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**The search for biomarkers of facial eczema, following a  
sporidesmin challenge in dairy cows, using mass  
spectrometry and nuclear magnetic resonance of serum,  
urine, and milk**

A thesis presented in partial fulfilment of the requirements for the degree of

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## Abstract

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Facial eczema (FE) is a secondary photosensitisation disease of ruminants that is significant in terms of both its economic importance to New Zealand and its impact on animal welfare. The clinical photosensitivity signs, caused by the retention of phytoporphyrin, occur secondarily to hepatobiliary damage caused by the mycotoxin sporidesmin.

Currently it is difficult to diagnose subclinical animals and those in the early stages of the disease. The project was aimed at applying new analytical and statistical techniques, to attempt the early diagnosis of FE in dairy cows following the administration of a single oral dose (0.24 mg/kg) of sporidesmin. Well-established traditional techniques including production parameters, liver enzyme (GGT, GDH) activity measurements, as well as measurements of phytoporphyrin by fluorescence spectroscopy were made for comparison.

Serum, urine, and milk were analysed using  $^1\text{H}$  Nuclear Magnetic Resonance (NMR), multivariate analysis (MVA), and time series statistics. Urine and milk did not prove useful for identification of sporidesmin intoxication. Serum metabolites differed between treated cows before and after administration of the toxin, and could distinguish samples belonging to the clinical group. The metabolites that were identified as being relevant to this classification were a mixture of glycoproteins, carboxylic acids, ketone bodies, amino-acids, glutamate, and glycerol, which were elevated for treated cattle, and acetate, choline, isoleucine, trimethylamine N-oxide, lipids, lipoproteins, cholesterol, and  $\alpha$ -glucose, which showed decreased concentrations. Citrate was found to be at higher concentration in non-responders and subclinicals only.

When serum was analysed using ultra performance liquid chromatography electrospray ionisation mass spectrometry (UPLC/ESI-MS) and UPLC tandem MS (MS/MS), only samples from clinical cows could be discriminated. The molecular ions involved could be tentatively identified as a combination of taurine- and glycine-conjugated bile acids. These bile acids all became elevated.

This study confirmed that liver enzyme activities (GGT, GDH) and phytoporphyrin concentrations are not effective as markers of early stage sporidesmin damage. Additionally, the new techniques were unable to detect early stage FE. However, some markers of treated cows were identified. The research does provide a strong foundation for future applications of metabolomics analysis, with MVA and time series statistics, for early stage FE diagnosis.

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“A goal without a plan is just a dream”

## PREFACE

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Histological analysis, was conducted by Dr Mark Collett, at the Institute of Veterinary and Biomedical Sciences (IVABS), Massey University, Palmerston North, New Zealand.

The statistical models for GAM and time series analysis were developed by Dr. Jonathan Marshall, Institute of Fundamental Sciences (IFS), Massey University, Palmerston North.

UPLC/MS metabolite measurements of serum were carried out at AgResearch Grasslands, Palmerston North under the supervision of Scott Harrison and Karl Fraser. Data pre-processing was undertaken by Mingshu Cao, AgResearch, Palmerston North.

The UPLC/MS/MS data was obtained by Dr. Ariane Khant of Auckland University, New Zealand, at the Danish Technical University, Lyngby, Denmark.

NB:

Throughout this report, the use of the term sporidesmin denotes the sporidesmin A variant produced by *Pithomyces chartarum*.

The use of the term phytoporphyrin is used synonymously to phylloerythrin irrespective of its use in the relevant published article.

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## LIST OF ABBREVIATIONS

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ACN	Acetonitrile
AGR	Albumin Globulin Ratio
AIC	Akaike information criterion
Alb	Albumin
ANIT	$\alpha$ -naphthylisothiocyanate
ANOVA	Analysis of variance
BA	Bile acid
BDL	Bile duct ligation
BHBA	$\beta$ -hydroxybutyric acid
Bil	Bilirubin
BSP	Bromsulphthalein
CAT	Correlation-adjusted t-score
CI	Chemical ionisation
CID	Collision induced dissociation
CPMG	Carr, Purcell, Meiboom and Gill
Da	Daltons
DSS	4, 4 – dimethyl-4-silapentane-1-sulfonic acid
EI	Electron ionisation
ESI	Electrospray ionisation
ETP	Epidithiodioxopiperazine
FE	Facial eczema
GAM	Generalised additive model
GC	Gas chromatography
GDH	Glutamate dehydrogenase

GGT	Gamma-glutamyl transferase or $\gamma$ -glutamyl transferase
Glo	Globulin
GPC	Glycerophosphocholine
GST	Glutathione S-transferase
H & E	Haematoxylin and Eosin
HDL	High density lipoproteins
HILIC	Hydrophilic interaction chromatography
HMDB	Human metabolome database
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single quantum correlation
HSS	High strength silica
IFS	Institute of Fundamental Sciences
IVABS	Institute of Veterinary, Animal and Biomedical Sciences
JRES	J-Resolved spectroscopy
kHz	kilohertz
LC	Liquid chromatography
LDH	Lactate dehydrogenase
LDL	Low density lipoproteins
LIC	Livestock Improvement Corporation
MALDI	Matrix-assisted laser desorption/ionisation
Mgcv	Mixed GAM computational model
MHC	Major histocompatibility complex
MPI	Ministry of Primary Industries
MS	Mass spectrometry
MS1	First mass spectrometer (in MS/MS series)
MS2	Second mass spectrometer (in MS/MS series)

MS/MS (MS <sup>2</sup> )	Tandem mass spectrometry
MW	Molecular weight
MVA	Multivariate analysis
m/z	Mass to charge ratio
NAD <sup>+</sup>	Nicotinamide adenine dinucleotide oxidised form
NADH	Nicotinamide adenine dinucleotide reduced form
NMR ( <sup>1</sup> H)	Proton nuclear magnetic resonance
NOESY	Nuclear overhauser effect spectroscopy
NP	Normal-phase
NZ	New Zealand
NZIER	New Zealand Institute for Economic Research
NZVP	New Zealand Veterinary Pathology
OCT	Ornithine carbamoyl transferase
OPLS-DA	Orthogonal partial least squares-discriminant analysis
PAR	Pareto scaling
PCA	Principal components analysis
PCV	Packed cell volume
PLS-DA	Partial least squares-discriminant analysis
ppm	parts per million
RF	Radio frequency
ROS	Reactive oxygen species
RP	Reverse-phase
RT	Retention time
SDA	Shrinkage discriminant analysis
SCC	Somatic cell count
Spp	Species pluralis

SPS	Sire proving scheme
SW	Spectral width
TCA	Tricarboxylic acid cycle
TMAO	Trimethylamine N-oxide
TOCSY	2D $^1\text{H} - ^1\text{H}$ total spin correlation spectroscopy
TP	Total protein
TSP	3-(trimethylsilyl) propanoic acid
UPLC	Ultra performance liquid chromatography
UV	Univariate scaling
VIPcv	Variable importance in projection cross validation
VLDL	Very low density lipoproteins

