Metabolomics: applications to food science & nutrition research

Rupa Mandal/David Wishart, TMIC, University of Alberta, Canada

Nov. 16, 2017 TEAGASC, Ireland

Outline

- The Metabolomics Innovation Centre (TMIC)
- Metabolomics for food analysis
- Alberta Food Composition Project
- Food and Metabolomics Databases
- Metabolomics in livestock analysis
- Conclusions

The Metabolomics Innovation Centre

Comprehensive, Quantitative
Metabolomics Services

www.metabolomicscentre.ca

TMIC
The Metabolomics Innovation Center

About TMIC

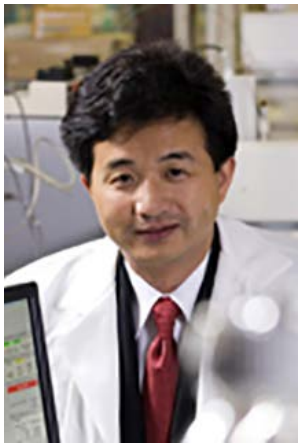
MTDC
Metabolomics Technology Demonstration Centre

- **Established in 2011, funded by Genome Canada to meet Canada's growing demand for high quality, high throughput metabolomic services**
- **TMIC is Canada's *national metabolomics laboratory and national metabolomics technology demonstration centre (MTDC)***
- **>$30 million in equipment distributed across 4 nodes at the UofA,1 node at UVic, 1 node at McGill, 1 node at McMaster -- 30 staff and trainees**
- **Accounts for 80% of Canada's metabolomics publications**

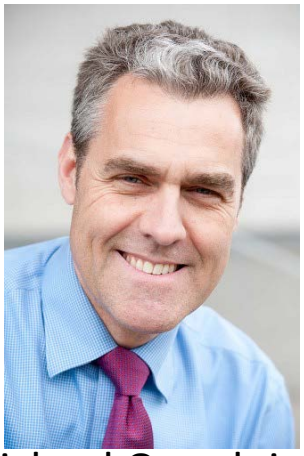
www.metabolomicscentre.ca

Leadership

Dr. David Wishart
Director, UofA



Dr. Liang Li, Core Scientist
UofA



Dr. Michael Overduin, Core
Scientist, UofA

Dr. Christoph Borchers,
Co-Director, UVic & McGill

Dr. James Harynuk, Core
Scientist, UofA

Dr. Philip Britz-McKibbin,
Core Scientist, McMaster

A Distributed Centre

The Metabolomics Innovation Centre (TMIC) - Comprehensive and Quantitative Metabolomics

November 16, APC Forum, UCC

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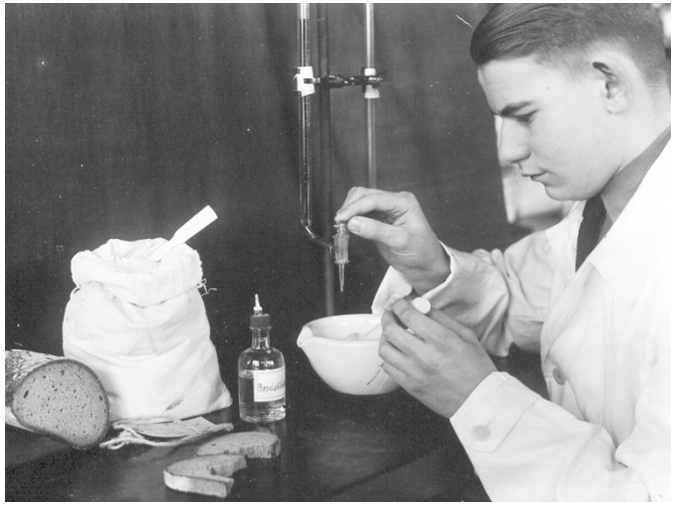
Some Definitions

- **Food Composition Analysis – The determination of the chemical (metabolite) components in food**
- **Food Biomarkers – Food components or food metabolites found in the metabolome that are characteristic of specific foods**
- **Metabolomics – The high throughput analysis and characterization of the chemicals constituting the metabolome**

Food Component Analysis

Traditional

- Protein
- Fat
- Ash
- Minerals
- Carbohydrates
- Calories
- Water content



Metabolomics

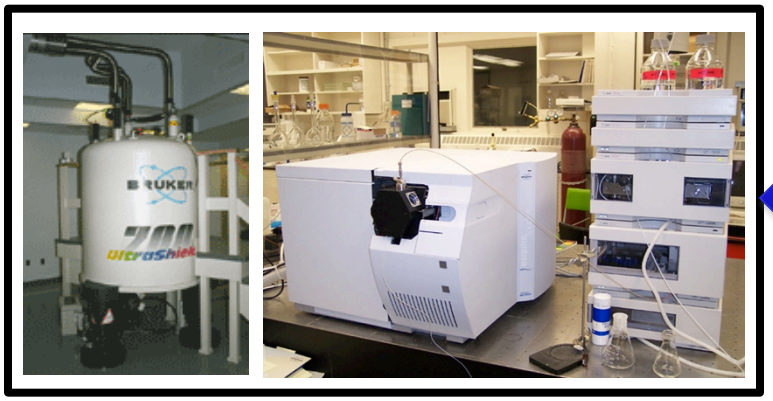
- Alanine
- Tryptophan
- Methyl-histidine
- PE(18:0/18:2)
- PE(16:0/18:1)
- TG(16:0/16:0/18:0)
- Phosphate
- Calcium
- Zinc
- Fructose
- Glucose
- N-acetylglucsoamine
- Apigenin
- Gallic acid
- Resveratrol
- Epigallocatechin Gallate
- Proline betaine
- …..

The Metabolomics Workflow

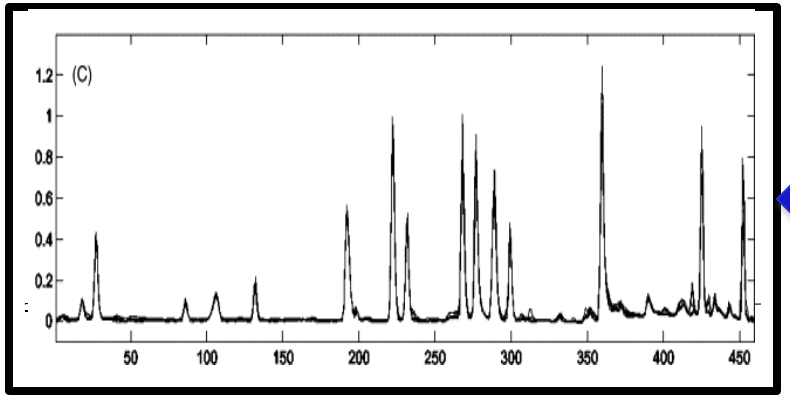
Biological or Tissue Samples

Extraction

Biofluids or Extracts



Chemical Analysis

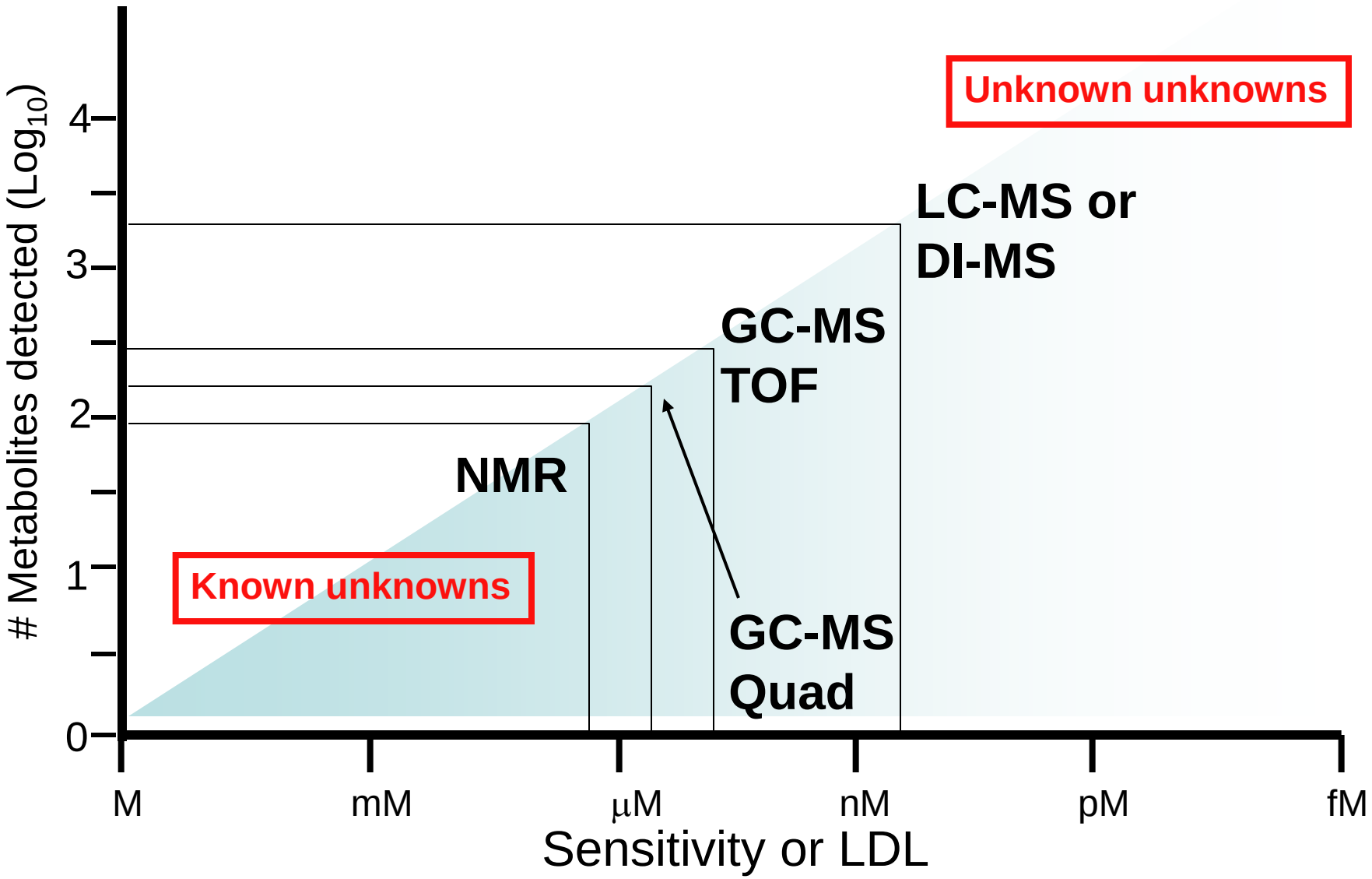


Data Analysis

Metabolomics Technologies

- UPLC, HPLC
- CE/microfluidics
- GC-MS
- LC-MS
- LC-MS/MS
- ICP-MS
- NMR spectroscopy
- X-ray crystallography
- FTIR

