Economic Development, Jobs, Transport and Resources

Prediction of Serum Metabolic Profile Biomarkers in Early Lactation Dairy Cows Using Mid-Infrared Spectroscopy of Milk

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DairyBio

MIRforPROFIT

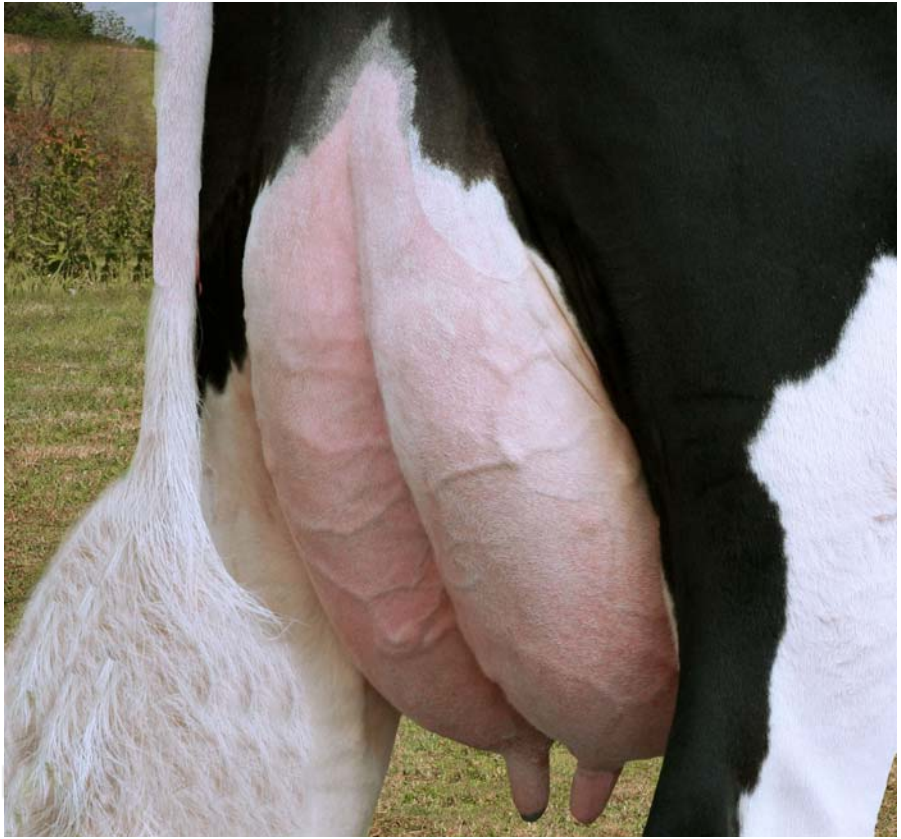
LA TROBE UNIVERSITY

AGRICULTURE VICTORIA

Spot the difference…


FALCON

Both are operating at 4x their resting energy requirements!



SPECIALIZED
GYTR


Sub-optimal performance?

The Bovine Equivalent is Metabolic Disease

- Hypocalcaemia (milk fever)
- Hypomagnesaemia (grass staggers or grass tetany)
- Ketosis (acetonaemia)

Clinical Disease


Sub-optimal performance?

Sub-Clinical Disease


Clinical Disease
Sub-Clinical Disease
For every case of clinical milk fever, you can expect 8+ cases of subclinical milk fever…

If we can’t see it, does it matter?

- ▼ Milk production
- ▼ Milk quality
- ▼ Repro performance
- ▲ Disease
- ▲ Risk of culling
- ▼ Animal health
- ▼ Animal welfare
- ▼ Profitability

So how can we tell if an animal has subclinical metabolic disease?

