EARLY *POST-MORTEM* DISCRIMINATION BETWEEN GRASS- AND GRAIN-FED BEEF USING MUSCLE LIPID BIOMARKERS

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EAAP European Federation of Animal Science

LIPID MARKER CIÊNCIAS ÔMICAS

Brazilian Livestock

153.79 million ha of pasture
Stocking capacity: 1.32 head/ha
Capacity: 1.02 AU/ha

Live animal imports
59 head

Herd
202.78 million head

Live animal exports
194,819 head

Carcass Average Weight: 255.13 kg
Male Carcass Average Weight: 297.45 kg

Slaughter
42.31 million head

Real offtake: 21.60%
Apparent offtake: 19.82%
Feedlots: 7.62 million head (18.20% of total slaughter)

Exports (27.96%)
3.02 million CWE

Meat production
10.79 million CWE

Domestic Market
7.78 million CWE (71.48% of production) + 80.61 thousand CWE via imports

Per capita consumption:
36.73 kg/year

Formal per capita consumption
(meat inspected by the federal, state and municipal systems):
23.54 Kg/year

85.70% fresh beef 2.59 million CWE	124 countries	China: 62.39% US: 4.47% Egypt: 4.30% Chile: 3.96% Others: 24.87%
8.85% Processed 267.10 thousand CWE	116 countries	USA: 40.91% United Kingdom: 18.06% European Union: 12.54% Canada: 2.39% Others: 26.10%
5.45% Offal and other products 164.50 thousand CWE	109 countries	Hong Kong: 35.13% Ivory Coast (Côte d'Ivoire): 7.68% European Union: 7.27% Russia: 7.22% Others: 42.69%

Source: ABIEC, with data from SECEX/Ministry of the Economy, IBGE, Rally da Pecuária, Athenagro

About
12%
of the world's cattle herd is in Brazil

Grass-fed beef

Brazil is naturally a **grass-fed beef producer**

Grass-fed beef is a niche market that has grown substantially in recent years.

Grass-fed beef certification adds a premium for meat from animals raised and finished on pasture with the ideology of improving animal welfare and human health.

Grass-fed beef certification

Currently, certification of grass-fed beef is carried out on farms based on auditable and non-auditable papers and protocols, which are **time-consuming** and, sometimes, **unreliable**

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Grass-fed beef certification

Currently, certification of grass-fed beef is carried out on farms based on auditable and non-auditable papers and protocols, which are **time-consuming** and, sometimes, **unreliable**

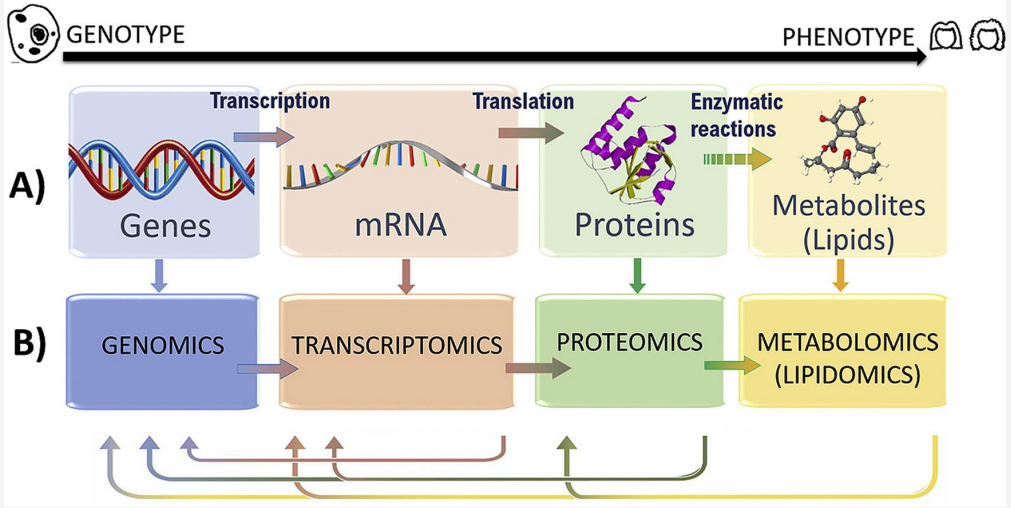
Due to the large number of producing farms and animals produced, it is unlikely that there will be a wide inspection on the farm to **guarantee what the animal was fed**, which opens a gap for **possible fraud/adulterations**.

A segregation between grass- and grain-fed beef within slaughter plants through a chemical muscle test (biomarker panel-based certification) may exclude any possibility of fraud/adulteration and guarantee what the animal was actually fed.

Hypothesis and objective

We hypothesized that early (30 min) post-mortem muscle lipidome segregates between feeding regimen classes.

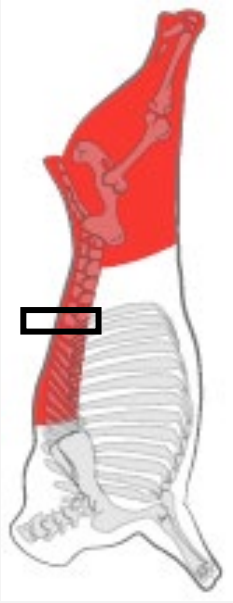
If so, we hypothesized that an early (30 min) *post-mortem* muscle lipid-based biomarker panel predicts feeding regimen.