Technology & Sensitivity

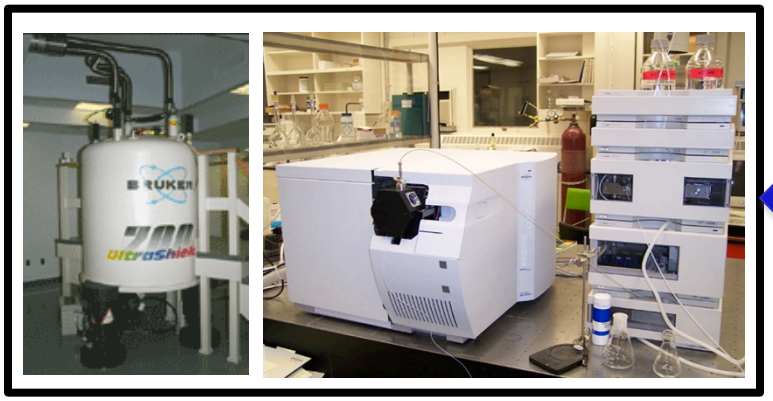


Metabolomics for Food Analysis

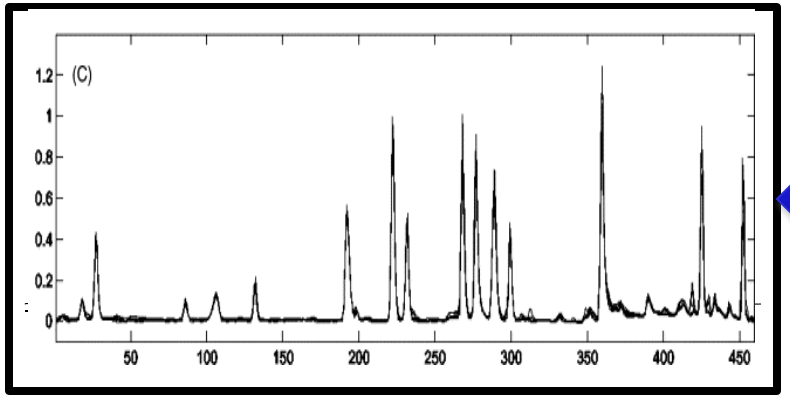
Food Samples

Extraction

Food Extracts



Chemical Analysis



Data Analysis

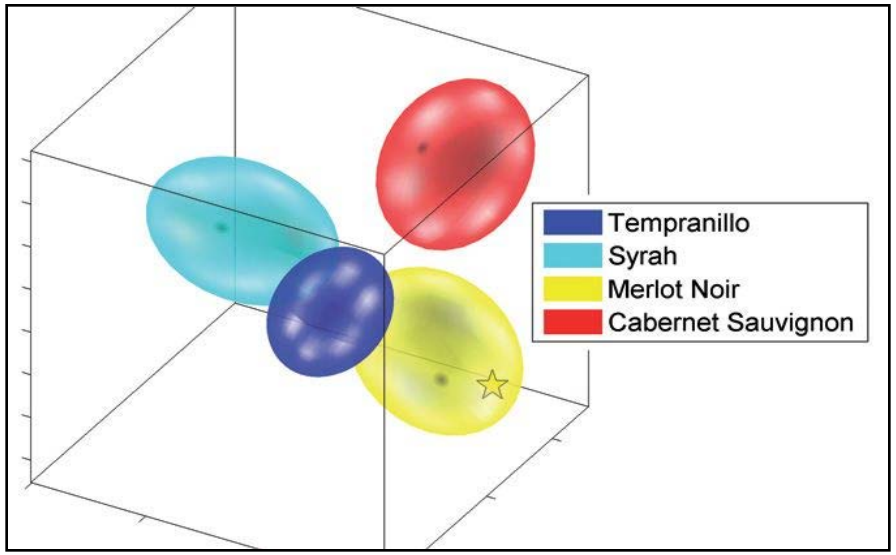
Advantages of Metabolomics

- **Applicable to food composition analysis and food biomarker analysis**
- **Uses rapid, high throughput methodologies (robotics, UPLC, MS/MS)**
- **Offers exquisite sensitivity (< 1 nM)**
- **Can be absolutely quantitative**
- **Can detect 100s to 1000s of compounds vs. only 10’s for conventional methods**

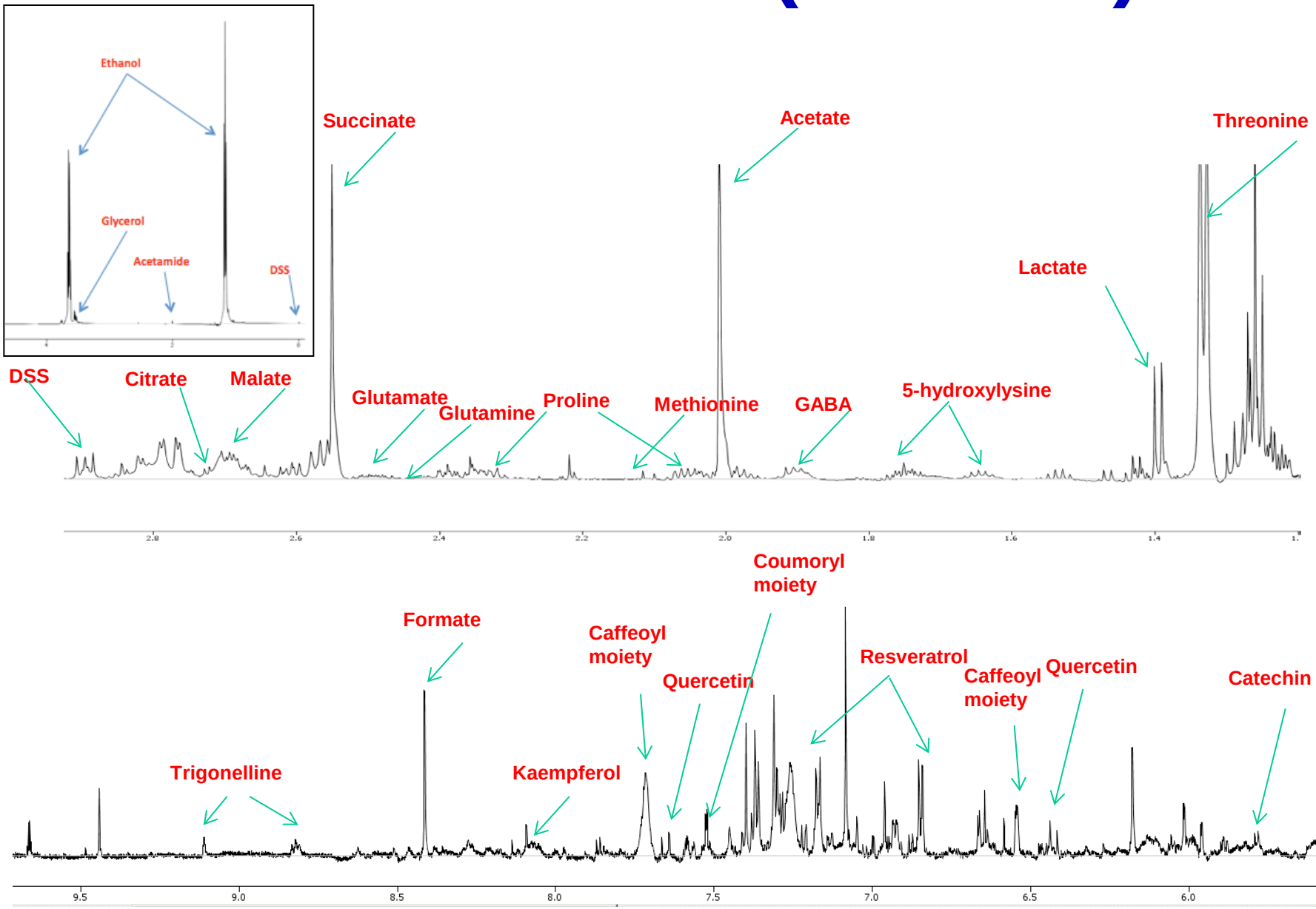
WineScreener & JuiceScreener

Bruker BioSpin ($600,000)

