75th annual meeting – European Federation of Animal Science- 9/1/2024 – 9/5/2024

Comparative transcriptomic profiling of mRNA isoforms linked to feed efficiency in Holstein dairy cattle

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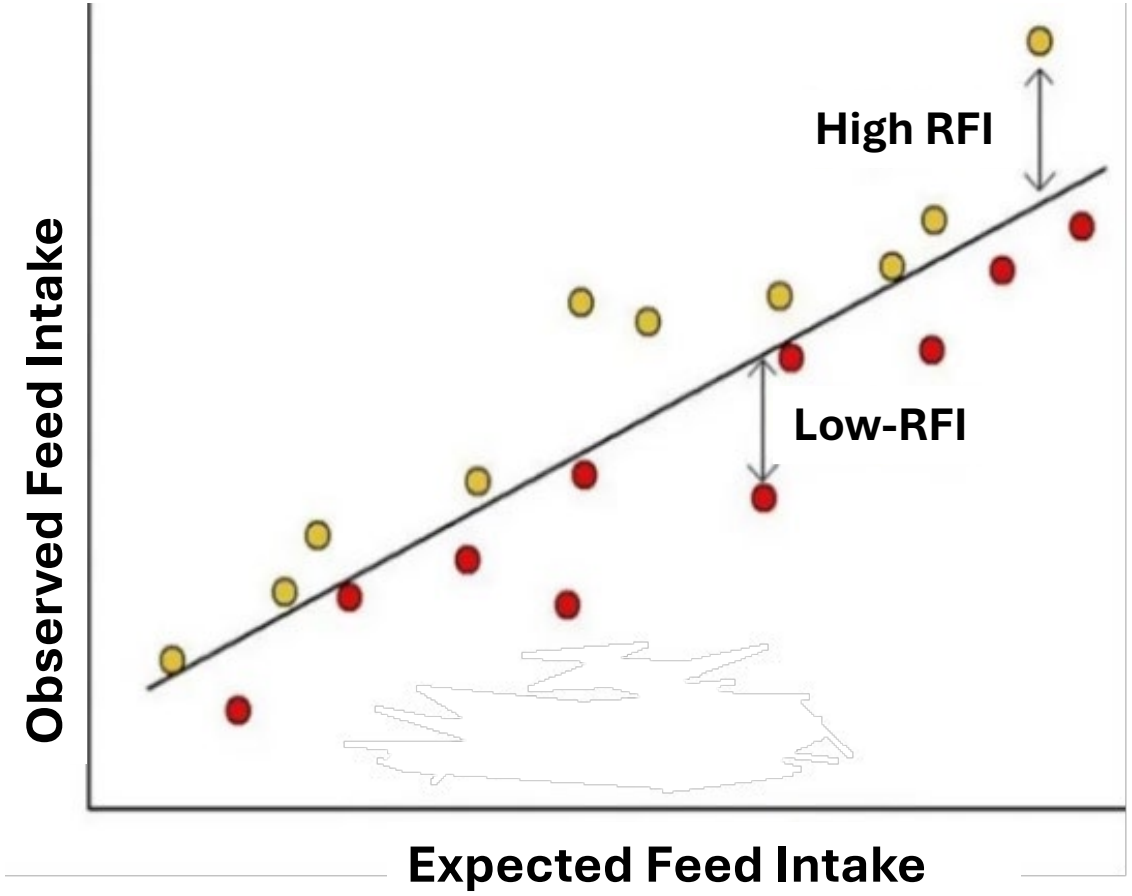
Feed Efficiency

What is Residual Feed Intake (RFI; kg DMI/day)?

- Difference between the animal's observed and expected feed intake while accounting for maintenance and growth needs

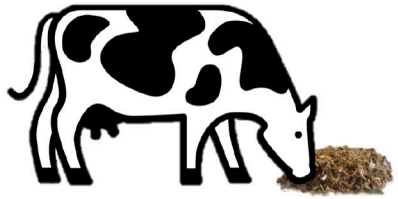
Factors affecting RFI:

- Genetics
- Diet and nutrition
- Animal health

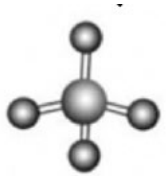


Adapted from: *How has selection for residual feed intake (RFI) affected the grow-finish pig's ability to cope with stress?*

Livestock methane emissions



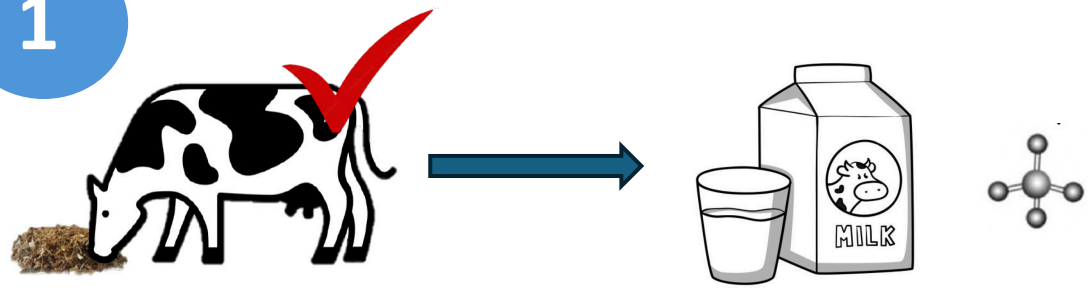
Enteric fermentation



~Energy loss: 2-12%

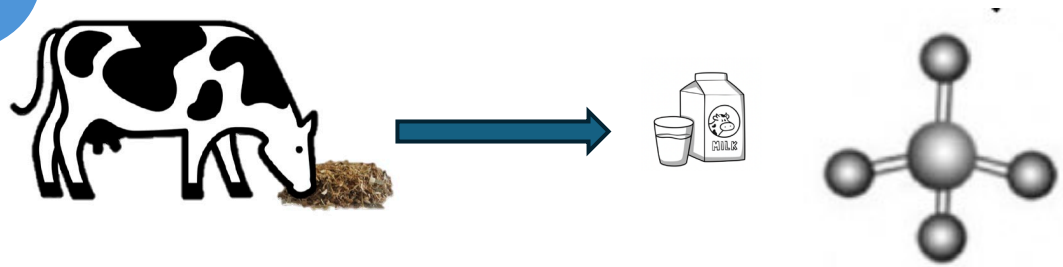
Feed represents on average up to 45% of Canadian dairy production costs