The aim of this study was to create an **early (30 min) post-mortem muscle lipid-based biomarker panel** to discriminate between grass- and grain-fed beef

Material and Methods

Eighty carcasses from **grass-fed** (n = 40; from 30 to 35 mo age) and **grain-fed** (n = 40; from 20 to 24 mo age) Nellore (*Bos Indicus*) bulls were obtained

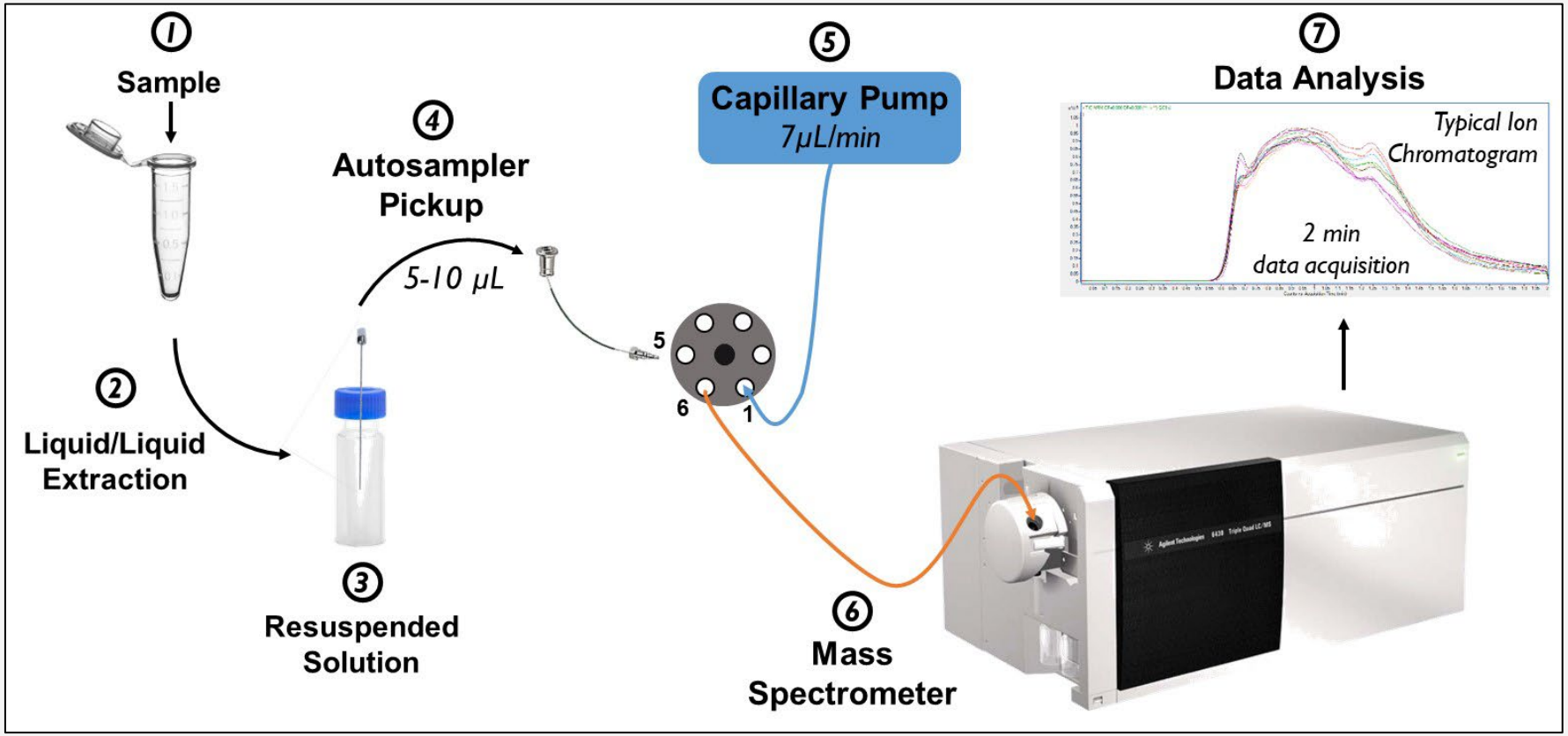


Longissimus thoracis (LT) muscle (11th rib level) samples (~10 g) were collected at 30 min post-mortem and snap-frozen in liquid nitrogen for further **lipidome analysis**.

Lipidomics workflow

N = 80

Bligh & Dyer (1959)



MS does the separation in the Multiple reaction monitoring (MRM) profiling mode

Multiple reaction monitoring (MRM)-profiling

3,437 MRMs related to lipids from 12 lipid classes

1,752 triglycerides (TG)

482 diglycerides (DG)

179 free fatty acids (FFA)

178 phosphatidylcholines (PC)

145 phosphatidylethanolamines (PE)

141 phosphatidylinositols (PI)

141 phosphatidylglycerols (PG)