Compound	Value	Unit	Official Reference Flag	Official Reference max. Value	Wine-Profiling™ Authentic NMR Database
acetic acid	525	mg/L	○	-	165 … 1007
benzoic acid	<5	mg/L	●	0 mg/L	<5 mg/L in reference set
citric acid	299	mg/L	●	1000 mg/L	<200 … 471
ethanol	91.7	g/L	○	-	56.2 … 108.0
fumaric acid	<5	mg/L	○	-	<5 … 12
glycerol	7.1	g/L	○	-	4.5 … 11.1
malic acid	5.5	g/L	○	-	<0.2 … 8.2
methanol	53	mg/L	●	250 mg/L	6 … 146
shikimic acid	88	mg/L	○	-	<20 … 100
sorbic acid	<5	mg/L	●	200 mg/L	<5 mg/L in reference set



NMR of Wine (at TMIC)



Milk Metabolomics

- **The Chemical Composition of Cow's Milk**
- **Skim milk, 1%, 2% and 3.25%**
- **Applied a combination of modern, quantitative metabolomics techniques along with state-of-the-art, computer-aided literature mining techniques to obtain the most comprehensive and up-to-date characterization of the chemical constituents in cow's milk**
- **NMR, GC-MS, ICP-MS, LC-MS/MS, GC-FAMEs**

Milk Metabolomics

Assays	No. of Metabolites
NMR	39
DI/LC-MS/MS	116
ICP-MS (metals)	32
HPLC (vitamins)	12
GC-MS	30
Text Mining	255

Total No. metabolites : > 470

Manuscript preparation in progress

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The Alberta Food Composition Project

- **A 5 year metabolomics project aimed at measuring the chemical composition of ~50 Alberta-grown food products (meat, poultry, dairy, cereals, oils, vegetables & fruits)**
- **Measuring 100's of compounds via *quantitative* metabolomics techniques**
- **Intent is to extend and validate literature-based composition data and identify candidate food biomarkers**

Using Multiple Platforms

TMIC
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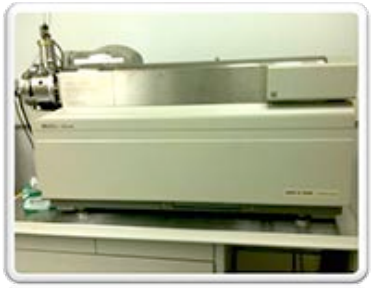
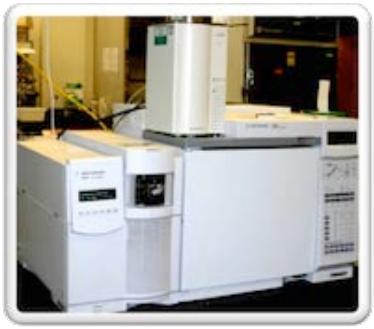
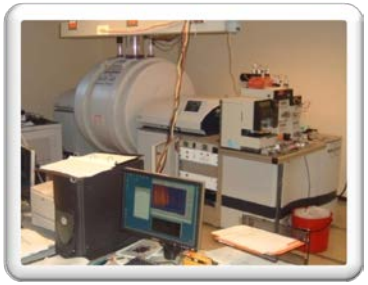
Type	Model
NMR	Bruker 700 MHz cryoprobe & autosampler
NMR	Varian/Agilent 600 MHz
ICP-MS	Perkin Elmer NexION 350
GC-MS	2x Agilent 7890A GC-MS with autosampler
GC-MS	Aglient/HP Series 5890 GC-MS
LC-MS	Bruker 9.4T FT-ICR MS w. cap HPLC
LC-MS	2x ABI Qtrap 4000 MS w. Turbo ESI
LC-MS	Agilent LC-ESI ToF MS
LC-MS	2x Bruker maXis II qTOF
HPLC	Agilent w. Fluorescent detector
UPLC	2x Agilent 1290 Infinity UPLC


www.metabolomicscentre.ca

Typical Results

- **~50 water-soluble metabolites by NMR**
- **~80 compounds by DFI/LC-MS/MS via the Biocrates AbsoluteIDQ™ kit**
- **~400 lipids and fatty acids via GC-MS/LC-MS**
- **53 trace elements by ICP-MS**
- **48 small metabolites via GC-MS**
- **17 polyphenols**
- **9 water- and lipid-soluble vitamins**
- ***Identification & quantification of ~600 cmpds***

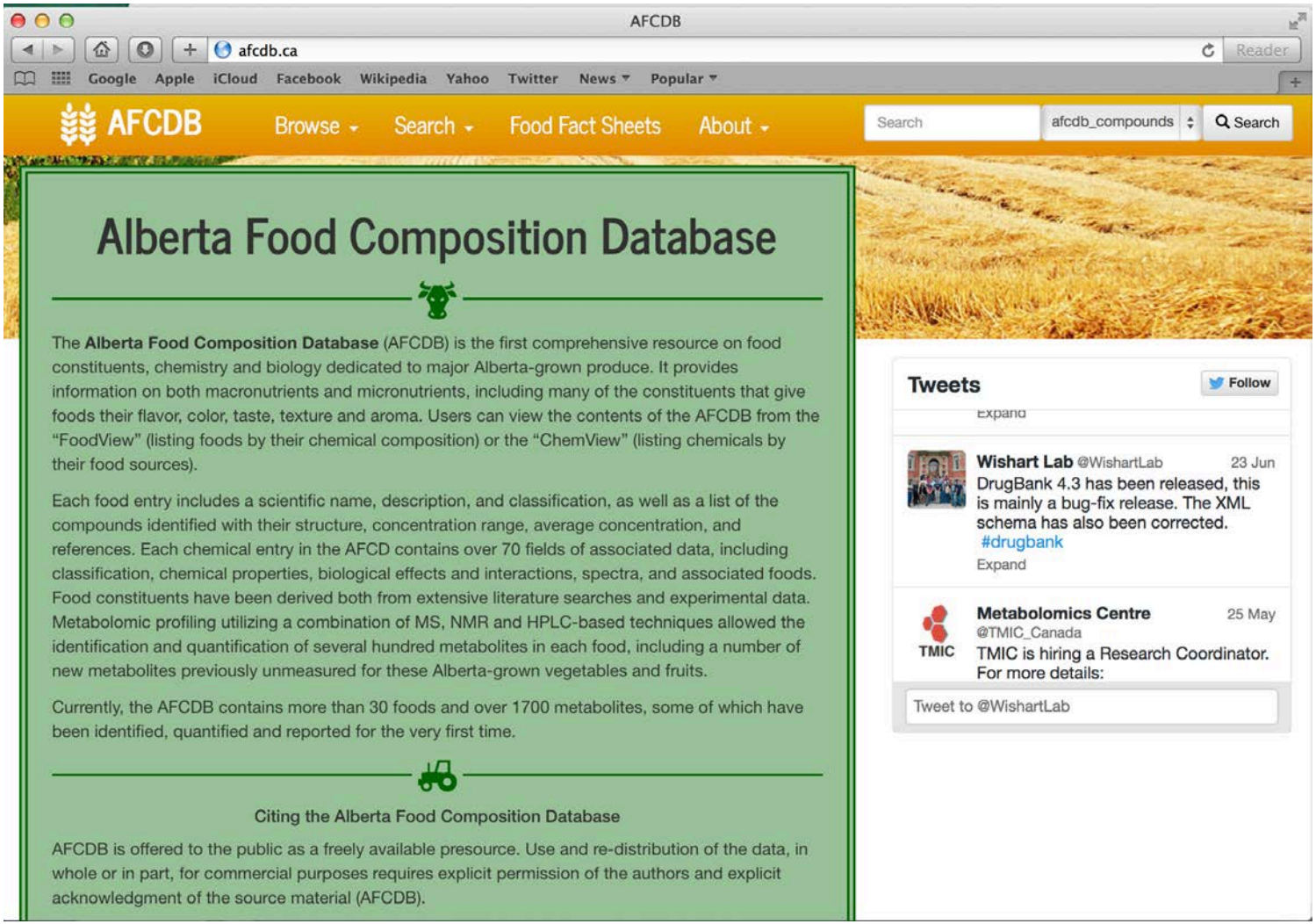
Metabolomics of Beet Root

Numbers of Quantified Compounds

HPLC	NMR	GCMS	GCMS (volatiles)	ICPMS	DIMS	Lipidomics
34	37	26	41	38	85	~400
Polyphenols, vitamins, chlorophylls, anthocyanins	Organic acids, amino acids, amines, sugars, polyols	Organic acids, amino, acids, fatty acids	Volatile organics, thiols, ketones, esters	Metals	Carnitines, amino acids, phospholipids, amines	Neutral lipids, cholesterols Phytosterols, fatty acids

