUM DRY
- RESUSPENSION IN PRESENCE OF INTERNAL STANDARDS

QC PREPARATION

- MIX AND ALIQUOT EXTRACTS FROM ALL PROCESSED SAMPLES
- VACUUM DRY
- RESUSPENSION IN PRESENCE OF INTERNAL STANDARDS

DATA ACQUISITION AND DATA PROCESSING

HILIC Chromatography — REVERSED PHASE Chromatography

HRMS

Full Scan 70-1000 m/z
70,000 resolution

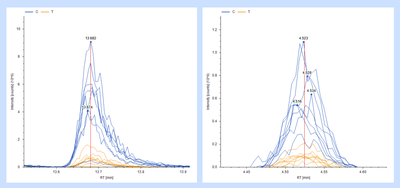
4 DATA SET

- HILIC ESI +
- HILIC ESI -
- C18 ESI +
- C18 ESI -

PROCESSING

FOR EACH DATASET:

- Chromatographic peaks allignment
- Frames evaluation & extraction
- Peaks integration

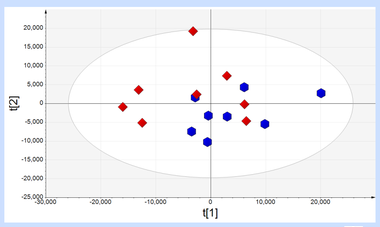


RESULTS

STATISTICAL HANDLING

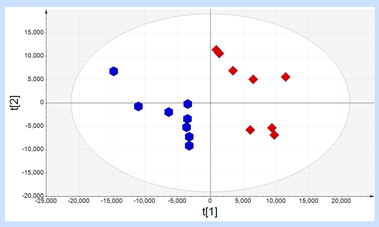
BEST PERFORMING DATA SET: C18 ESI -

PCA derived from EXP 1 (bulls)



Animal grouping and separation

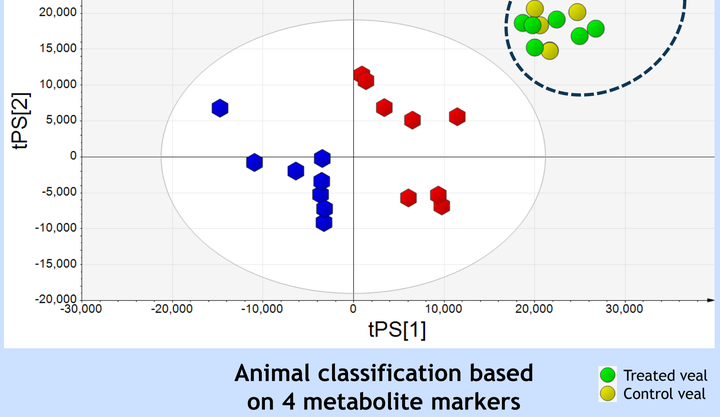
PLS derived from EXP 1 (bulls)



Treated bull
Control bull

Selection of metabolite markers

PREDICTION ON EXP 2 (CALVES: 8T / 4C)



Animal classification based on 4 metabolite markers

Treated veal
Control veal

	T	C
T	8	4
C	0	0

Confusion matrix derived from prediction model applied to veal calves would account for 100% FALSE POSITIVE result, BUT…

VEAL Nb	Condition (T/C)	CBT conc in retina [ng/g]
Animal 1	C	0,97
Animal 2	C	2,58
Animal 3	C	2,42
Animal 4	C	0,95
Animal 5	T	645,75
Animal 6	T	277,27
Animal 7	T	712,37
Animal 8	T	802,69
Animal 9	T	217,78
Animal 10	T	408,48
Animal 11	T	399,26
Animal 12	T	187,43

… low residual CBT concentrations were detected in retina samples from C group:

The model seems to be strong enough to detect subjects probably low contaminated by CBT

CONCLUSIONS

The predictive model based on 4 metabolites selected thanks to PLS-DA on adult bovines, enables to correctly classify 100 % treated veal calves as «Treated subjects». Most importantly these 4 metabolites classify control veal calves, possibly undergoing some accidental contamination with CBT, as «Treated subjects». highlighting a great capability to detect very low dosages of CBT.
These results are under further evaluation to verify their reliabilty.

Authors thank Italian Ministry of Health for funding (RC IZS VE 03/2016, CUP B22F17000340001)