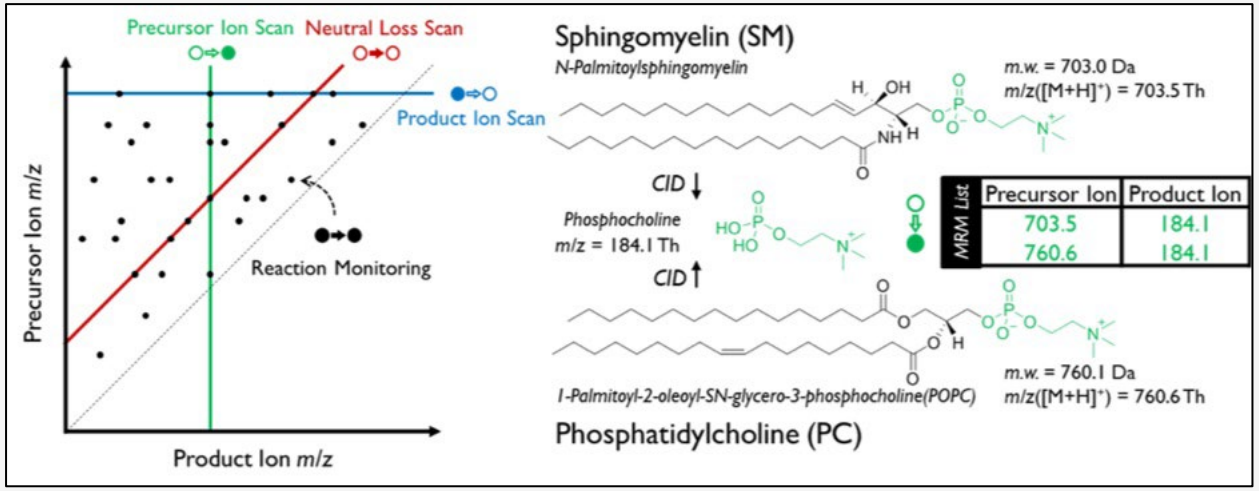
139 phosphatidylserines (PS)

120 ceramides (CER)

84 acyl carnitines (CAR)

42 sphingomyelins (SM)

34 cholesterol esters (CE)



Out of the 3,437 MRMs scanned in a pooled sample for identifying the detectable lipids, **272** had intensities of at least 1.3-fold higher than the blank and were used for interrogating individual samples.

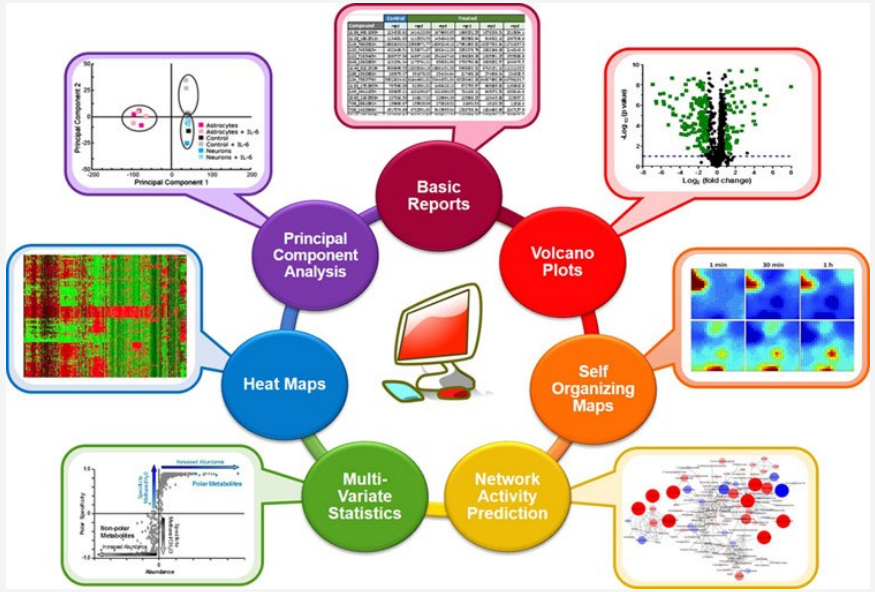
Data Analysis

Lipidome data were uploaded to Metaboanalyst 6.0

Data were Pareto-scaled prior to statistical and bioinformatics analyses.