METHOD

Alberta Food Composition Database



www.afcdb.ca

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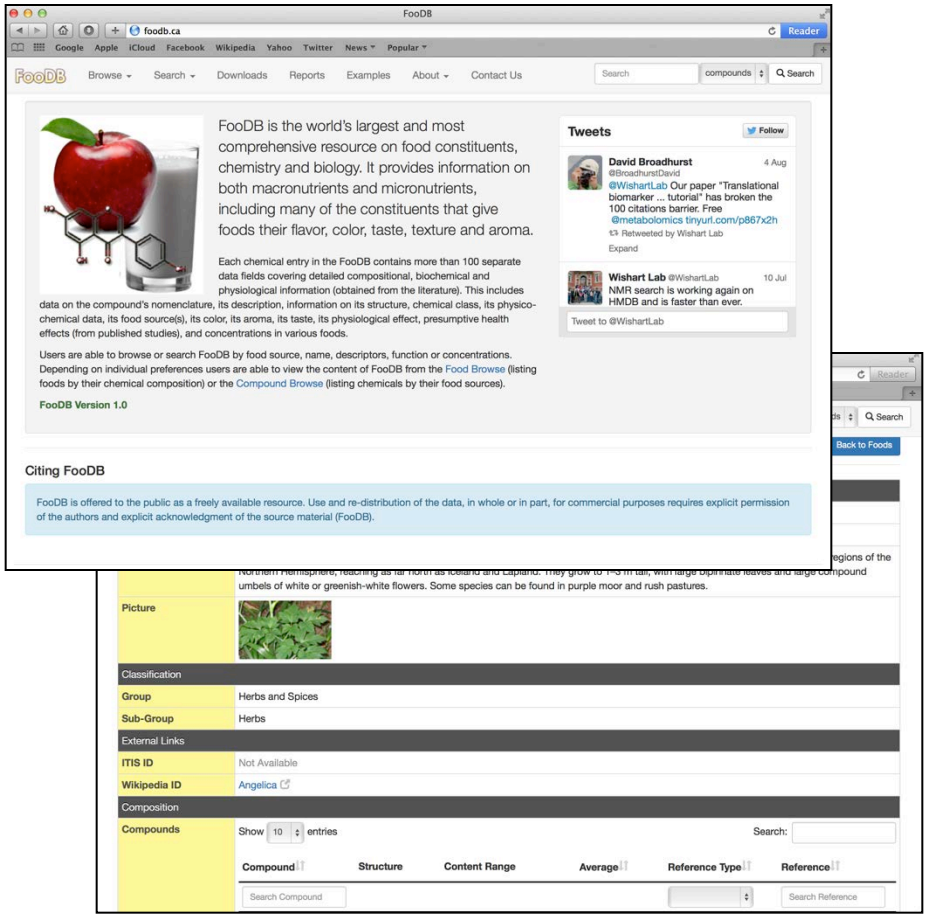
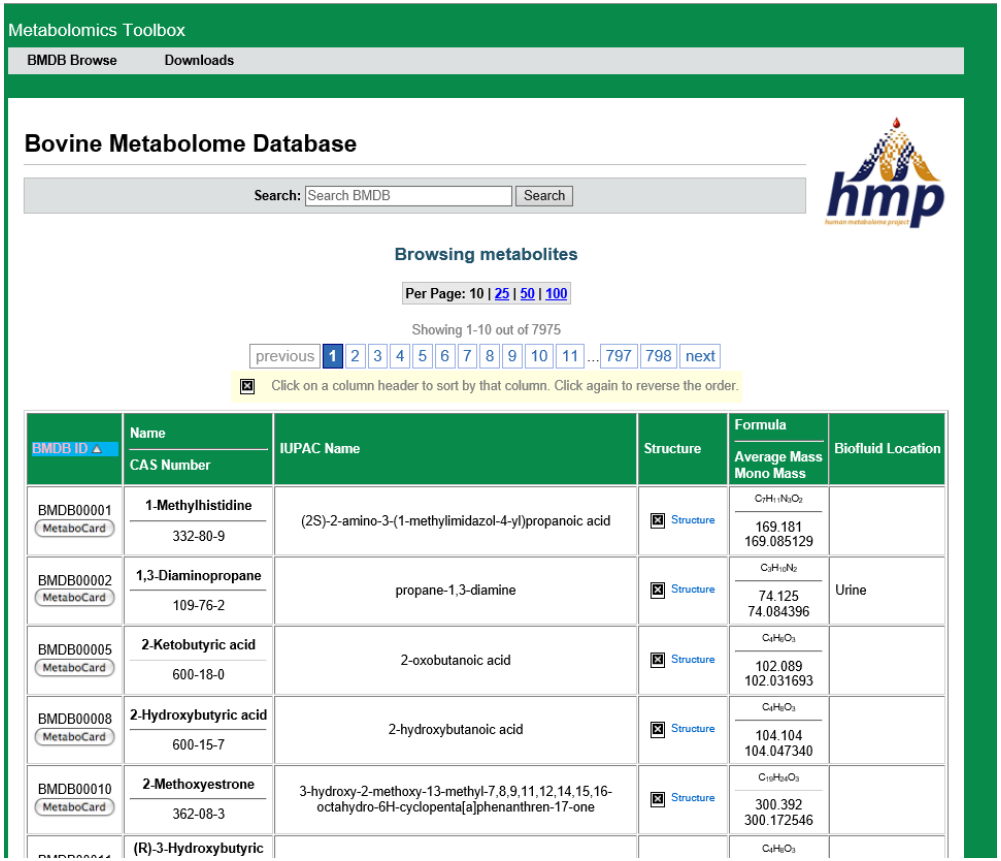
The Food Database (FooDB)



www.foodb.ca

- 26,619 compounds, 25,579 structures with 24,843 descriptions
- 171,359 synonyms
- ~700,000 concentration values
- 31,791 references
- 1376 cmpds with health effects
- 2692 cmpds with flavour data
- Content data on 907 raw or processed foods
- Supports structure & text searches
- >100 data fields/compound
- Full data downloads

Bovine Metabolome Database

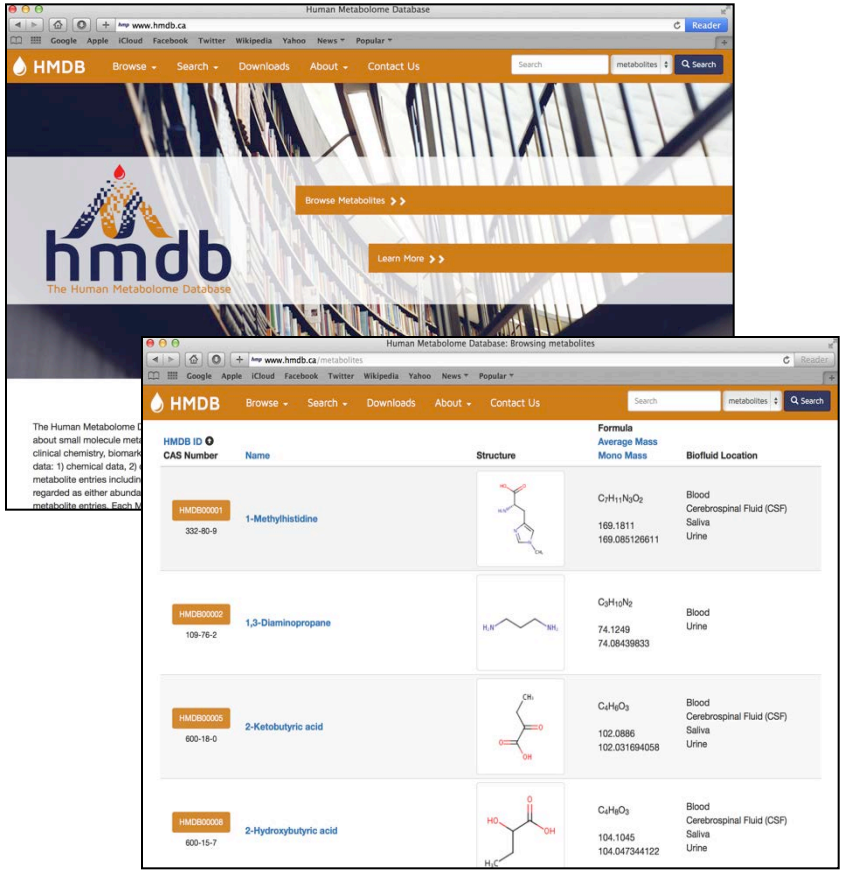


BMDB ID	Name / CAS Number	IUPAC Name	Structure	Formula / Average Mass / Mono Mass	Biofluid Location
BMDB00001 MetaboCard	1-Methylhistidine / 332-80-9	(2S)-2-amino-3-(1-methylimidazol-4-yl)propanoic acid	Structure	$C_7H_{11}N_3O_2$ / 169.181 / 169.085129	
BMDB00002 MetaboCard	1,3-Diaminopropane / 109-76-2	propane-1,3-diamine	Structure	$C_3H_{10}N_2$ / 74.125 / 74.084396	Urine
BMDB00005 MetaboCard	2-Ketobutyric acid / 600-18-0	2-oxobutanoic acid	Structure	$C_4H_6O_3$ / 102.089 / 102.031693	
BMDB00008 MetaboCard	2-Hydroxybutyric acid / 600-15-7	2-hydroxybutanoic acid	Structure	$C_4H_8O_3$ / 104.104 / 104.047340	
BMDB00010 MetaboCard	2-Methoxyestrone / 362-08-3	3-hydroxy-2-methoxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthren-17-one	Structure	$C_{19}H_{24}O_3$ / 300.392 / 300.172546	
BMDB00011	(R)-3-Hydroxybutyric			$C_4H_8O_3$	

- ~ 8000 compounds
- Supports structure & text searches
- >100 data fields/compound
- Full data downloads

http://www.cowmetdb.ca

The Human Metabolome Database (HMDB)



http://www.hmdb.ca

- Comprehensive database of human metabolites found in biofluids or tissues (Version 4 released in 2017)
- Old version had 41,993 metabolites, new version has 114,100 “quantified”, “detected”, “expected” and “predicted” metabolites
- Old version had 442 biological pathways, new version has 26,515
- New version has >200,000 MS/MS spectra at multiple collision energies
- New version has 5200 metabolite-SNP interactions
- Supports sequence, spectral, structure and text searches as well as compound browsing

Livestock metabolomics and the livestock metabolome: A systematic review

Seyed Ali Goldansaz, An Chi Guo, Tanvir Sajed, Michael A. Steele, Graham S. Plastow, David S. Wishart