1



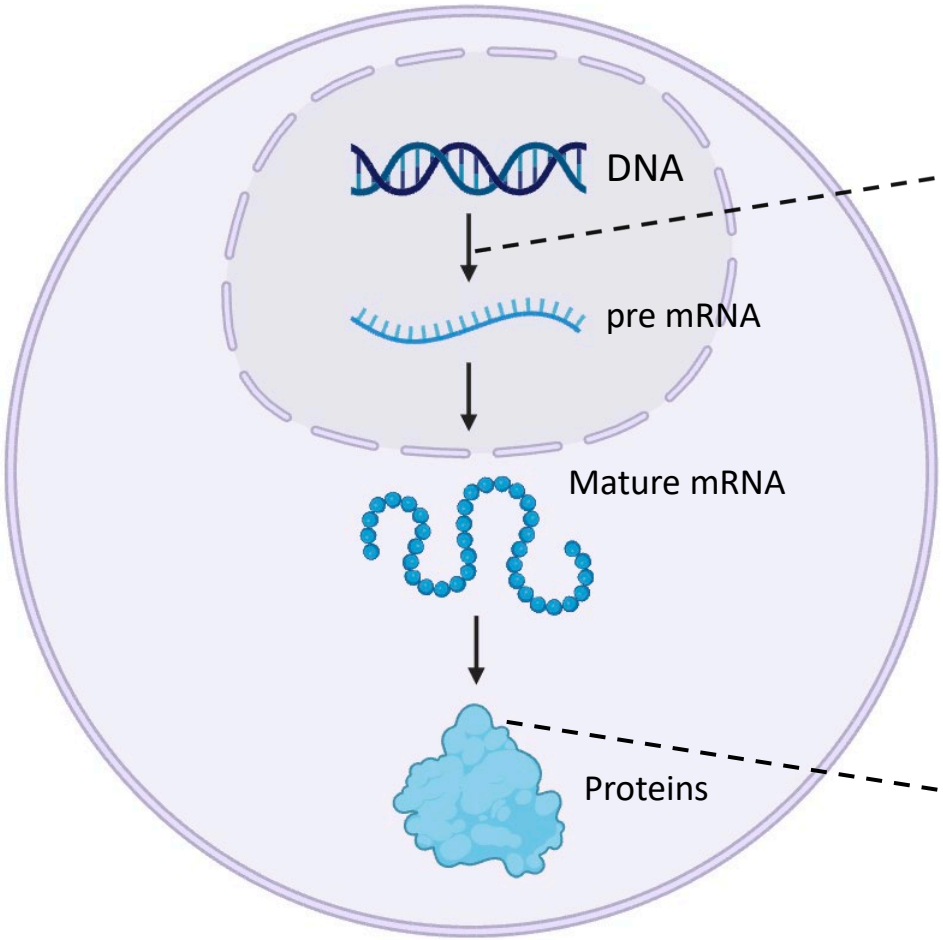
Low-RFI = High feed efficiency

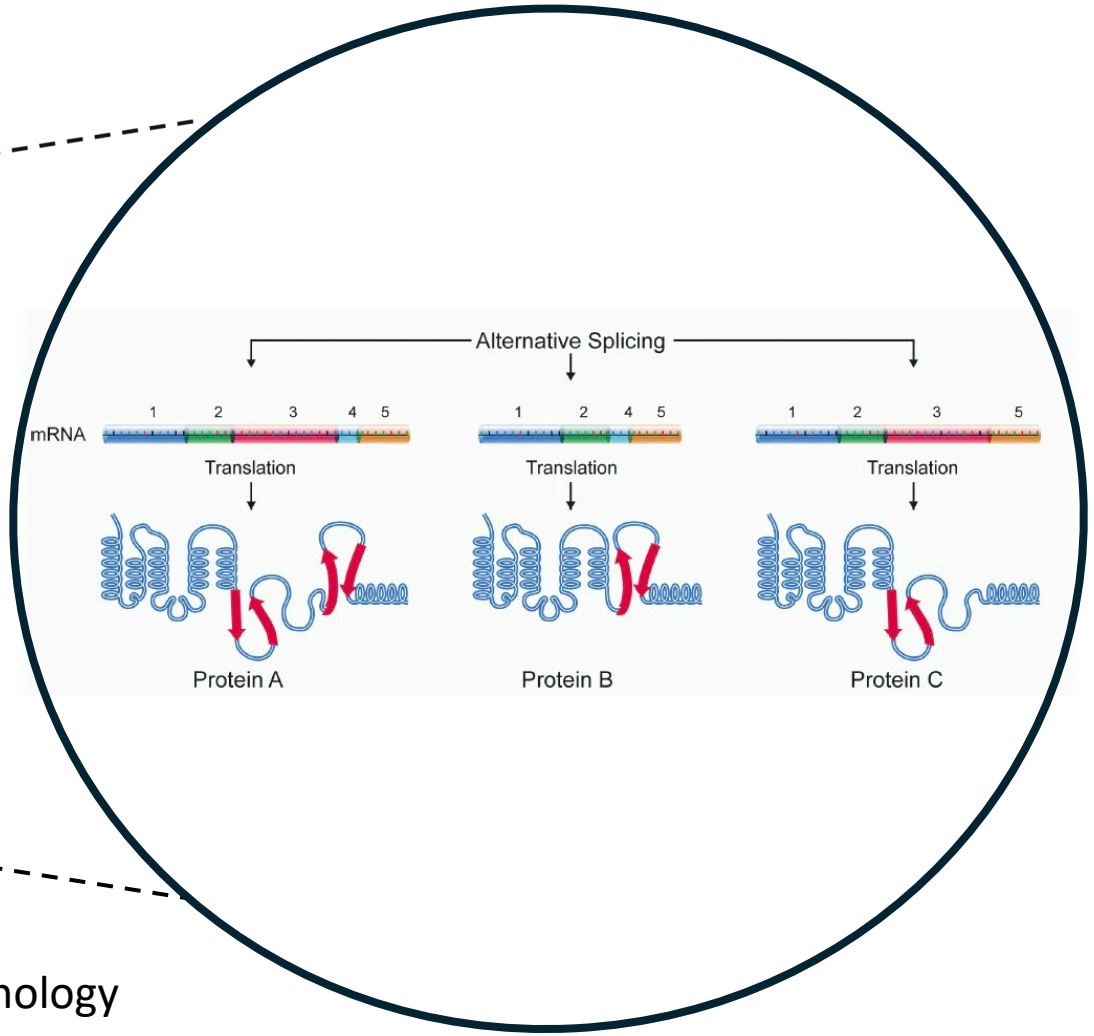
2



High-RFI = Low feed efficiency

Transcriptomics





Achieved using RNA-seq technology

https://statomics.github.io/SGA/sequencing_intro.html

Research goals

Objectives

Identify differentially expressed **mRNA isoforms** indicative of genes associated with **feed efficiency** in **divergent RFI** animals fed **different diet** treatments**.**

Research Questions

- **Impact of RFI** and **diet** on the expression of mRNA isoforms associated with **feed efficiency**.
- mRNA isoforms **commonly expressed** between the groups.
- **Functional annotations**.

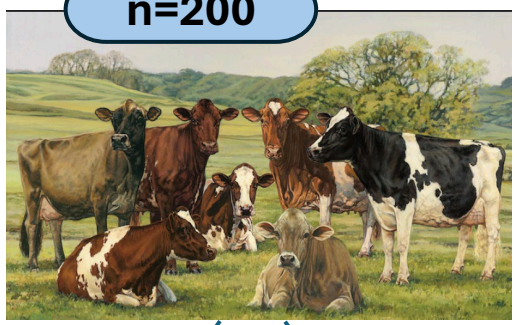
Importance

Allow for interpretations in the gene regulatory network mechanisms for feed efficiency.

RESEARCH ARTICLE Open Access
RNA-Seq transcriptomics and pathway analyses reveal potential regulatory genes and molecular mechanisms in high- and low-residual feed intake in Nordic dairy cattle
M. S. Salleh[1], G. Mazzoni[1], J. K. Höglund[2], D. W. Olijhoek[2,3], P. Lund[3], P. Løvendahl[2] and H. N. Kadarmideen[4*]

Experimental design: RFI and Diet

Categorizing RFI

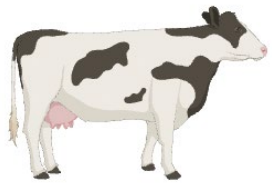


Extremes of the population selected for RFI

Δ RFI

1.07kg DMI/d (± 0.29)