Multivariate analysis

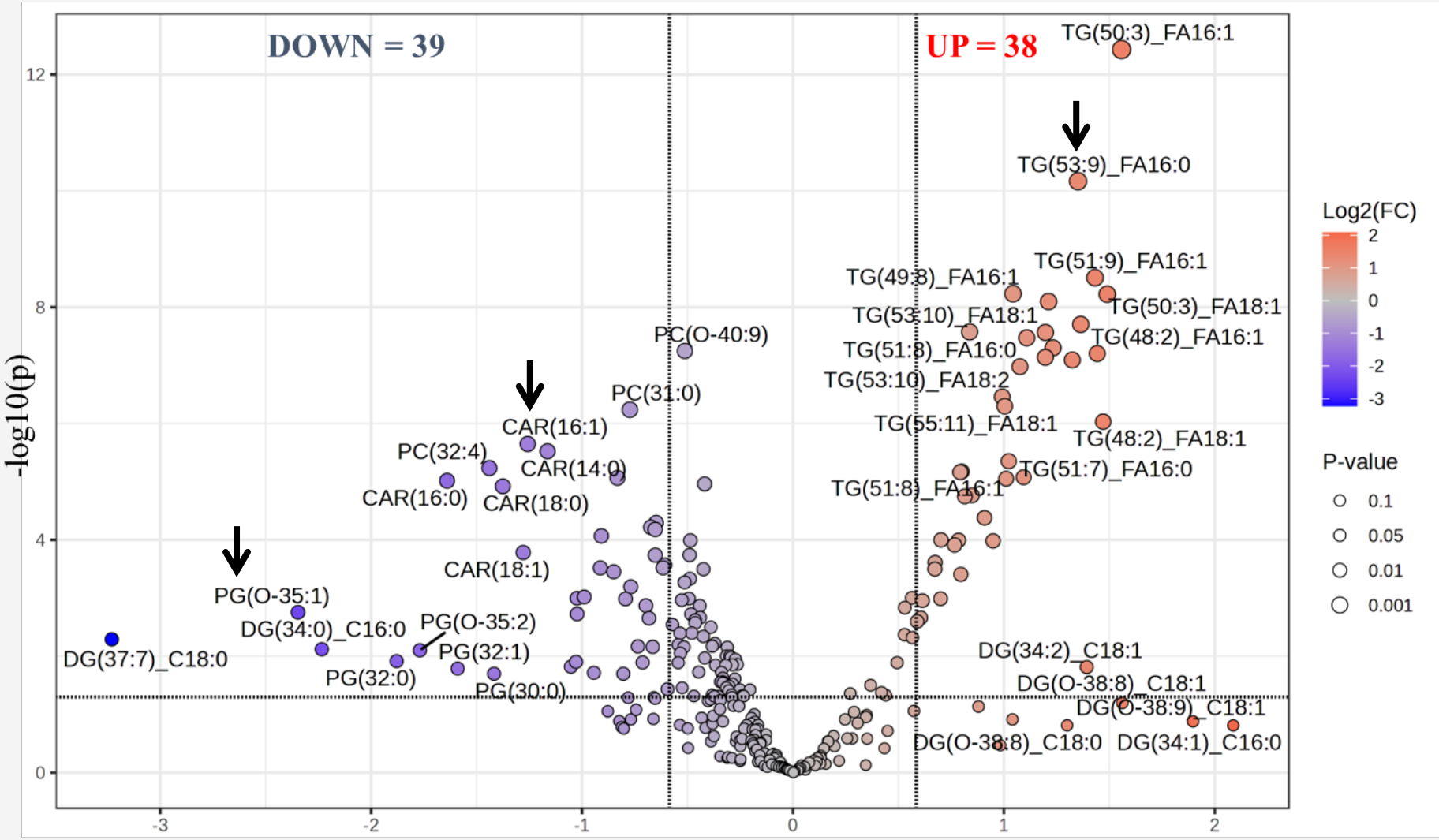
1. Volcano plot; 2.Principal component analysis (PCA); and 3. Orthogonal partial least squares discriminant analysis (OPLS-DA) were performed



Machine learning – Biomarker analysis

Receiver operating characteristic (ROC) curve analysis was performed to create a predictive model for evaluating the performance of biomarkers

Volcano Plot (P ≤ 0.05; FC ≥ 1.5)



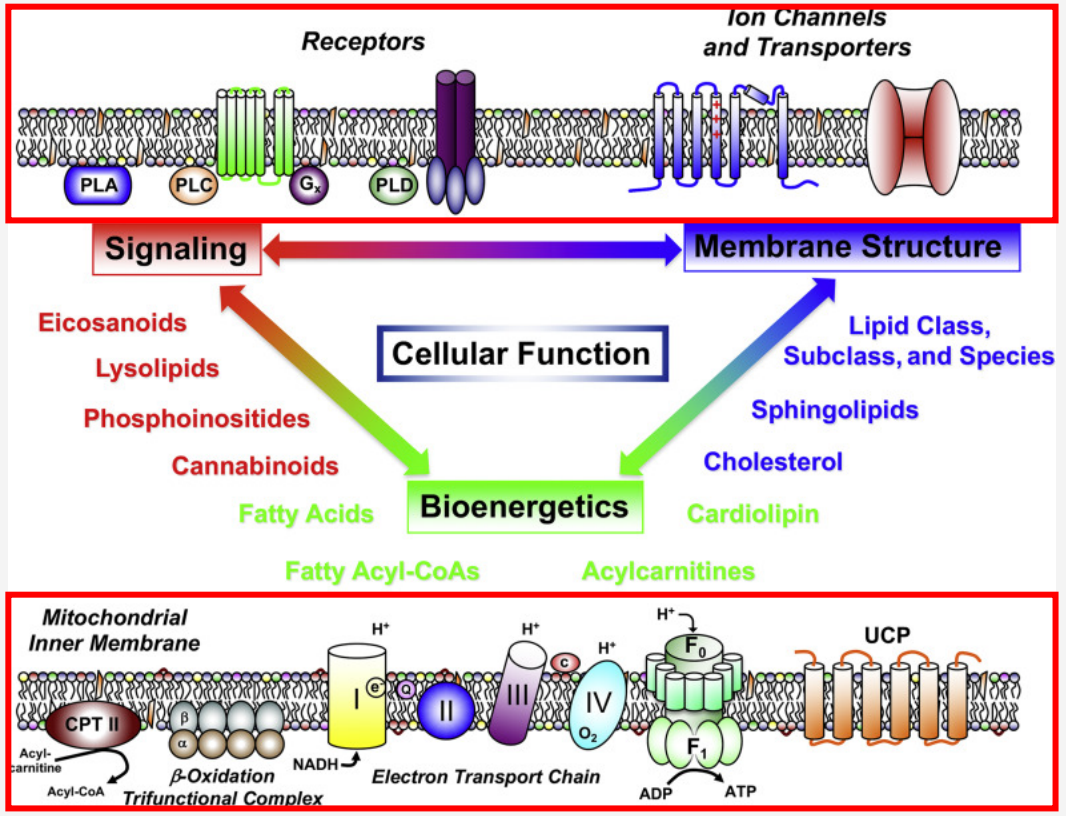
TG, CAR and PG affect muscle metabolism

Hypothesis for TG (upregulated in grain-fed beef), CAR (upregulated in grass-fed beef) and PG (upregulated in grass-fed beef) to have an impact on the feeding regimen discrimination