Published: May 22, 2017 • https://doi.org/10.1371/journal.pone.0177675

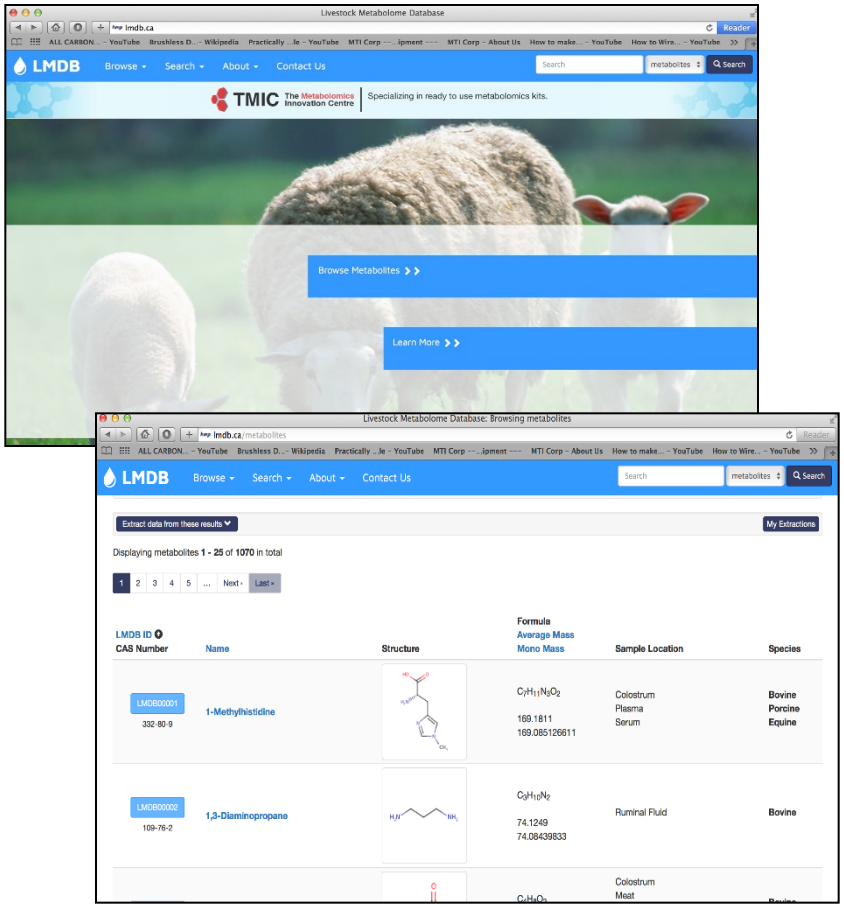
Abstract
Introduction
Materials and methods
Results and discussion
Conclusion
Supporting information
Author Contributions
References

Reader Comments (0)
Media Coverage (0)
Figures

Abstract

Metabolomics uses advanced analytical chemistry techniques to comprehensively measure large numbers of small molecule metabolites in cells, tissues and biofluids. The ability to rapidly detect and quantify hundreds or even thousands of metabolites within a single sample is helping scientists paint a far more complete picture of system-wide metabolism and biology. Metabolomics is also allowing researchers to focus on measuring the end-products of complex, hard-to-decipher genetic, epigenetic and environmental interactions. As a result, metabolomics has become an increasingly popular "omics" approach to assist with the robust phenotypic characterization of humans, crop plants and model organisms. Indeed, metabolomics is now routinely used in biomedical, nutritional and crop research. It is also being increasingly used in livestock research and livestock monitoring. The purpose of this systematic review is to quantitatively and objectively summarize the current status of livestock metabolomics and to identify emerging trends, preferred technologies and important gaps in the field. In conducting this review we also critically assessed the applications of livestock metabolomics in key areas such as animal health assessment, disease diagnosis, bioproduct characterization and biomarker discovery for highly desirable economic traits (i.e., feed efficiency, growth potential and milk production). A secondary goal of this critical review was to compile data on the known composition of the livestock metabolome (for 5 of the most common livestock species namely cattle, sheep, goats, horses and pigs). These data have been made available through an open access, comprehensive livestock metabolome database (LMDB, available at http://www.lmdb.ca). The LMDB should enable livestock researchers and producers to conduct more targeted metabolomic studies and to identify where further metabolome coverage is needed.

Livestock Metabolome Database (LMDB)



http://lmdb.ca/

- **Comprehensive database of bovine, porcine, equine, ovine, caprine metabolomic data**
- **1070 metabolites**
- **33 different biofluids**
- **3234 concentration entries**
- **15,750 NMR & MS spectra**
- **616 references**
- **Fully searchable**

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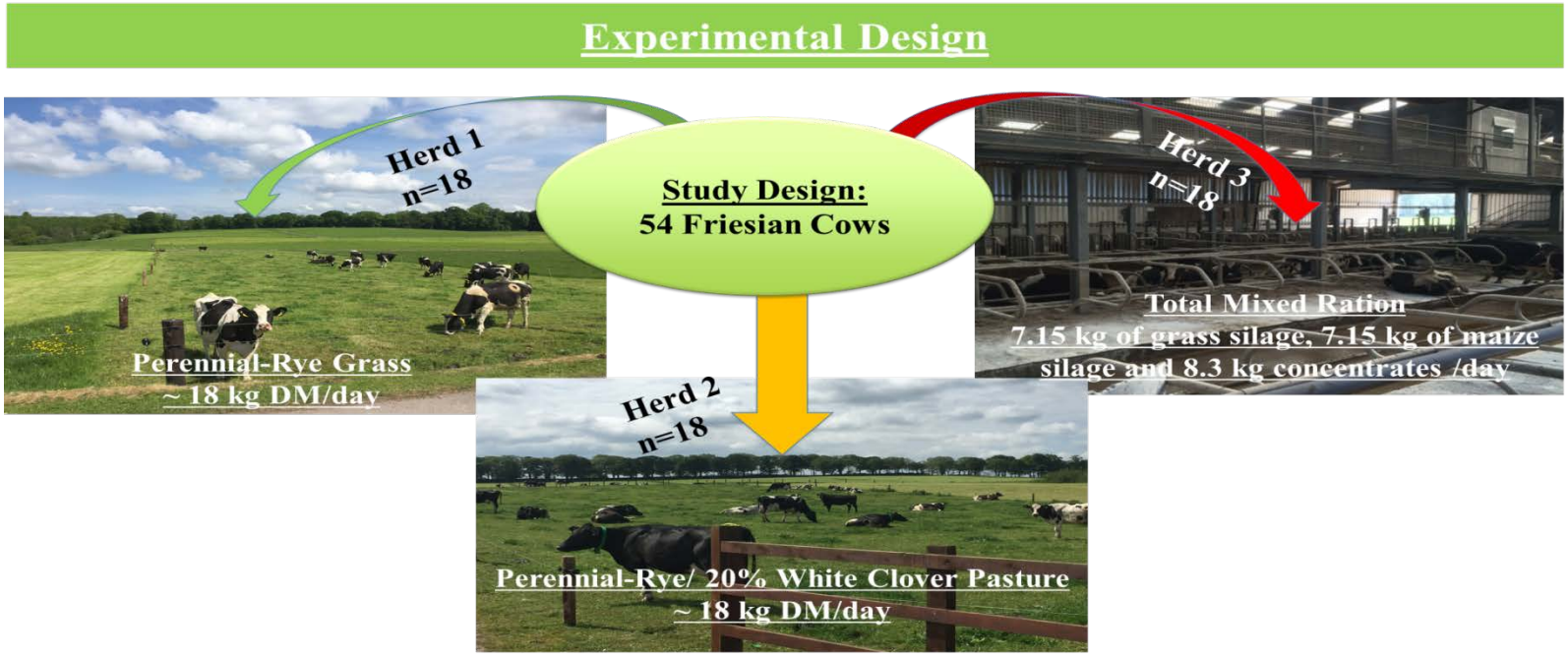
Current Collaboration

Tom O'Callaghan
Catherine Stanton



Examining the effects of Pasture and Conventional indoor TMR cow feeding systems on the rumen and milk metabolomes