Metabolic Profiling

Energy → **Protein** → **Mineral**

- NEFA
  - Fat mobilisation
- BHB
  - Ketosis risk

- Urea
- Albumin
- Globulin

- Calcium
- Magnesium
- Phosphorus
- Trace elements
  - Cu, Co, Se

Serum Metabolic Profiling

Metabolite	Lower Threshold	Upper Threshold
BHB	-	1.2 mmol/L
NEFA	-	0.7 mmol/L
Urea	1.7 mmol/L	6.78 mmol/L
Albumin	35 g/L	-
Globulin	-	50 g/L
Calcium	2.0 mmol/L	-
Magnesium	0.82 mmol/L	-

ENERGY

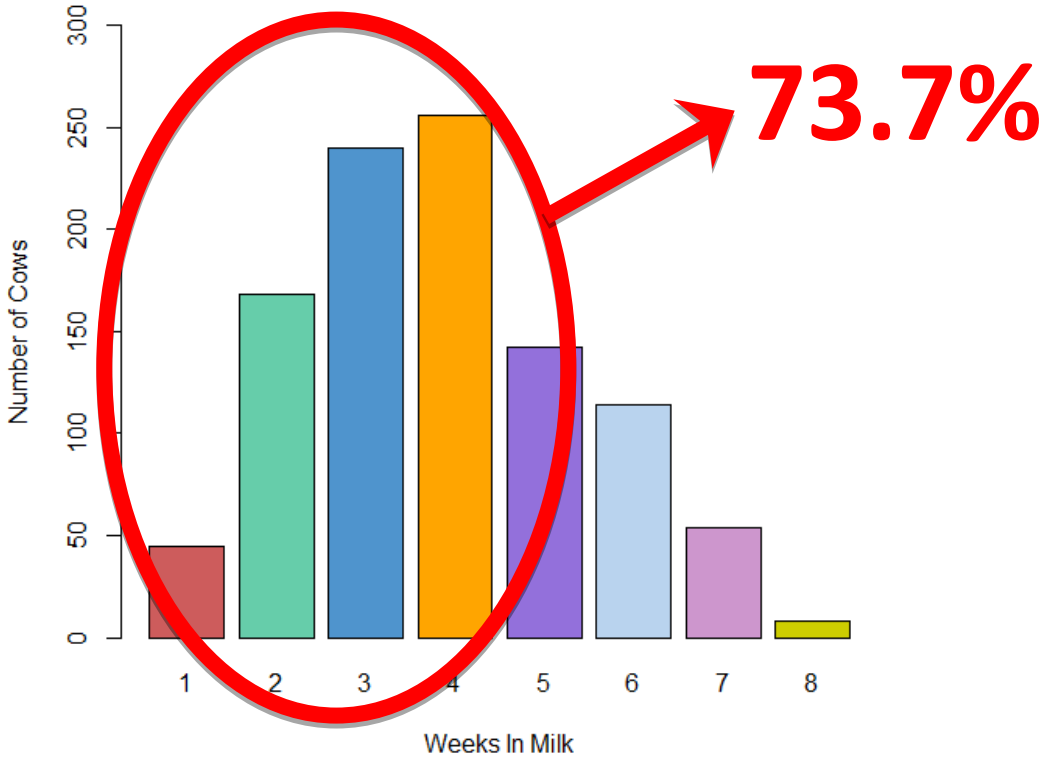
PROTEIN

MINERAL

Experimental Design

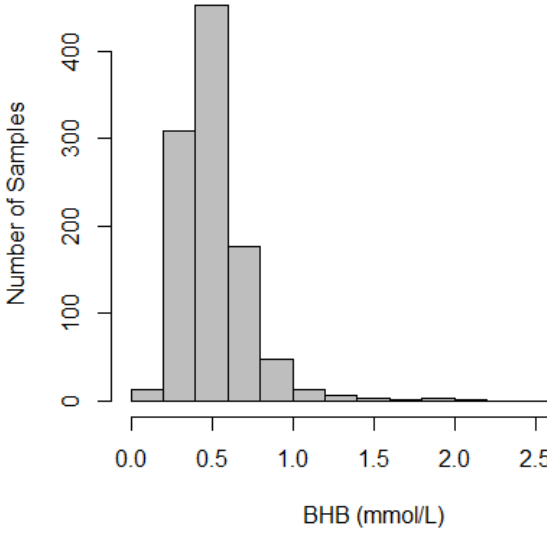
- 1027 genotyped Holstein-Friesian cows
- Blood and milk on same day in Spring 2017
- 5 Farms in Gippsland region, Victoria, Australia (Ginfo)
- First 8 weeks of lactation
- Metabolic profile at commercial lab
- Milk collected by HICO
- MIR by Tasherd
  - Bentley Instruments FTS Combi