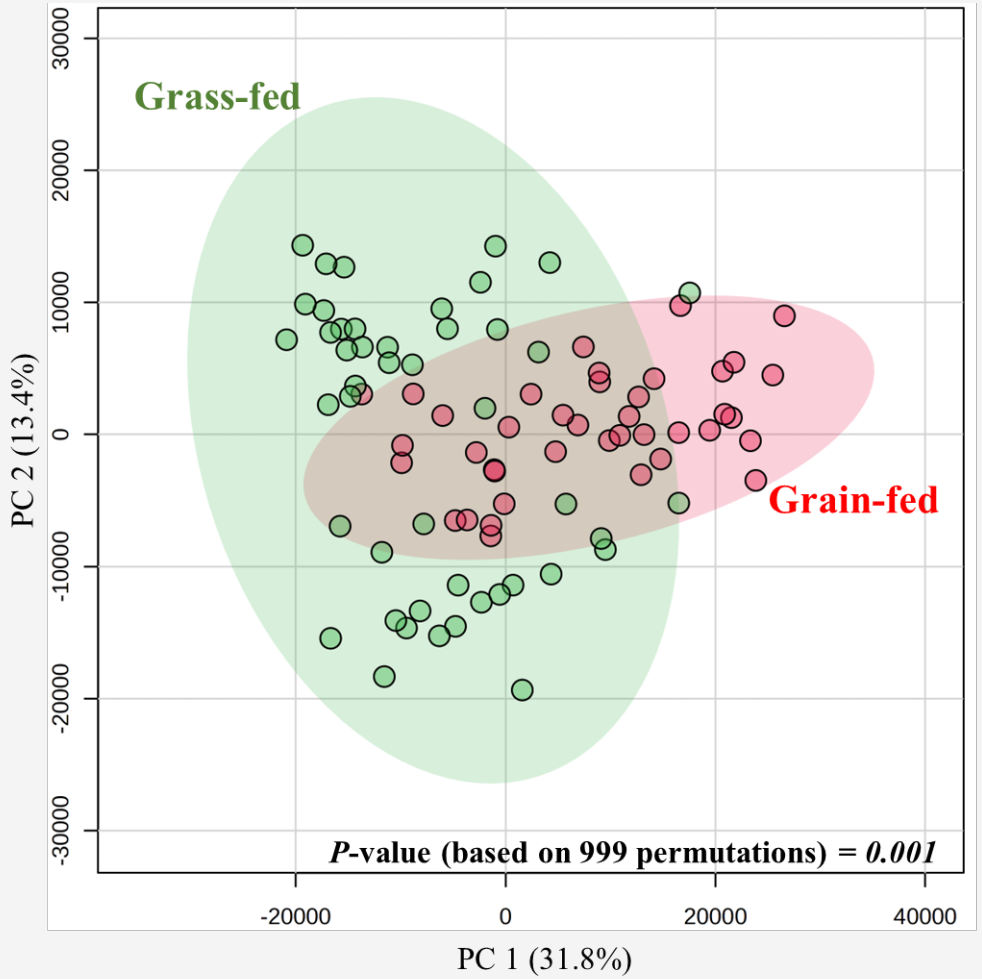
Grass-fed beef oxidized more fatty acids (**from TG through CAR in beta-oxidation**) to generate ATP for maintaining energy homeostasis.

Grass-fed beef showed **higher abundance of PG**, which is a precursor of cardiolipin and an indicative of **oxidative phosphorylation**