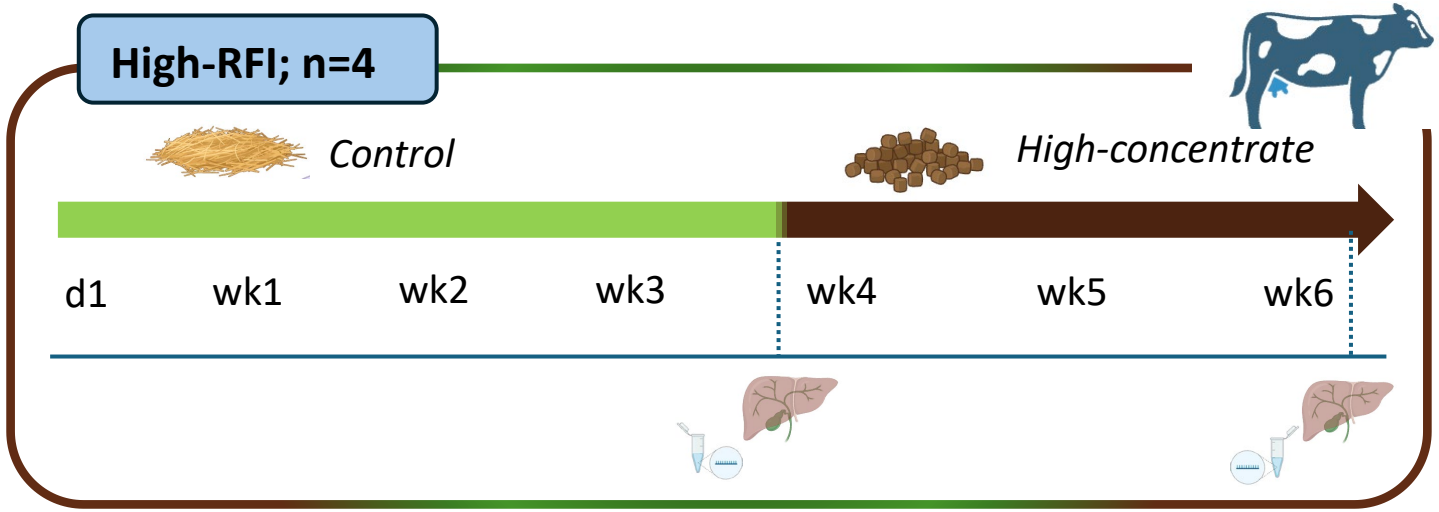
High-RFI; n=4

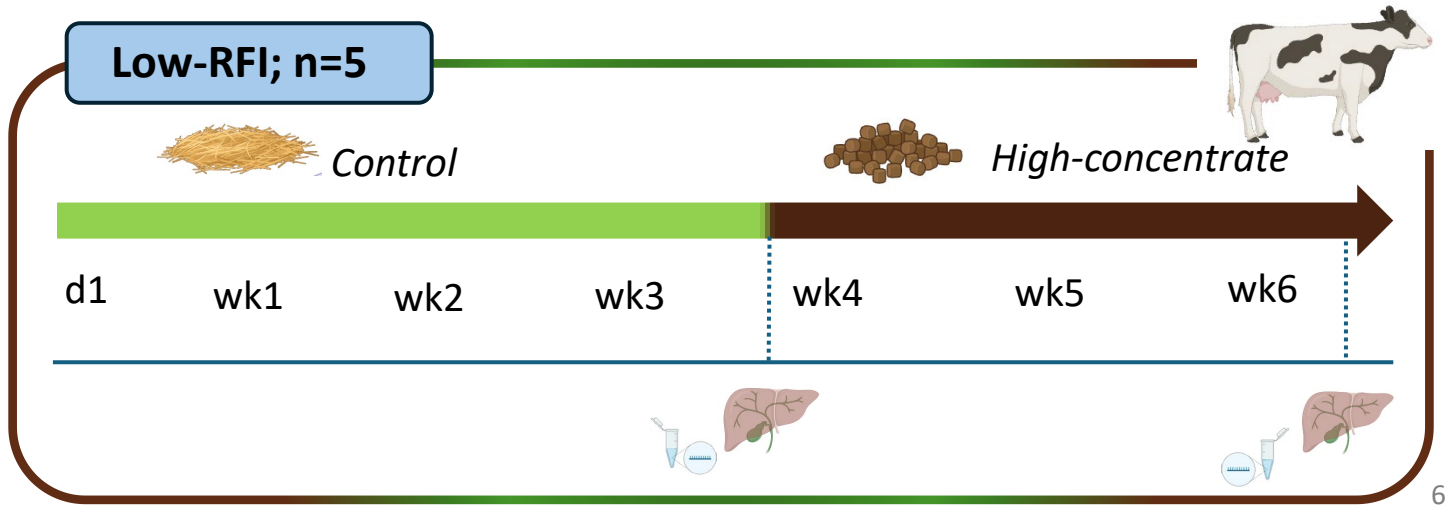


Low-RFI; n=5

Each cow was fed 2 diets

Categorizing diet





Experimental design: Analysis workflow

Raw RNA-seq data

Quality control

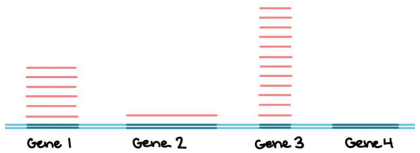
Alignment to Bovine reference genome
ARS-UCD 1.3
Large gap read mapping-de novo assembly

Compiled

Novel genes w/novel transcript lengths

Annotated genes w/annotated transcript lengths

Annotated genes w/novel transcript lengths



Differentially expressed transcripts

Transcript discovery and RNA-seq analysis

Functional annotations
GALLO (*Fonseca et al., 2020*)

OXFORD (GIGA)n SCIENCE
GigaScience, 9, 2020, 1–9
doi: 10.1093/gigascience/giaa149
Technical Note

TECHNICAL NOTE

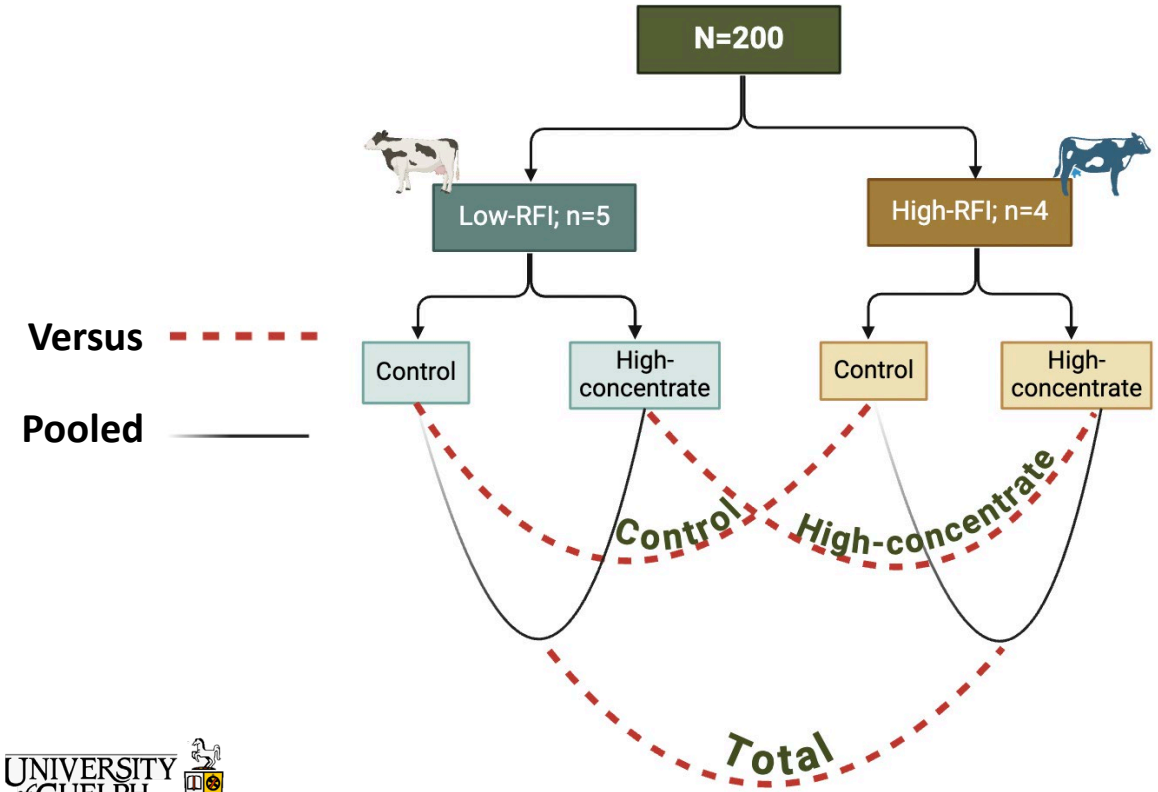
GALLO: An R package for genomic annotation and integration of multiple data sources in livestock for positional candidate loci