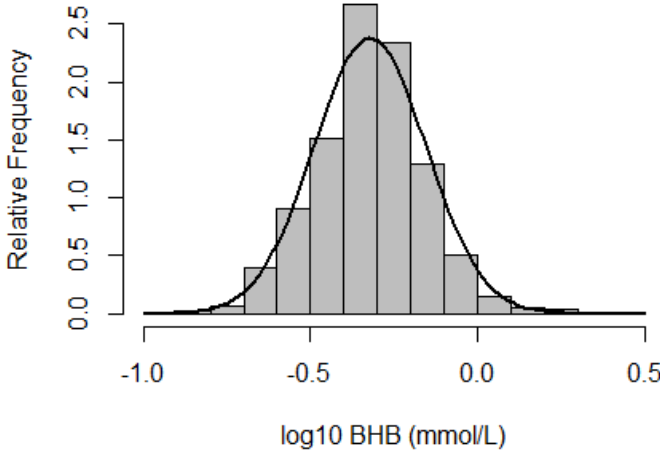
Data Analysis: Descriptive Statistics

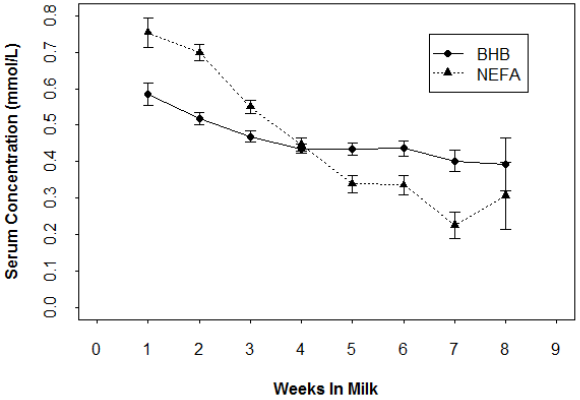


Data Analysis: Metabolic Profile Results

- Metabolite concentrations checked for normal distribution
- NEFA & BHB skewed → log10 transformation