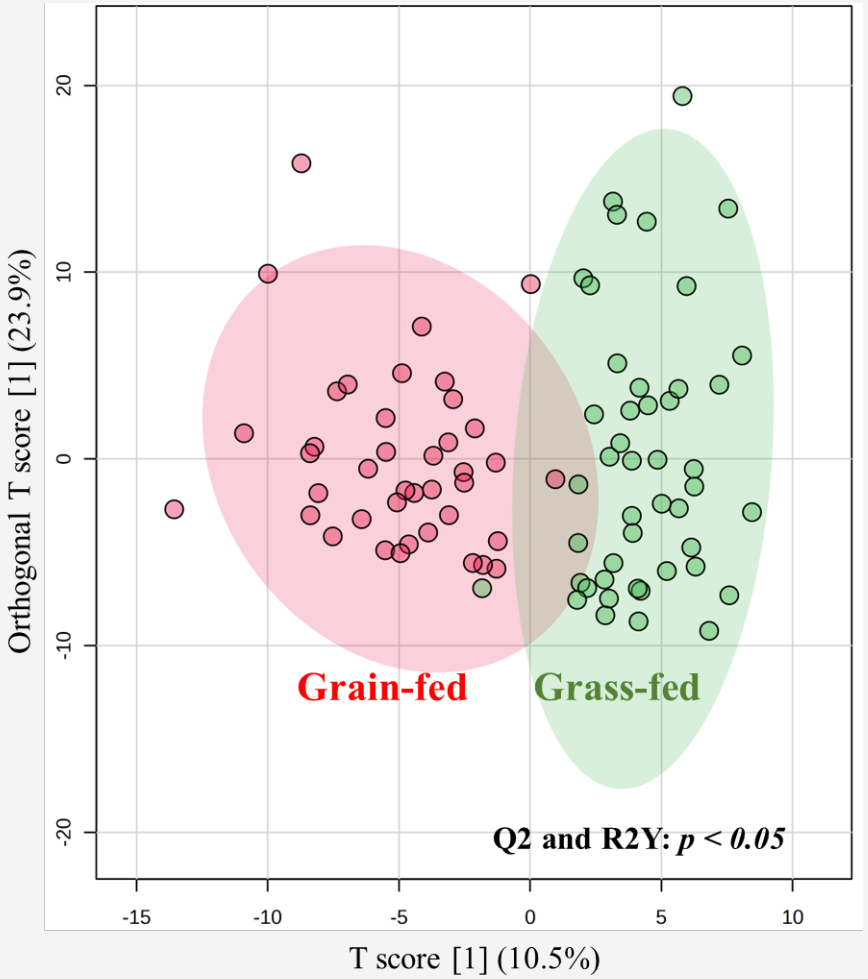
Grass-fed beef experienced a **more oxidative muscle metabolism** than grain-fed beef.



PCA



OPLS-DA



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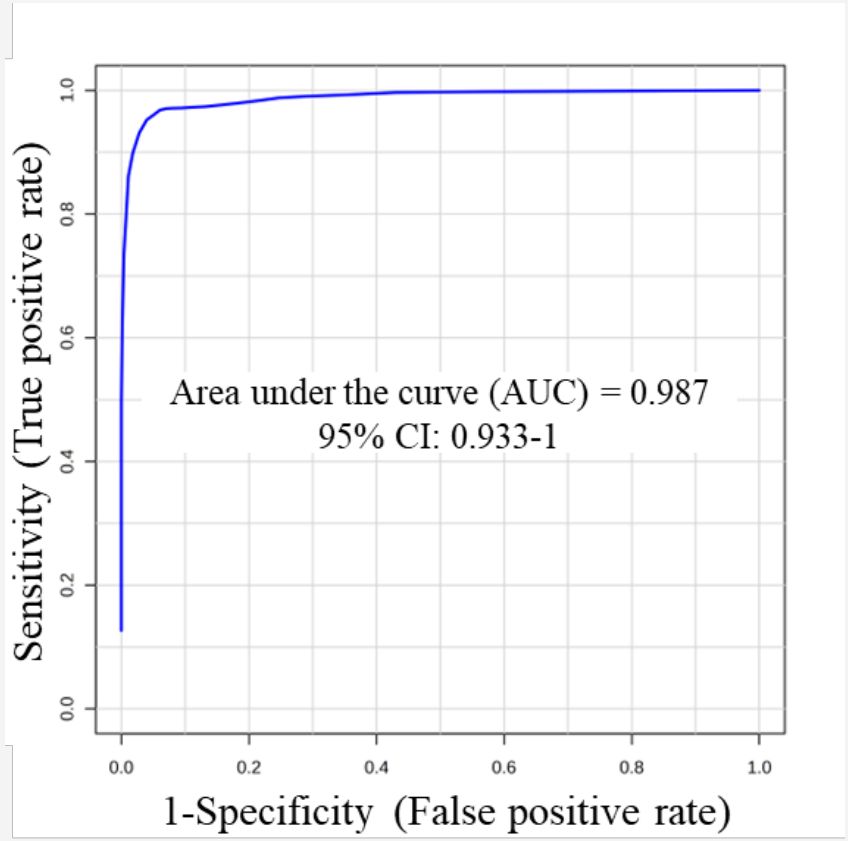
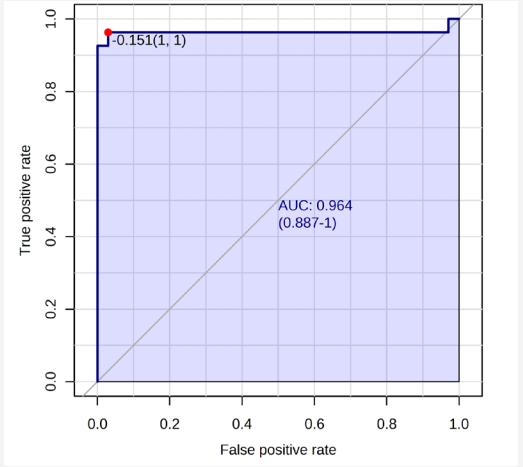
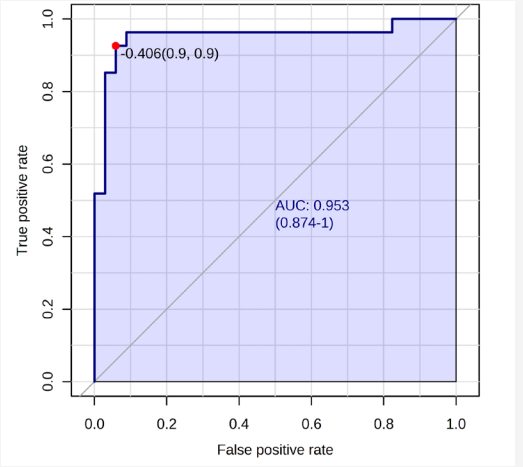
Multivariate exploratory ROC analysis



