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New Technologies to Screen Multiple Chemical Contaminants in Foods

A biomarker-based approach to identify hormone abuse in livestock

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Introduction

The European Union (EU) prohibits the use of growth-promoting agents in cattle and in an attempt to guarantee food safety and maintain a zero tolerance policy, monitoring for the presence of substance abuse is essential. Conventional technologies that currently perform this task are largely prohibited by cost, speed of analysis, ability to perform multi-analyte detection at a high-throughput rate and the capability of detecting the vast array of substances used.

Aim

The aim of Work Package 2 of the BioCop project is to develop novel "fingerprint" or indirect methods of detecting hormone abuse in livestock. The circulating levels of proteins change in animals in response to growth-promoter treatments and these proteins have potential to be utilised as *biomarkers* of abuse.

Work Package 2 aims to use proteomic profiling techniques to identify suitable plasma biomarkers of hormone exposure and to develop biosensor-based assays suitable for large scale screening of multiple biomarker concentrations.

It is envisaged that on the basis of alterations in the profiles of chosen biomarkers, illegally treated animals may be distinguished from untreated animals.

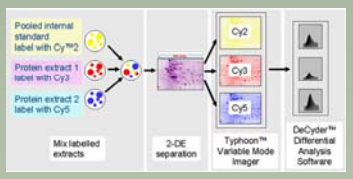
Hormone treatment studies

Plasma samples were collected from 18-week old male and female calves undergoing a growth-promoting regime typically associated with veal production.

Hormone-treated animals received multiple 19-nortestosterone decanoate (150mg), 17β-oestradiol benzoate (25mg) and dexamethasone (4mg) injections, whilst matching control animals were given placebo administrations. Throughout the course of the 42-day study period blood samples were obtained at regular intervals to facilitate the identification of biomarkers present within plasma which could be used to identify hormone treatment.

Biomarker identification

Suitable biomarkers of hormone treatment within plasma from treated animals were identified via electrophoretic and mass spectrometric analytical techniques. Two-dimensional fluorescence difference gel electrophoresis (DIGE) of high abundant protein-depleted plasma from control (extract 1) and treated (**extract 2**) animals enabled the identification of differentially expressed proteins within the 2 sets of experimental animals. The identify of proteins of interest were then determined via MALDI-TOF mass spectrometry.



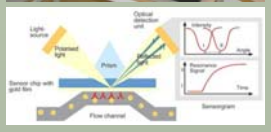
Additional biomarkers of hormone treatment were identified using conventional assay techniques such as ELISAs, radioimmunoassays, western blotting and ligand-binding assays. Through this process, Work Package 2 has chosen 8 biomarkers which may be used to identify hormone treated animals.

IDENTIFIED BIOMARKERS
Alpha-1 antitrypsin
Serotransferrin
Fetuin-A
Alpha-2 antiplasmin
IGF binding protein-2
Osteocalcin
C-terminal PIIINP
Sex-hormone binding globulin

These biomarkers are representative of a range of *in vivo* metabolic activities which may be impacted by exogenous hormone administrations including bone/collagen formation, the IGF-1/growth hormone axis and sex hormone regulatory pathways. The detection and measurement of these biomarkers will be performed with assays developed on a biosensor platform using antibodies and proteins sourced from BioCop consortium partners.

Biosensor analysis

The multi-assay Surface Plasmon Resonance (SPR) biosensor platform used within Work Package 2 was developed by Biacore (GE Healthcare, Sweden). This unique prototype biosensor is capable of simultaneously measuring up to 16 different protein concentrations within a single sample. Biosensor protein assays are divided over four flow cells of a single biosensor chip thus allowing samples to be analyzed under different cycle conditions.



Biosensor assays are performed using an indirect inhibition assay format, in which (diluted) bovine plasma samples are combined with specific antibodies, produced by CER (Belgium), and run over a chip with immobilised target protein.

Conclusion

Work Package 2 of the BioCop project has established a panel of biomarkers of exposure to growth-promoting agents and developed biosensor assays and technology to enable measurement within plasma. Validation of this novel "fingerprint" strategy will be initiated in the near future and will lead to a cost-effective, rapid and sensitive screening tool which can be used in the fight against hormone abuse across the EU.

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