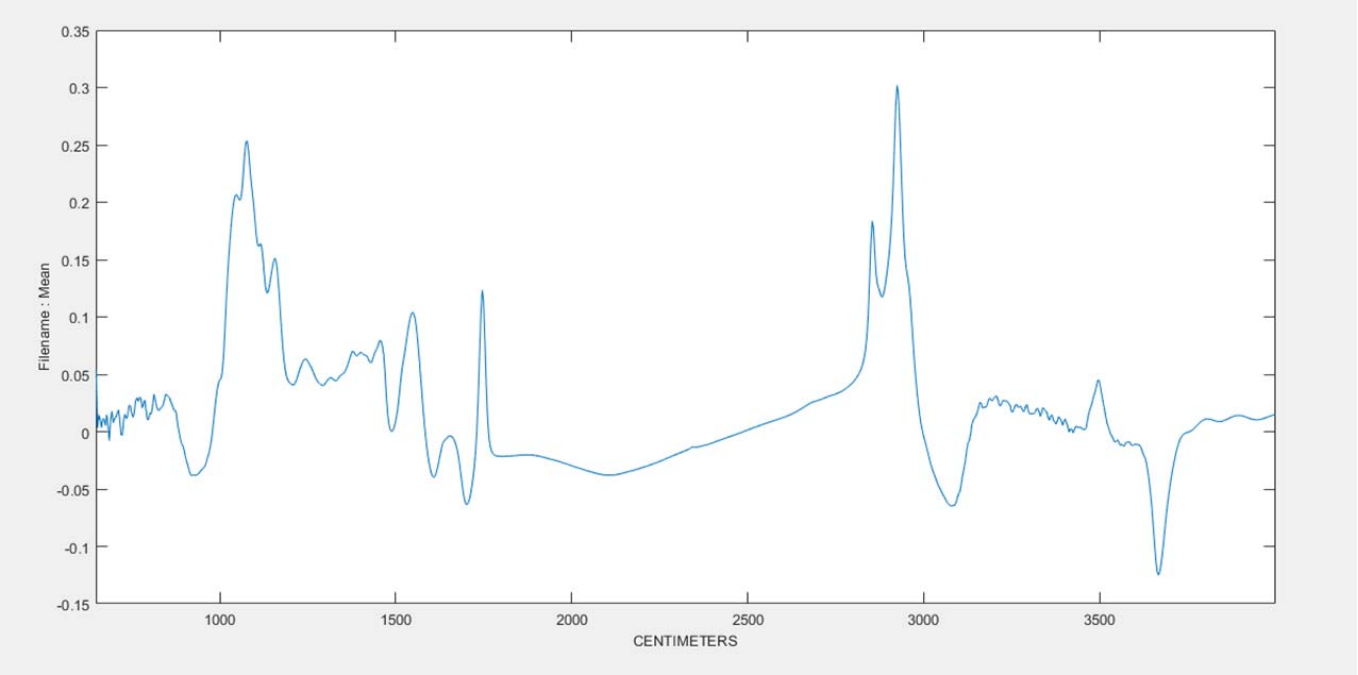






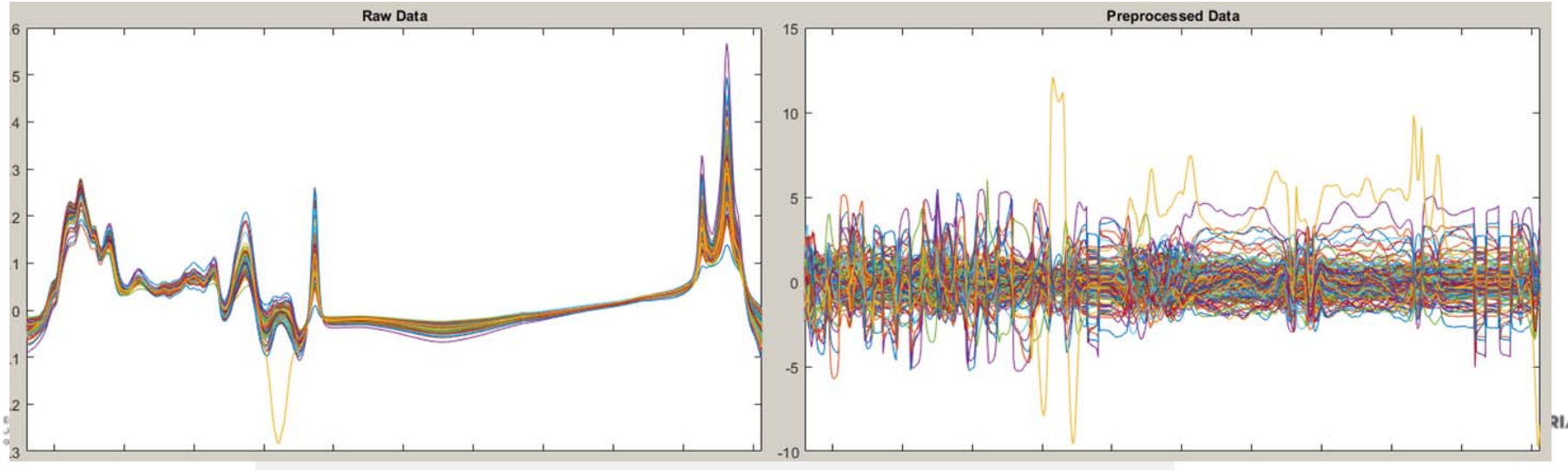
Data Analysis: Raw MIR Spectral Data

- 899 Spectral wavelengths between 625cm^{-1} & 3999 cm^{-1}
- Single outlier identified via PCA