Pablo A. S. Fonseca [1,*], Aroa Suárez-Vega [1], Gabriele Marras [1,2] and Ángela Cánovas [1,*]

Methodology: Comparison objective 1

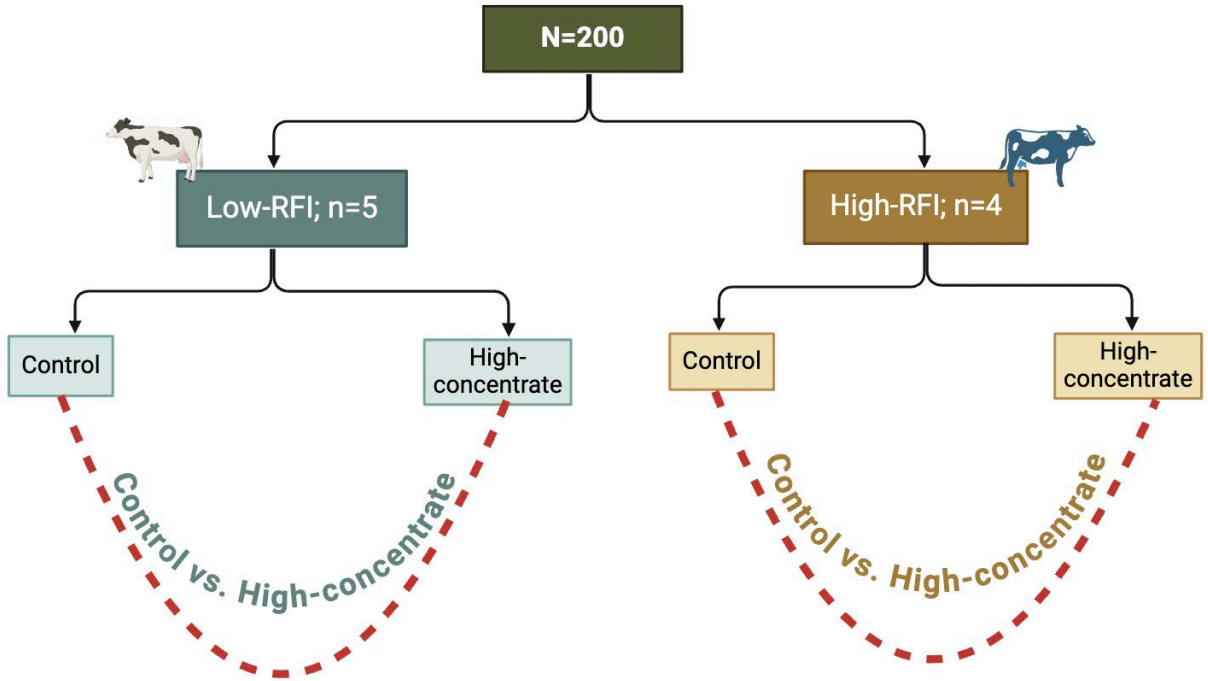
High vs. Low-RFI = Impact **of RFI** on mRNA isoform expression

1. Control
2. High-concentrate
3. Total (Control + High-concentrate)



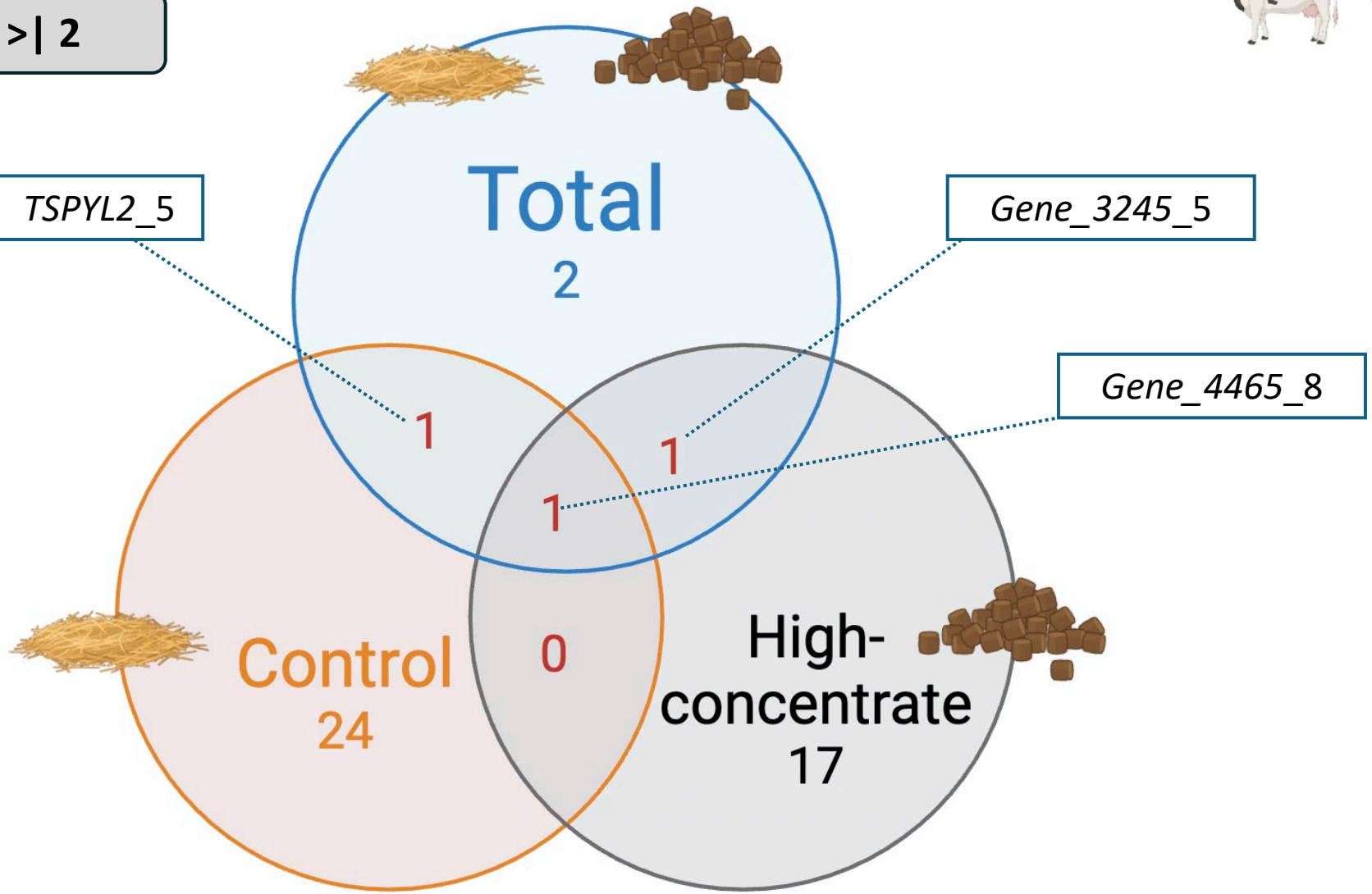
Control vs. High-concentrate diet = Impact **of diet** on mRNA isoform expression