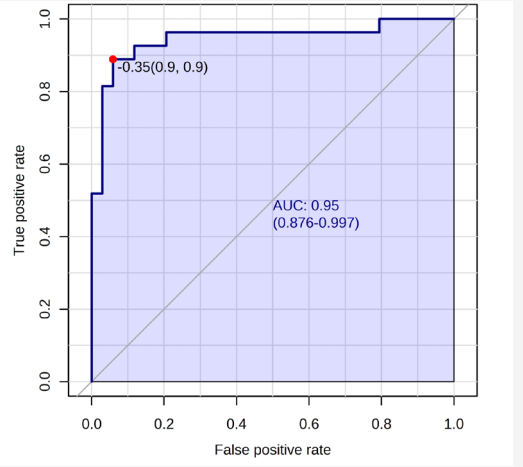
PG(31:1) = 0.964



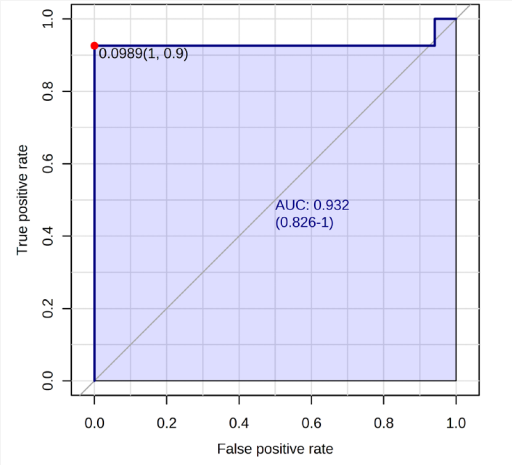
CAR(16:1) = 0.953



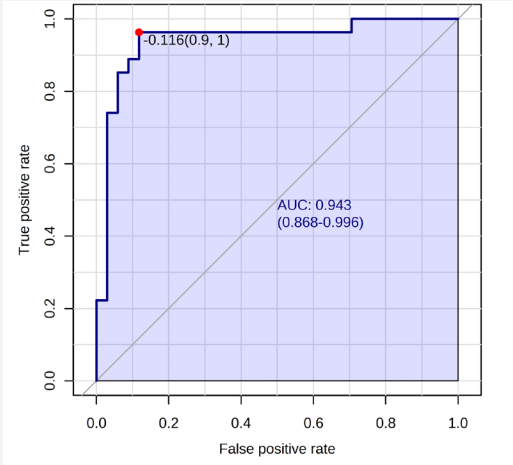
CAR(16:0) = 0.95



PG(33:4) = 0.932



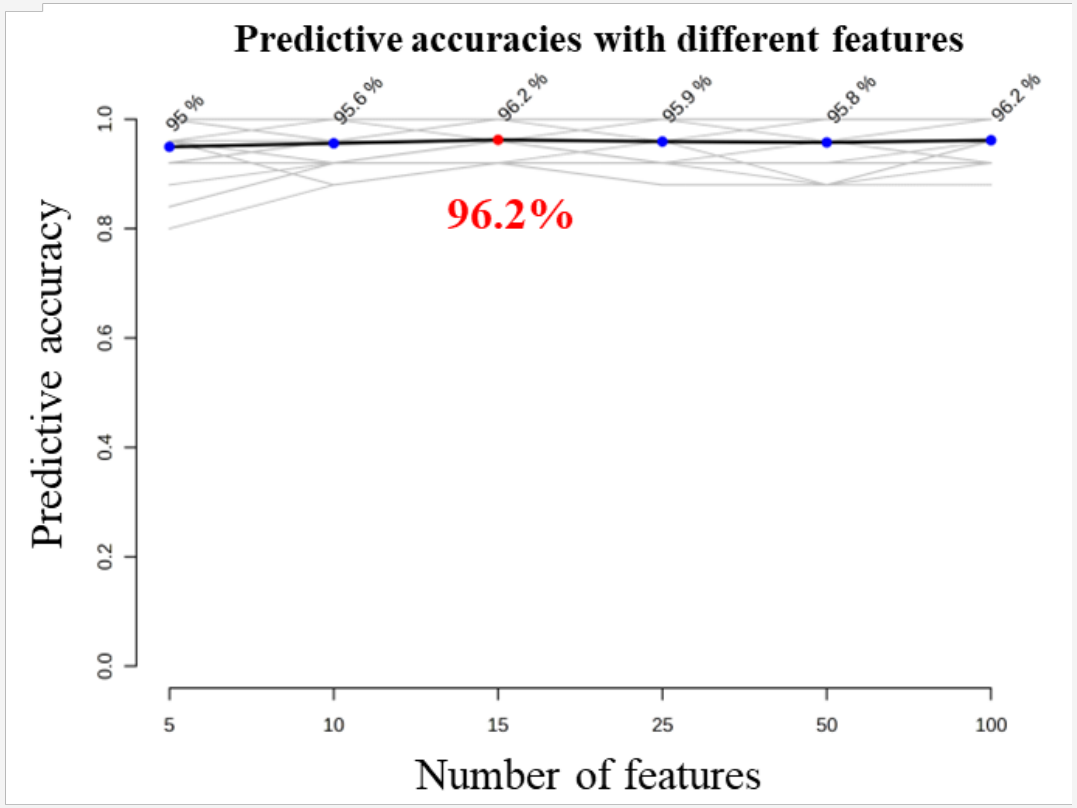
TG(48:2)_FA16:0 = 0.943

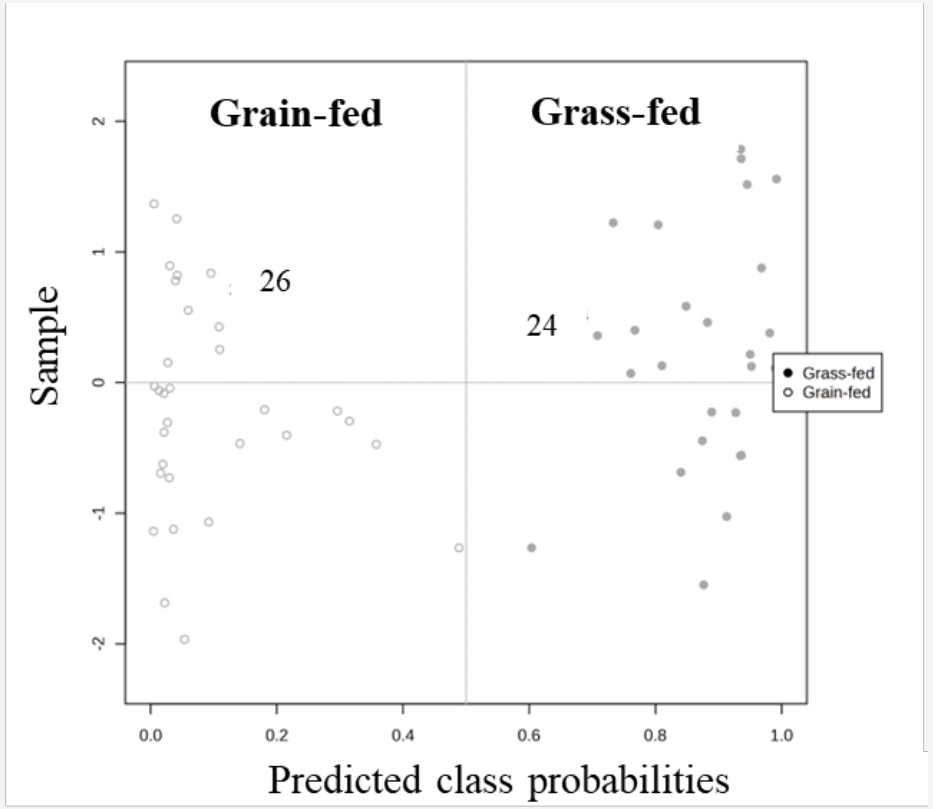


Classification method: Random Forest

Feature ranking method: Random Forest built-in

Probability view





2 misclassification in 80 samples

Blind-test

24 new samples were interrogated using the predictive model (15 best biomarkers)

Sample	Probability	Observed class	Predicted class
1	0.95	Pasture	Pasture
2	0.69	Pasture	Pasture
3	0.91	Pasture	Pasture
4	1.0	Pasture	Pasture
5	0.69	Pasture	Pasture
6	0.88	Pasture	Pasture
7	0.99	Pasture	Pasture
8	0.97	Pasture	Pasture
9	0.98	Pasture	Pasture
10	0.80	Pasture	Pasture
11	1.0	Pasture	Pasture
12	0.81	Pasture	Pasture

Sample	Probability	Observed class	Predicted class
47	0.91	Feedlot	Feedlot
48	0.66	Feedlot	Feedlot
49	0.87	Feedlot	Feedlot
50	0.93	Feedlot	Feedlot
51	0.86	Feedlot	Feedlot
52	0.87	Feedlot	Feedlot
53	0.91	Feedlot	Feedlot
54	0.94	Feedlot	Feedlot
55	0.92	Feedlot	Feedlot
56	1.0	Feedlot	Feedlot
57	0.98	Feedlot	Feedlot
58	1.0	Feedlot	Feedlot

We hypothesized that an early (30 min) post-mortem muscle lipid-based biomarker panel predicts feeding regimen.

Conclusion

Muscle lipidome from grass- and grain-fed beef has a discriminating profile, which allowed creating a **preliminary predictive model** to discriminate them during early *post-mortem*.

Therefore, it can be used as a tool to replace a time-consuming and unreliable paper-based system with a biomarker panel-based certification, **excluding any possibility of fraud/adulteration** and increasing the reliability of the grass-fed beef certification.

Next steps

Increasing the **number of samples** to improve model prediction

Developing the device and software (under intellectual property application) to be used in the **slaughter line** (non-destructive instrumental method) with a **real-time response** to allow carcasses segregation and grass-fed beef certification.

- Using this device, we can perform an individual assessment instead of a batch assessment, which may be useful for other predictions (beef tenderness)

Acknowledgements

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Thanks for your attention!

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