Data Analysis: Pre-processing of Spectra

- “Noise” associated with water regions excluded
- 563 wavelengths between 928cm^{-1} & 3025cm^{-1} included
- Savitzy-Golay 2nd derivative transformation + smoothing + removal of linear trend + auto scaling



Data Analysis: Calibration & Validation

- Partial Least Square (PLS) regression models
- Accuracy reported as coefficient of determination (R^2)
- Error reported as Root Mean Square Error (RMSE)
- Cross-validation (CV) using “Venetian Blind” method
  - 20 Data splits
  - CV on 2 samples per subset
- Number of LVs: max variance min RMSECV

Data Analysis: External Validation (Random)

Calibration Data Set

Validation Data Set

Data Analysis: External Validation (Farm Exclusion)

Results: R^2 for CV & Random EV

Metabolite	R^2 Cross-validation (n=822)	R^2 External Validation (n=205)
BHB	0.49	0.49
NEFA	0.46	0.51
Urea	0.89	0.88
Albumin	0.26	0.28
Globulin	0.20	0.31
Alb:Glob	0.23	0.27
Calcium	0.18	0.18
Magnesium	0.17	0.23

Results: R^2 for Farm Exclusion External Validation

	Farm 1 (n=480)	Farm 2 (n=137)	Farm 3 (n=81)	Farm 4 (n=149)	Farm 5 (n=180)	Random (n=205)
BHB	0.30	0.37	0.35	0.26	0.25	0.49
NEFA	0.13	0.60	0.08	0.37	0.24	0.51
Urea	0.53	0.66	0.51	0.47	0.35	0.88
Albumin	0.06	0.12	0.10	0.16	0.17	0.28
Globulin	0.11	0.14	0.21	0.17	0.03	0.31
Alb:Glob	0.08	0.10	0.20	0.09	0.11	0.27
Calcium	0.05	0.15	0.02	0.17	0.16	0.18
Magnesium	0.01	0.06	0.24	0.09	0.02	0.23

Data Analysis: PLS-DA on Binary Variables

- Metabolite concentration classified as **WITHIN** or **OUTSIDE** optimum range
- Partial Least Square Discriminant Analysis (PLS-DA)

or

Results: PLS-DA of Binary Traits (Random EV)

Metabolite	Sensitivity	Specificity	Class. Error	p-value
BHB	0.67	0.93	0.20	1
NEFA	0.85	0.75	0.20	0.00
Urea	0.82	0.93	0.12	0.00
Calcium	1	0.91	0.05	0.90
Magnesium	0.83	0.79	0.19	0.94
Albumin	0.33	0.72	0.47	0.82
Globulin	0.0	0.77	0.61	0.84

What are the possible applications of these predictions?

Urea Concentration & Nitrogen Excretion

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Cows and seep

Dairy far
water

Government dat

GIPPSLAND Times & MAFFRA SPECTATOR

Sunday February 11, 2018

News | Local News

A warning over Lake Guthridge algae bloom

11 Jan 2018, 2 p.m.

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Urea Concentration & Nitrogen Excretion

J. Dairy Sci. 93:2377–2386
doi:10.3168/jds.2009-2415

Prediction of ammonia emission from dairy cattle manure based on milk urea nitrogen: Relation of milk urea nitrogen to ammonia emissions

S. A. Burgos,[1] N. M. Embertson,[2] Y. Zhao, F. M. Mitloehner, E. J. DePeters, and J. G. Fadel[3]
Department of Animal Science, University of California, Davis 95616

J. Dairy Sci. 96:4310–4322
http://dx.doi.org/10.3168/jds.2012-6265

Prediction of urinary nitrogen and urinary urea nitrogen excretion by lactating dairy cattle in northwestern Europe and North America: A meta-analysis

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†Wageningen UR Livestock Research, PO Box 65, 8200 AB, Lelystad, the Netherlands

Using blood urea nitrogen to predict nitrogen excretion and ...
Kohn, R A;Dinneen, M M;Russek-Cohen, E
Journal of Animal Science; Apr 2005; 83, 4; ProQuest
pg. 879

Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats[1]