1. High-RFI
2. Low-RFI



Low RFI vs. High RFI: Differentially expressed mRNA isoforms

p-val<0.01, FC |>| 2

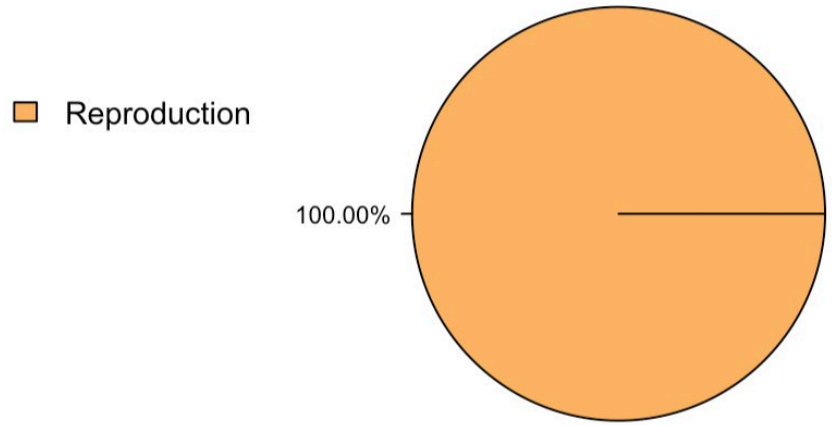


Low RFI vs. High RFI: Common differentially expressed mRNA isoforms and functional analysis

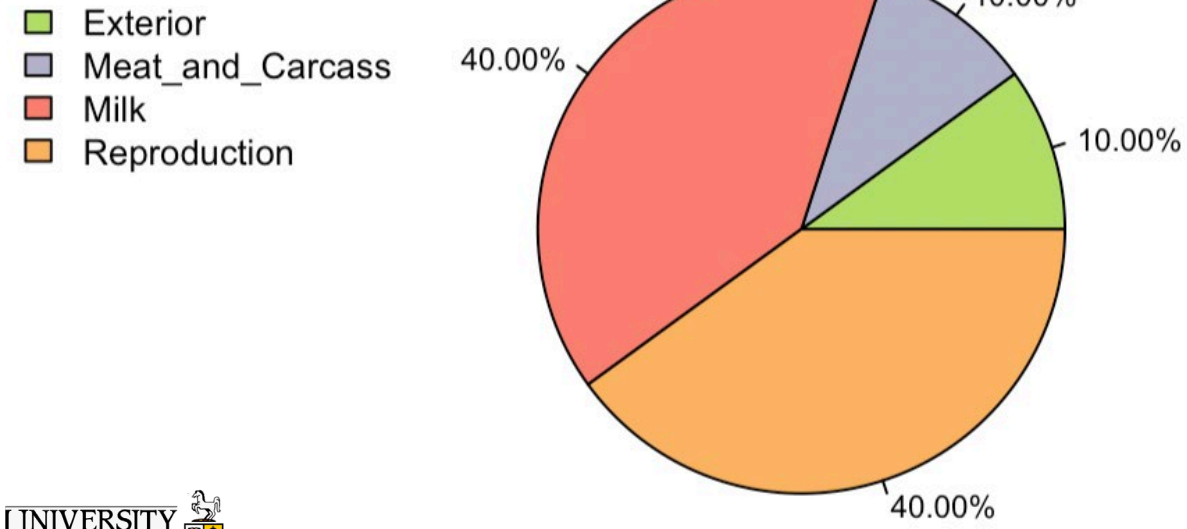
Associated gene names	mRNA isoforms	Gene Ontology: Biological process	p-value
TSPYL2	*TSPYL2_5* ↑	Nucleosome assembly **Negative regulation** of DNA replication **Negative regulation** of cell growth	2.32x10-4
Novel genes		**Orthologs**	
Gene_4465	*Gene_4465_8* ↓	Lipid metabolic process Prostaglandin metabolic process Steroid biosynthetic process	*AKR1C2* *AKR1C21*	1.76x10-5
Gene_3245	*Gene_3245_5* ↓	Proteolysis Spermatogenesis Spermatid development Cell differentiation	*ADGB*	5.60x10-3

Low-RFI vs High-RFI: Overlapping QTLs using list of differentially expressed mRNA isoforms