Current Collaboration

Teagasc
Agriculture and Food Development Authority

Tom O'Callaghan
Catherine Stanton

+

TMIC

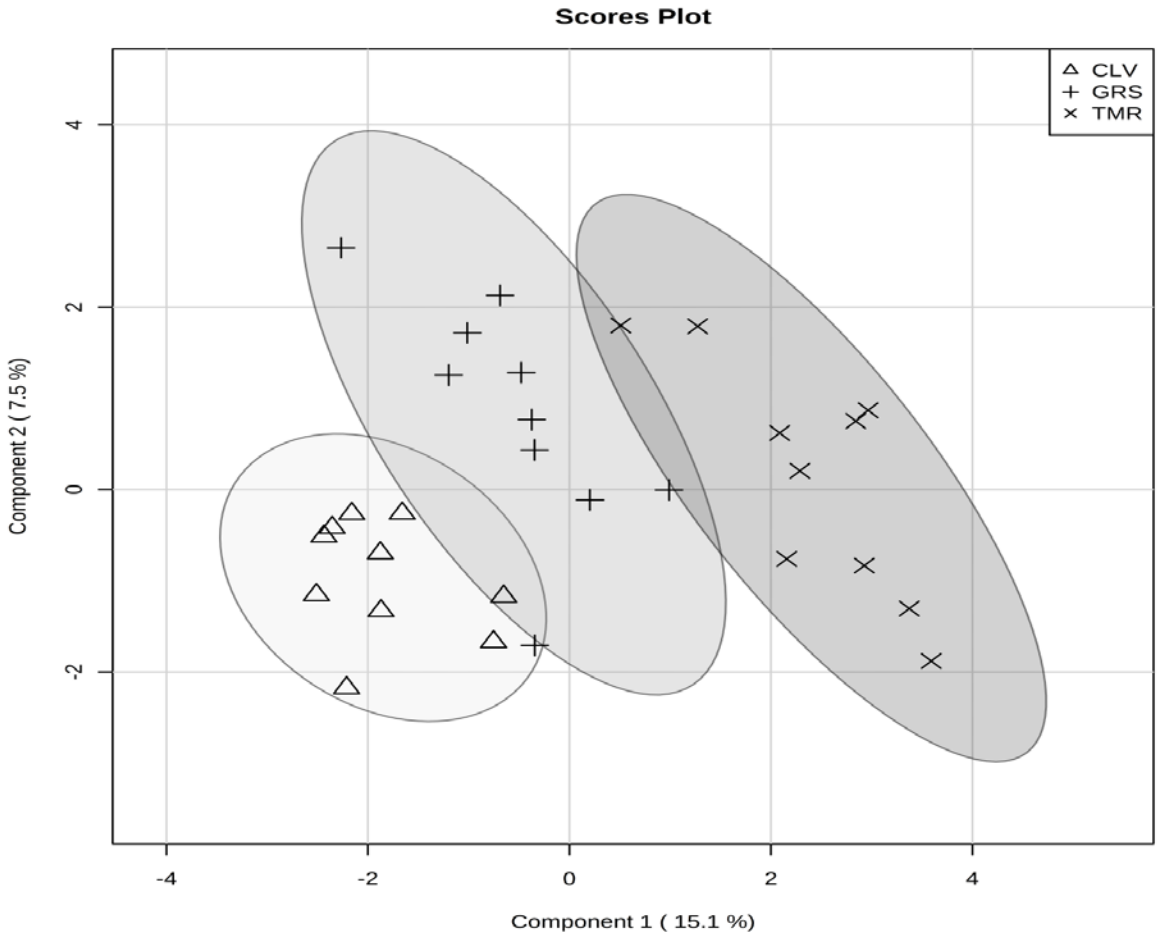
Impact of feeding system on the metabolome of bovine bio-fluids

Preliminary Results indicate:

- The rumen and milk metabolomes from each of the feeding systems are quite diverse and distinctive.
- NMR metabolomic profiling of milks and rumen sample
- CLV feeding system resulted in increased concentrations of formate, a substrate compound for methanogenesis. Milks and rumen-fluids were shown to have varying levels of dimethyl sulfone in each feeding system and was found to be an important compound for distinguishing between diets.
- CLV and GRS feeding systems were found to have increased concentrations of *p*-cresol
- This study has highlighted that ^{1}H-NMR is capable of distinguishing both rumen-fluids and milk samples based on feeding system, which could offer potential as a tool for milk verification purposes in the future

Current Collaboration

Tom O'Callaghan
Catherine Stanton



Manuscript in preparation

Collaboration

Teagasc
Agriculture and Food Development Authority

Paul Ryan
Catherine Stanton

+

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www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Recombinant Incretin-Secreting Microbe Improves Metabolic Dysfunction in High-Fat Diet Fed Rodents

Received: 13 July 2017
Accepted: 2 October 2017
Published online: 19 October 2017

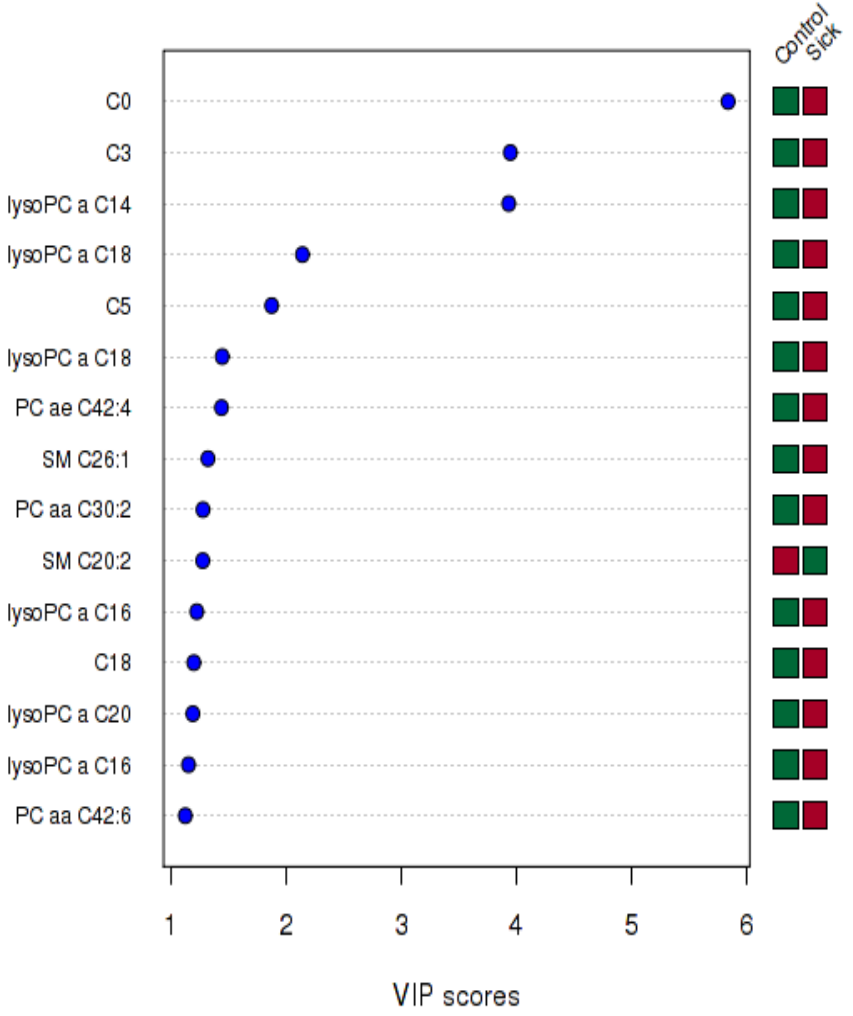
Paul M. Ryan[1,2,3], Elaine Patterson[1,3], Robert M. Kent[1,2], Helena Stack[4], Paula M. O'Connor[1,2], Kiera Murphy[1], Veronica L. Peterson[5], Rupasri Mandal[6], David S. Wishart[6,7,8], Timothy G. Dinan[3,9], John F. Cryan[3,5], Randy J. Seeley[10], Catherine Stanton[1,3] & R. Paul Ross[1,3]

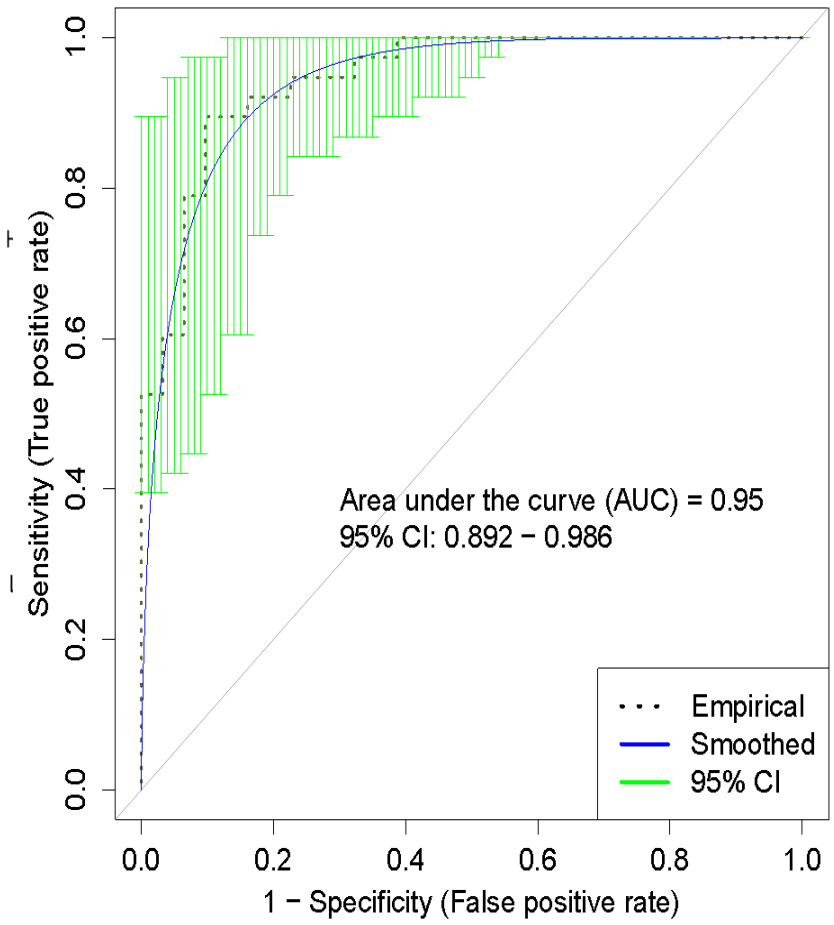
The gut hormone glucagon-like peptide (GLP)-1 and its analogues represent a new generation of anti-diabetic drugs, which have also demonstrated propensity to modulate host lipid metabolism. Despite this, drugs of this nature are currently limited to intramuscular administration routes due to intestinal degradation. The aim of this study was to design a recombinant microbial delivery vector for a GLP-1 analogue and assess the efficacy of the therapeutic in improving host glucose, lipid and cholesterol metabolism in diet induced obese rodents. Diet-induced obese animals received either Lactobacillus paracasei NFBC 338 transformed to express a long-acting analogue of GLP-1 or the isogenic control microbe which solely harbored the pNZ44 plasmid. Short-term GLP-1 microbe intervention in rats reduced serum low-density lipoprotein cholesterol, triglycerides and triglyceride-rich lipoprotein cholesterol substantially. Conversely, extended GLP-1 microbe intervention improved glucose-dependent insulin secretion, glucose metabolism and cholesterol metabolism, compared to the high-fat control group. Interestingly, the microbe significantly attenuated the adiposity associated with the model and altered the serum lipidome, independently of GLP-1 secretion. These data indicate that recombinant incretin-secreting microbes may offer a novel and safe means of managing cholesterol metabolism and diet induced dyslipidaemia, as well as insulin sensitivity in metabolic dysfunction.