R. A. Kohn[2], M. M. Dinneen, and E. Russek-Cohen

Department of Animal and Avian Sciences, University of Maryland, College Park 20742

Asian-Aust. J. Anim. Sci.
Vol. 21, No. 8 : 1159 - 1163
August 2008
www.ajas.info

Evaluation of Urinary Nitrogen Excretion from Plasma Urea Nitrogen in Dry and Lactating Cows

S. Kume*, K. Numata, Y. Takeya, Y. Miyagawa, S. Ikeda, M. Kitagawa
K. Nonaka[1], T. Oshita[2] and T. Kozakai[2]
Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Urea Concentration & Fertility

Reduction of Fertility and Alteration of Uterine pH in Heifers Fed Excess Ruminally Degradable Protein[1]

C. C. Elrod[2] and W. R. Butler[3]

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Plasma and Milk Urea Nitrogen in Relation to Pregnancy Rate in Lactating Dairy Cattle

W. R. Butler, J. J. Calaman[1], and S. W. Beam

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J. Dairy Sci. 87:1001–1011

Relationships Between Milk Urea and Production, Nutrition, and Fertility Traits in Israeli Dairy Herds

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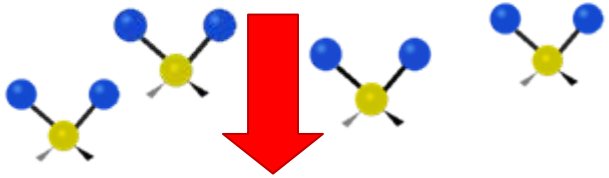
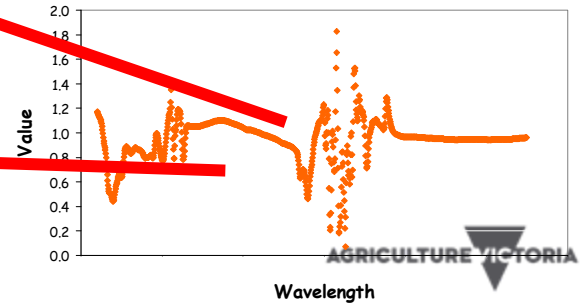
Is MIR better than Milk Fat to Protein Ratio for estimating fat mobilisation and ketosis risk?





NEFA prediction

BHB prediction

Linear Models to Compare with True Results from Serum

Is MIR better than Milk Fat:Protein?





Sample ID	BHB Actual	BHB MIR	NEFA Actual	NEFA MIR	Fat:Protein
Cow 1003	0.56	0.559301	0.34	0.547037	1.192998
Cow 456	0.81	0.44897	0.49	0.448191	1.169719
Cow 9235	0.68	0.810528	0.2	0.412734	1.182278
Cow 450	0.23	0.320906	0.27	0.645807	1.028745

MIR vs Fat:Protein Ratio

Fat Mobilisation

BHB_{Serum} ~F:P

BHB_{Serum} ~ BHB_{MIR}

Ketosis Risk

BHB_{Serum} ~F:P

BHB_{Serum} ~ BHB_{MIR}

Fixed effects: WIM + Age Category + Farm ID

MIR vs Fat:Protein - Accuracy (Adjusted R^2) of Prediction Models

	Risk of Ketosis (Serum BHB)		Fat Mobilisation (Serum NEFA)	
Data	**Fat:Prot**	**MIR_{BHB}**	**Fat:Prot**	**MIR_{NEFA}**
Random	0.21	0.47	0.44	0.59
Farm 1	0.19	0.32	0.34	0.36
Farm 2	0.21	0.40	0.43	0.61
Farm 3	0.00	0.35	0.00	0.03
Farm 4	0.17	0.27	0.31	0.41
Farm 5	0.06	0.29	0.20	0.29

MIR vs Fat:Protein - Accuracy (R^2) of Prediction Models

	Farm 1 (n=480)	Farm 2 (n=137)	Farm 3 (n=81)	Farm 4 (n=149)	Farm 5 (n=180)	Random (n=205)
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Calcium	0.05	0.15	0.02	0.17	0.16	0.18
Magnesium	0.01	0.06	0.24	0.09	0.02	0.23