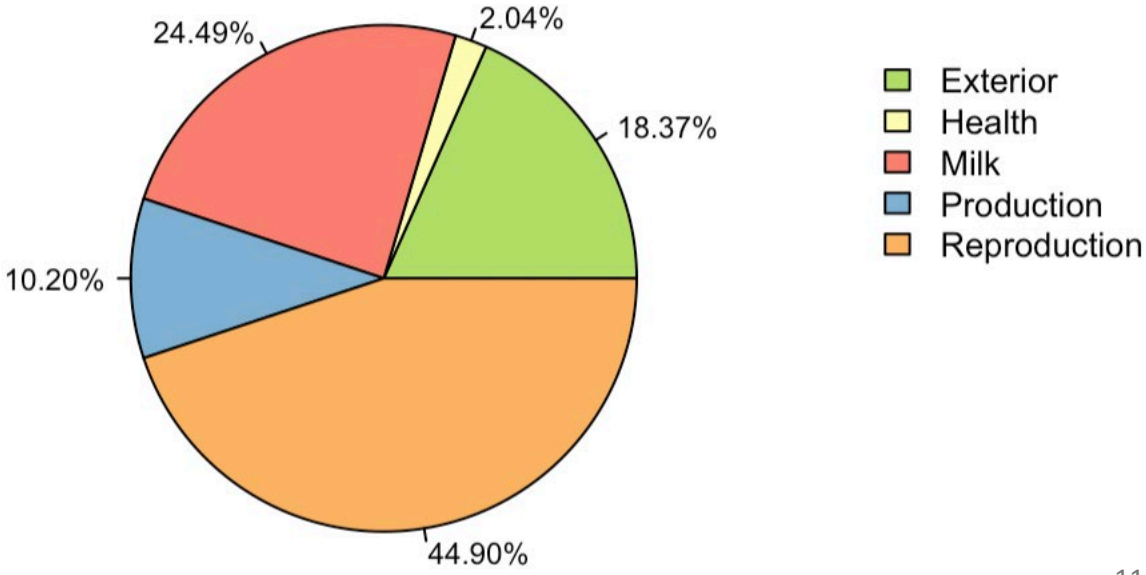
Total



Control



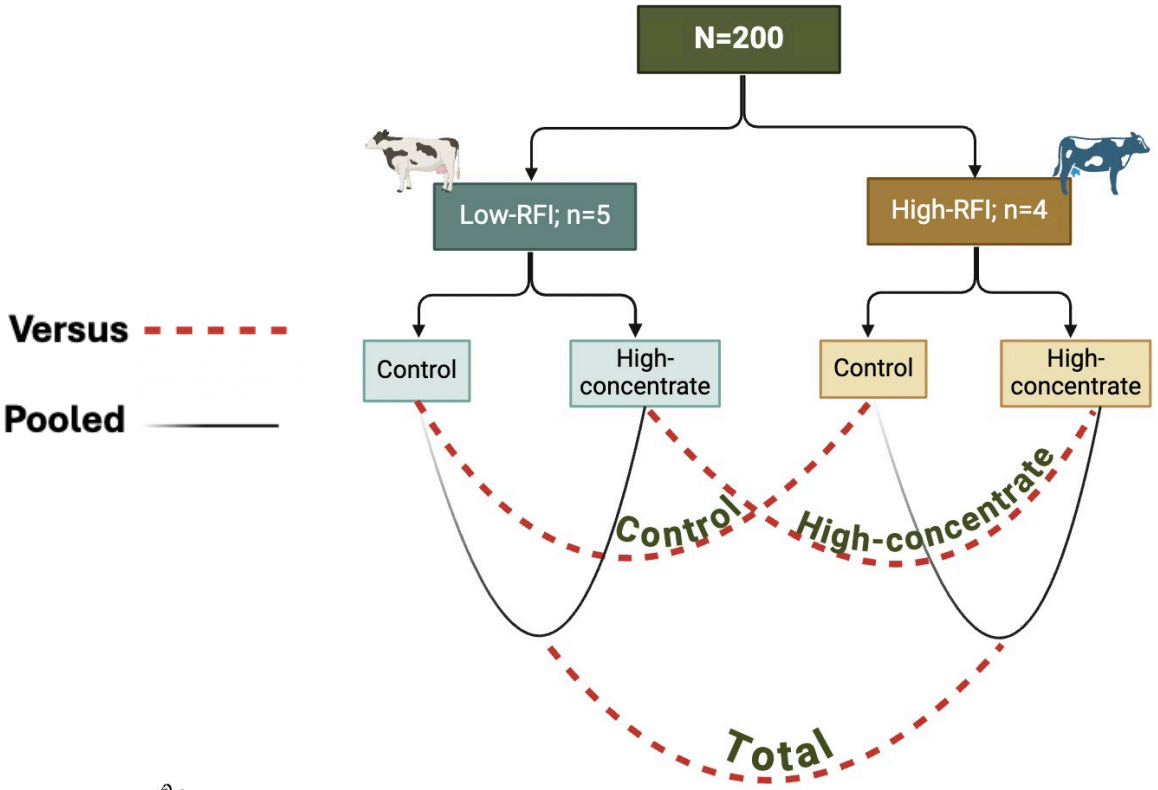
High-concentrate



Methodology: Comparison objective 2

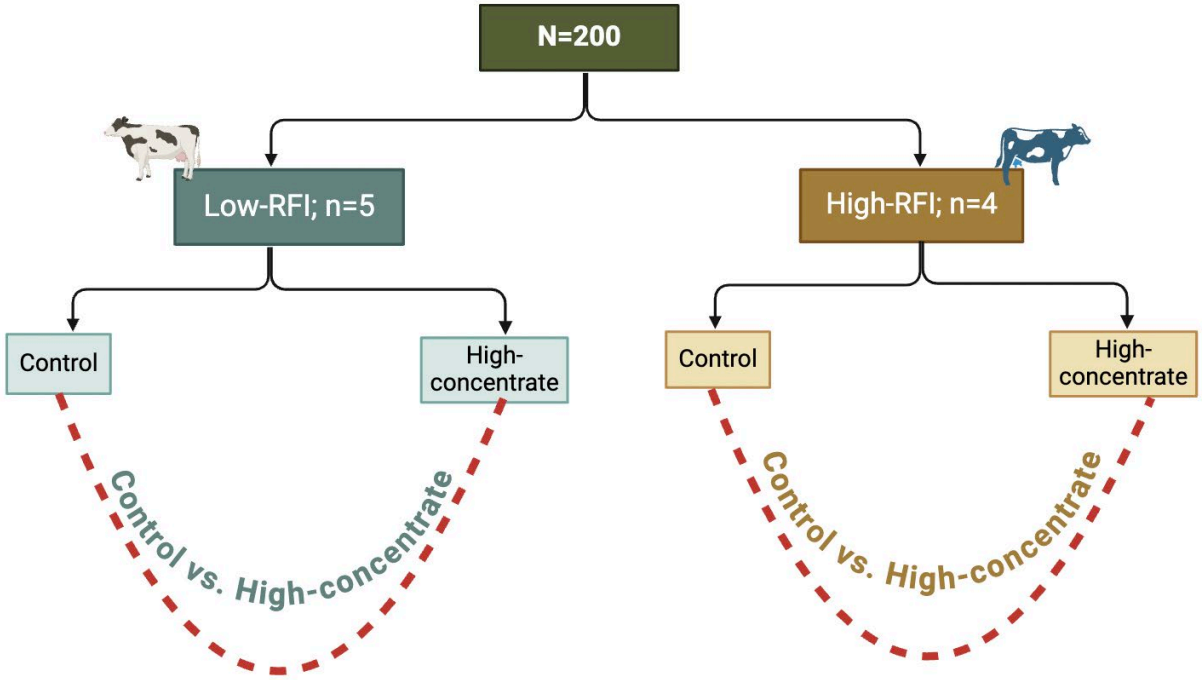
High vs. Low-RFI = Impact of RFI on mRNA isoform expression

1. Control
2. High-concentrate
3. Total (Control + High-concentrate)



Control vs. High-concentrate diet = **Impact of diet** on mRNA isoform expression

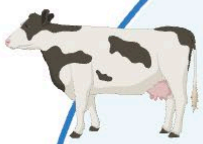
1. High-RFI
2. Low-RFI



Control vs. High concentrate: Differentially expressed mRNA isoforms

p-val<0.01, FC |>| 2

DYNLRB2_4
ENSBTAT00000054111
LTBP2_1

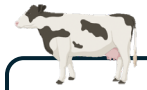


CHAMP1_1
ISOC1_1
GLYR1_202

Control vs. High concentrate: Top 3 significant mRNA isoforms and functional analysis

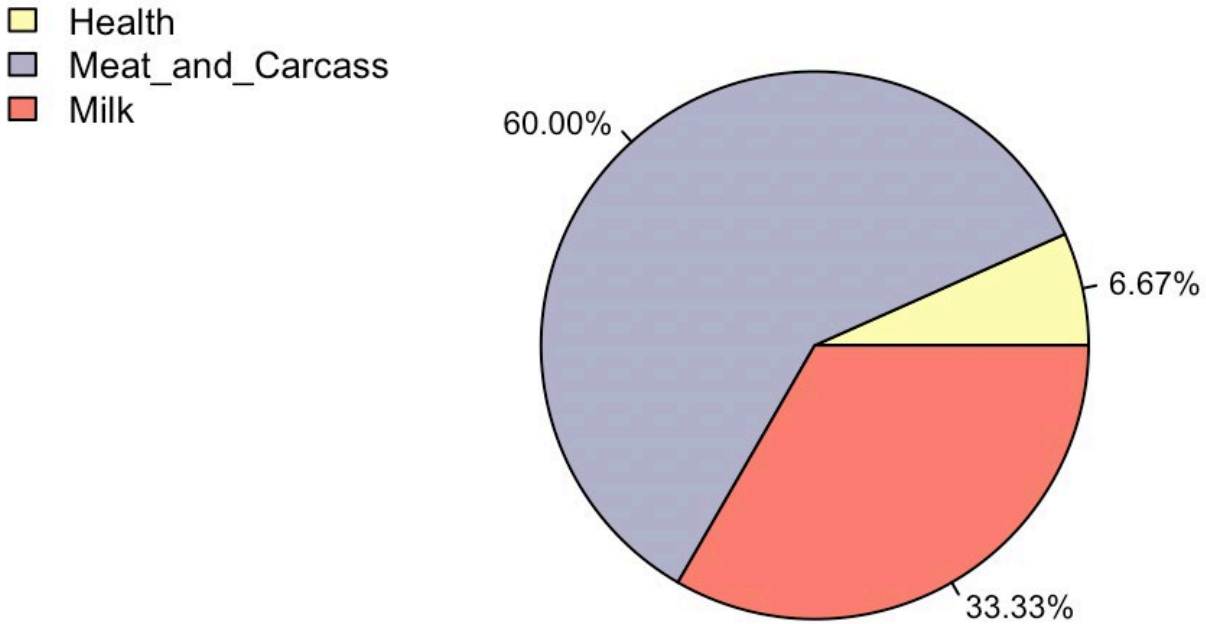
RFI	mRNA isoforms	Associated gene names	Gene Ontology: Biological process	p-value
 **Low-RFI**	*DYNLRB2*_4 ↓	*DYNLRB2*	Microtubule-based movement	3.00x10-4
	ENSBTAT00000054111 ↑	*ENSBTAG00000039130*	Protein folding	8.00x10-4
	*LTBP2*_1 ↑	*LTBP2*	Supramolecular fiber organization	1.30x10-3
 **High-RFI**	*CHAMP1*_1 ↓	*CHAMP1*	Sister chromatid biorientation	3.98x10-6
	*ISOC1*_1 ↑	*ISOC1*	Cytoplasm	6.00x10-4
	GLYR1-202 ↑	*GLYR1*	Transcription initiation	1.10x10-3