Predicting Dairy Cattle Disease Before Parturition

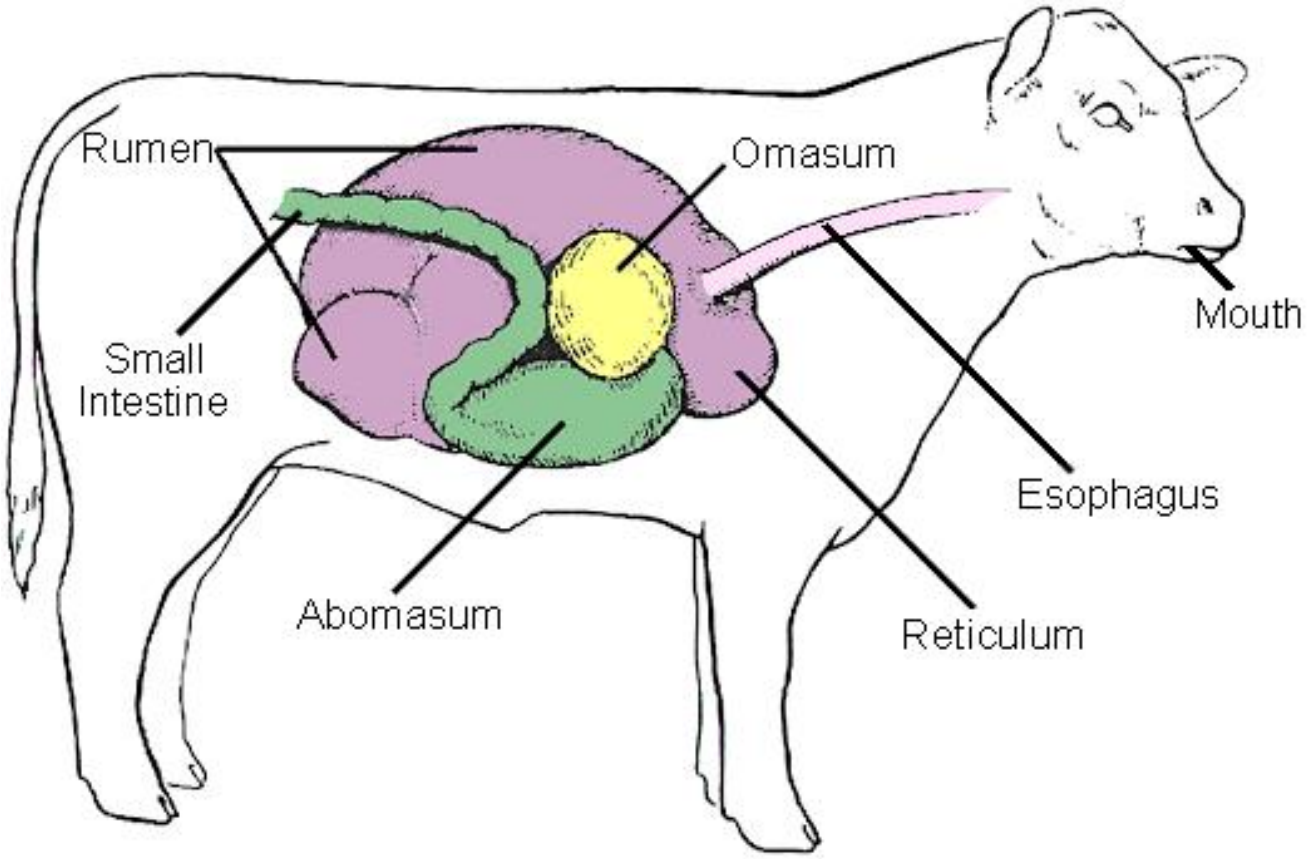
- 30-50% of dairy cows are affected by mastitis, metritis, ketosis and milk fever during transition
- 100,000 dairy cows culled/yr because of these conditions ($200 million in losses)
- Looked at serum from 12 dairy cows (-4 weeks, -1 week, +1 week, + 4 weeks) during the transition period
- 6 developed diseases at +1-+3 weeks, the other 6 stayed healthy

Results (Serum Metabolomics)





Characterizing Ruminal Fluid



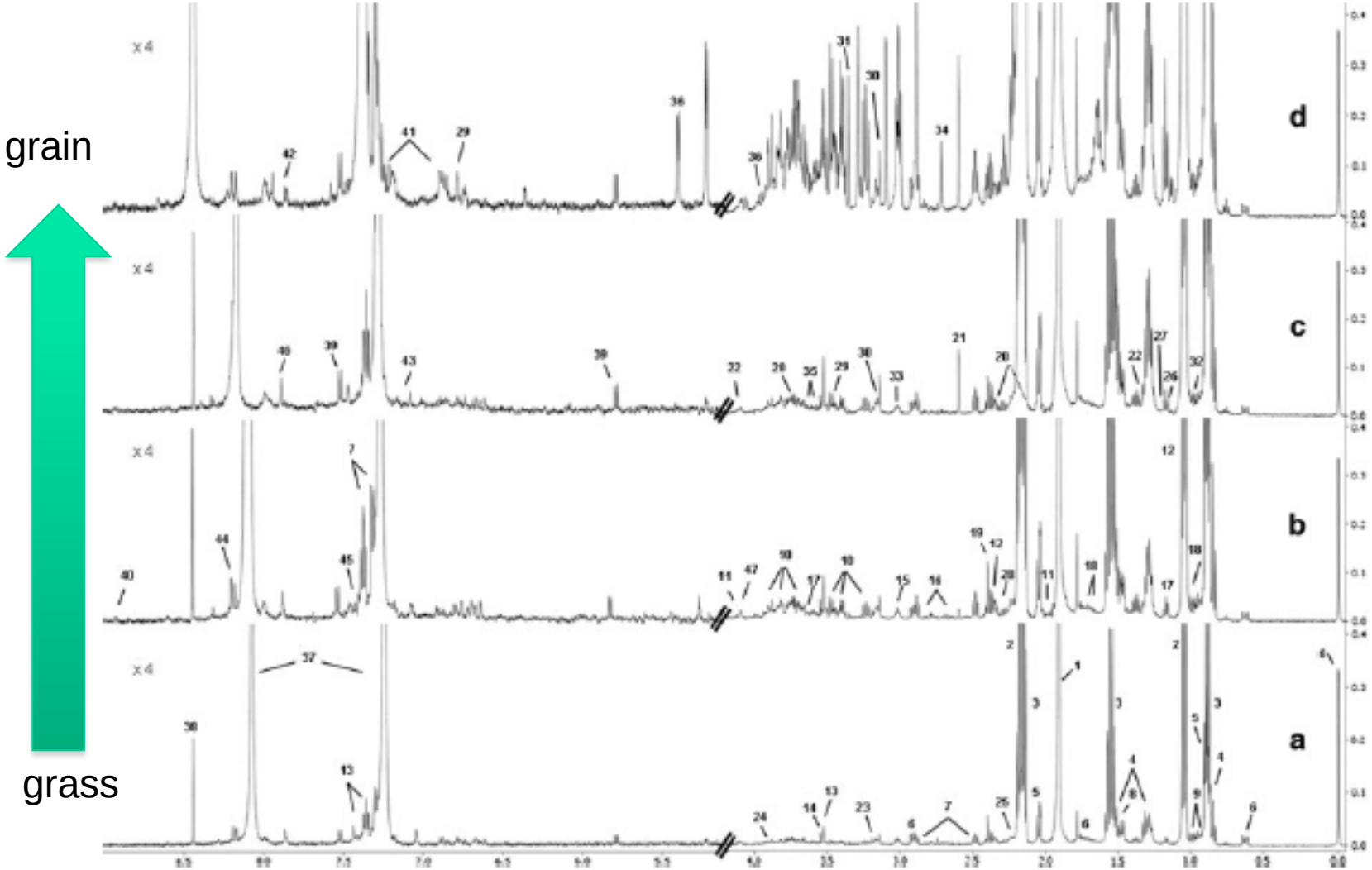


Fig. 1 Typical 500 MHz ^{1}H-NMR rumen fluid spectra (0–9 ppm) from dairy cows fed 0% (**a**), 15% (**b**), 30% (**c**), and 45% (**d**) barley grain. Numbers indicate metabolites, as follows: 1, acetate; 2, propionate; 3, butyrate; 4, valerate; 5, isovalerate; 6, DSS; 7, 3-phenylpropionate; 8, alanine; 9, isoleucine; 10, glucose; 11, proline; 12, isobutyrate; 13, phenylacetate; 14, glycine; 15, cadaverine; 16, aspartate; 17, ethanol; 18, leucine; 19, succinate; 20, glutamate; 21, methylamine; 22, lactate; 23, choline; 24, fumarate; 25, acetone; 26, isopropanol; 27, 3-hydroxybutyrate; 28, acetoacetate; 29, 3-hydroxyphenylacetate; 30, N-nitrosodimethylamine; 31, methanol; 32, valine; 33, lysine; 34, dimethylamine; 35, glycerol; 36, maltose; 37, imidazole; 38, formate; 39, uracil; 40, nicotinate; 41, tyrosine; 42, benzoate; 43, histidine; 44, hypoxanthine; 45, phenylacetylglycine; 46, xanthine; 47, ribose. The intensity of the aromatic region (5.1–9.0 ppm) is 4 times higher than that of the aliphatic one (0.0–4.2 ppm)

The Bovine Ruminal Fluid Metabolome

..., R Mandal, SM Dunn, BN **Ametaj**, DS **Wishart** - Metabolomics, 2013 - Springer

Abstract The rumen is a unique organ that serves as the primary site for microbial fermentation of ingested plant material for domestic livestock such as cattle, sheep and goats. The chemical composition of ruminal fluid is thought to closely reflect the healthy/ ...