MIR vs Fat:Protein - Accuracy (R^2) of Prediction Models

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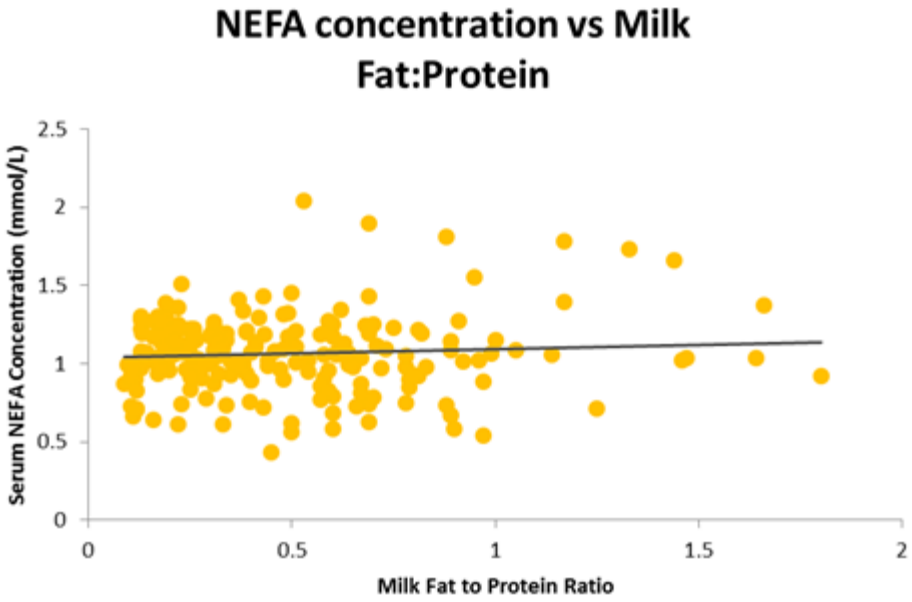
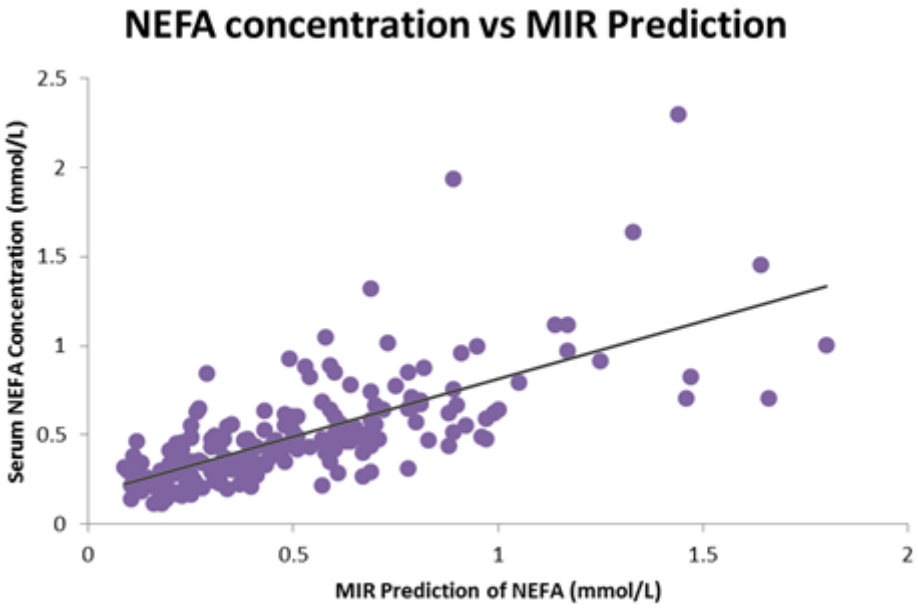
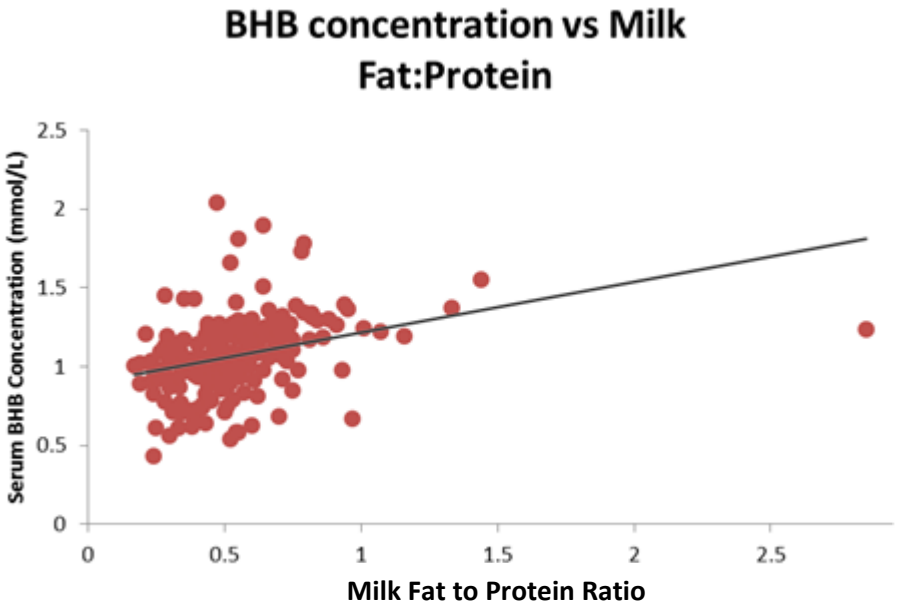
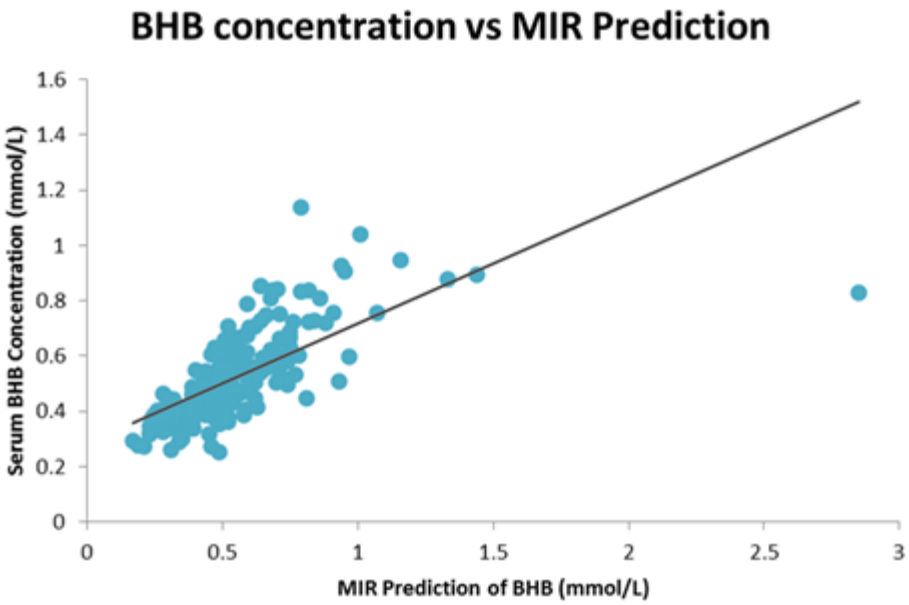
MIR vs Milk Fat:Protein for Prediction of Fat Mobilisation





MIR vs Milk Fat:Protein for Prediction of Ketosis Risk





Conclusions

- MIR provides superior prediction of degree of fat mobilisation and risk of ketosis than milk fat to protein ratio
- PLS-DA is a promising analysis technique for identifying animals at risk of excessive fat mobilisation & protein intake
- The make-up of the reference/calibration population strongly influences the accuracy of external prediction
- MIR shows real promise for prediction of serum urea concentrations

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MIR for Profit

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- Michelle Axford

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- Dr Phuong Ho

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