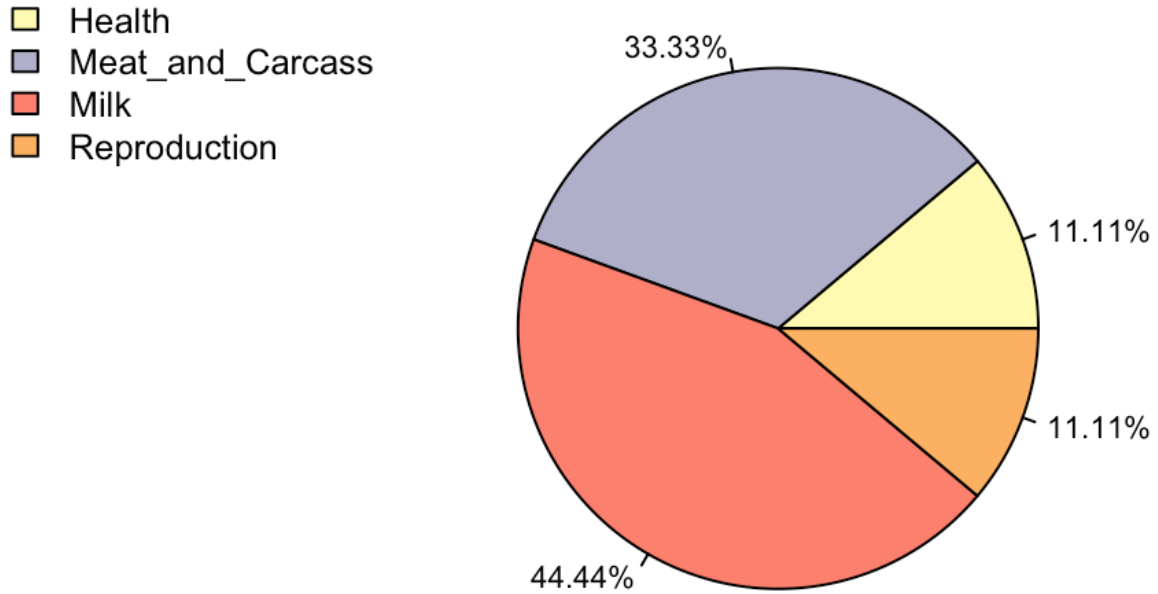
Control vs. High concentrate: Overlapping QTLs using list of differentially expressed mRNA isoforms



Low-RFI

High-RFI





Diverging RFI groups fed control and high concentrate diets

- RFI is a **major determinant** of how animals utilize nutrients.
- Low-RFI animals **activates** mechanisms involved in **cellular reproduction, growth and metabolism**.
- More feed efficient animals have **greater co-localizations with meat and carcass** traits potentially due to the **negative activity** associated with **cellular growth and metabolism** leading to **allocation of nutrients towards milk production**.

Acknowledgements

Food from Thought

Ontario
Ministry of Agriculture, Food and Rural Affairs

THE Resilient Dairy GENOME PROJECT

THE Efficient Dairy GENOME PROJECT

BCRC
BEEF CATTLE RESEARCH COUNCIL

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NSERC CRSNG
NSERC Alliance grants

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Canada

Cánovas lab at Red rose farm; 747710 Township Road 4, Drumbo, Ontario, N0J 1G0

Thank you!

Fatimatzahra Muhammad - **muhammaf@uoguelph.ca**

Ration composition of experimental diets

Ingredients	Low Concentrate	High Concentrate
Forage:Concentrate	68:32	39:61
Grass/clover silage (g/kg DM)	684	391
Barley (g/kg DM)	189	377
Rapeseed cake (g/kg DM)	25.7	51.4
Soybean mean (g/kg DM)	85.7	171
Urea (g/kg DM)	4.7	2.7
Mineral premix (g/kg DM)	9.3	5.3
Vitamin premix (g/kg DM)	2.1	1.2
Gross energy (MJ/kg DM)	18.7	19.2
DM (g/kg)	513	620
Ash (g/kg DM)	72	57.3
Crude protein (g/kg DM)	170	204
Crude fat (g/kg DM)	31.8	33.6
Starch (g/kg DM)	105	218
Crude fiber (g/kg DM)	179	127
NDF (g/kg DM)	335	271
iNDF (g/kg DM)	45.3	41.8

- ✓ Crossover design with 2 periods
- ✓ ~30% difference in the concentrate proportion of dry matter

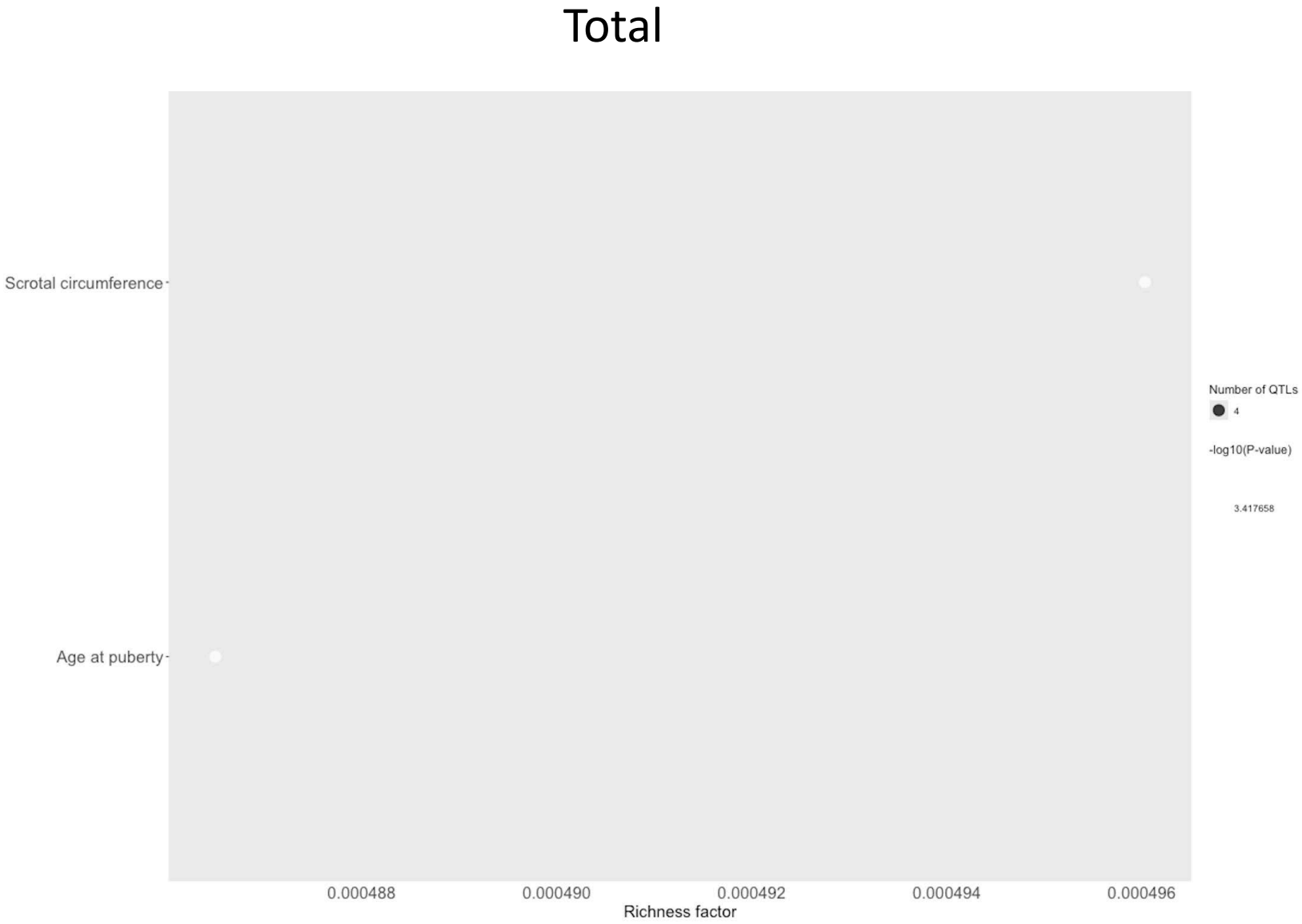
Number of QTLs: Low-RFI vs. High-RFI

Total	
QTL type	No. of QTLs
Reproduction	4
Control	
Exterior	1
Meat_and_Carcass	1
Milk	4
Reproduction	4
High Concentrate	
Exterior	9
Health	1
Milk	12
Production	5
Reproduction	22