Applications of NMR in Dairy Research

AD Maher, SJ Rochfort - Metabolites, 2014 - mdpi.com

... subclinical ketosis. J. Dairy Sci., in press. 47. Saleem, F.; Bouatra, S.; Guo, AC; Psychogios, N.; Mandal, R.; Dunn, SM; **Ametaj**, BN; **Wishart**, DS The bovine ruminal fluid metabolome. Metabolomics 2013, 9, 360–378. 48. **Wishart**, DS ...

A metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows

..., R Mandal, Q Zebeli, SM Dunn, DS **Wishart** - Journal of dairy ..., 2012 - Elsevier

Dairy cows fed high-grain diets during early lactation have a high incidence of metabolic disorders. However, the precise mechanism (s) of how grain feeding causes disease is not clear. In an effort to understand how this diet transition alters the rumen environment and

Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in the diet of dairy cows

..., N Psychogios, MJ Lewis, SM Dunn, J Xia, DS **Wishart** - Metabolomics, 2010 - Springer

Abstract This study presents the first application of metabolomics to evaluate changes in rumen metabolites of dairy cows fed increasing proportions of barley grain (ie, 0, 15, 30, and 45% of diet dry matter). 1 H-NMR spectroscopy was used to analyze rumen fluid samples ...

Predicting Feeding Efficiency

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Plasma metabolites associated with residual feed intake and other productivity performance traits in beef cattle

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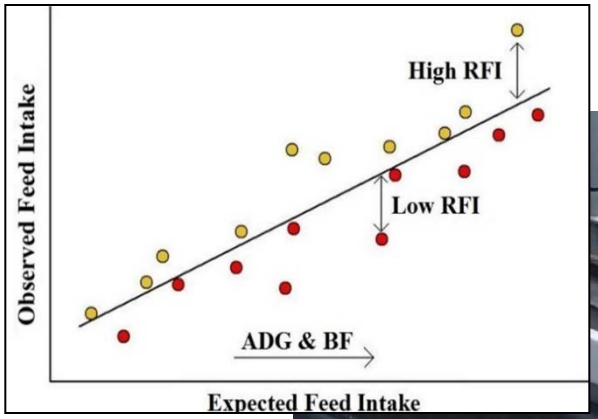
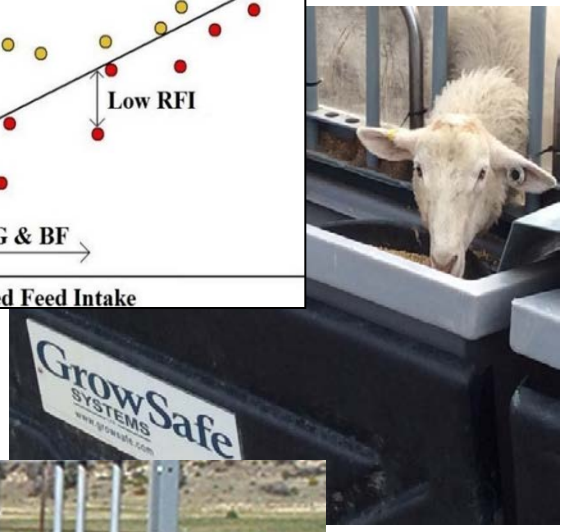
Residual Feed Intake

- Measure of feed efficiency & metabolism
- Defined as the difference between an animal's actual feed intake and its expected feed intake based on its size and growth
- The lower the value, the more efficient the animal
- Moderate heritability, affected by metabolites





Predictive Plasma Metabolites

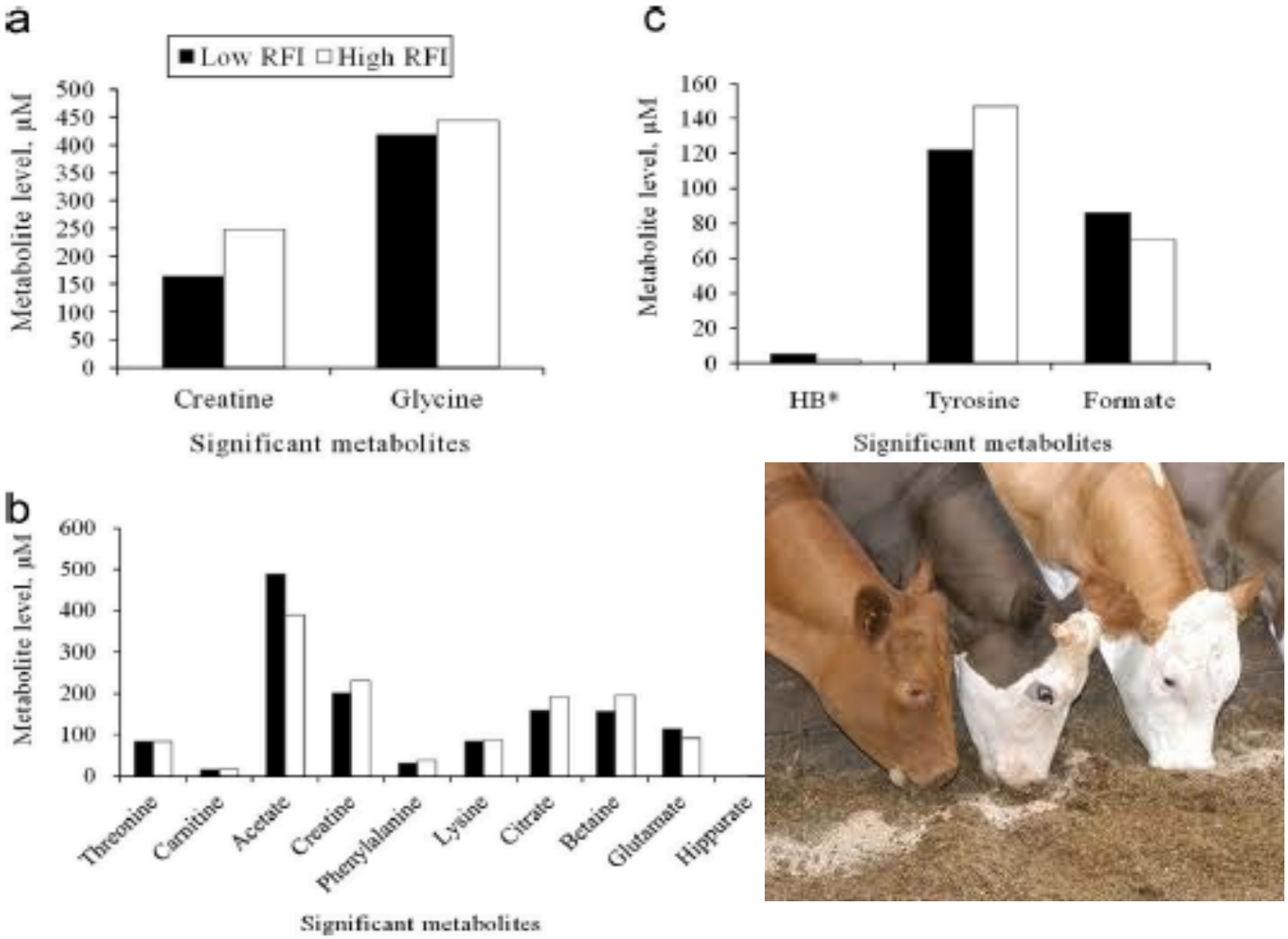


Fig. 1. Average concentrations of significant metabolites in the discovery population in periods 1(A), 2(B) and 3(C).

Predictive Plasma Metabolites

Table 2
Metabolites significantly associated with RFI in the validation population in period 1 (2 weeks into the feeding trial).

Metabolite	Partial *R* square	*P*-value
Valine	0.30	0.003
Creatinine	0.26	0.021
Choline	0.16	0.008
Uridine	0.09	0.004
Histidine	0.08	0.025
Dimethylamine	0.03	0.048
Trimethylamine	0.03	0.023
2-Hydroxybutyrate	0.02	0.044
3-Hydroxybutyrate	0.02	0.005
Total	0.99	

Predictive Plasma Metabolites

Table 3
Metabolites significantly associated with variation in dry matter intake (DMI) in the validation population of beef cattle in period 1 (2 weeks into the feeding trial).

Metabolite	Partial *R*-square	Model *R*-square	*P*-value
Fumarate	0.39	0.39	0.003
Pyroglutamate	0.17	0.56	0.018
Choline	0.14	0.70	0.016
Creatinine	0.13	0.83	0.003
Ornithine	0.09	0.92	0.001
HIB	0.02	0.94	0.035
Dimethylamine	0.02	0.96	0.034
Trimethylamine	0.01	0.97	0.054
Methyloxovalerate	0.01	0.98	0.050
Malate	< 0.01	0.98	0.025
Glucose	< 0.01	0.98	0.036
Citrate	< 0.01	0.98	0.050

Conclusions

- **Metabolomics is transforming food composition analysis**
- **Food composition databases have grown considerably in depth and breadth thanks to metabolomics**
- **Quantitative metabolomics is leading to the identification of dozens of new and useful food biomarkers**
- **Metabolomics is transforming crop and livestock research and agriculture practice**

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