Number of QTLs: Control vs. High concentrate

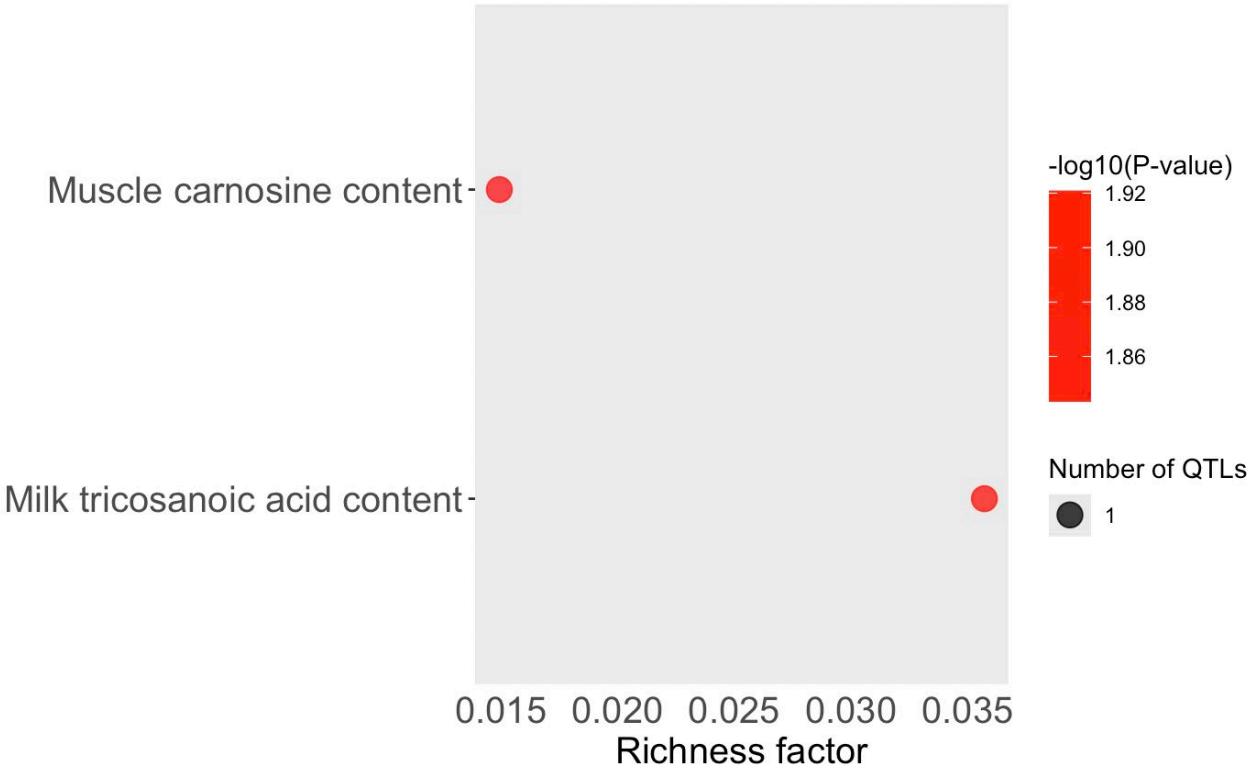
Low-RFI	
QTL type	No. of QTLs
Health	1
Meat_and_Carcass	9
Milk	5
High-RFI	
Health	1
Meat_and_Carcass	3
Milk	4
Reproduction	1

Impact of RFI: QTL enrichment analysis

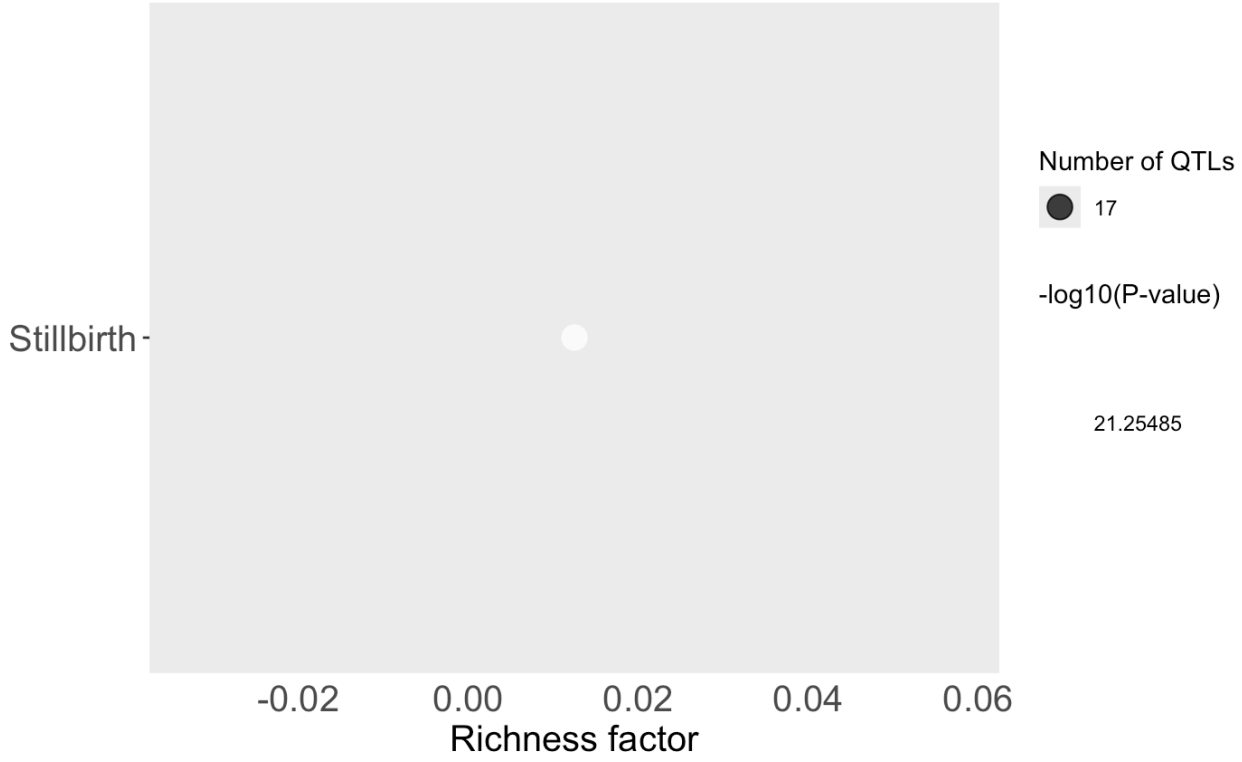


Impact of RFI: QTL enrichment analysis

Control



High-concentrate



Impact of diet: QTL enrichment analysis