Milk Quality Workshop 2024

Impact of a grass-based system on milk fat and micronutrient composition

Tailoring milk protein profile through genetic selection

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Dairy Research Ireland
funded by Irish Dairy Farmers

Milk supply

2 supply patterns

1. Liquid milk pool:
Winter milk schemes
Approx. 15 % of total supply

2. Manufacturing milk pool:
Spring-calving herds
Approx. 85 % of total supply

- Prevalence of seasonal production - focus on long shelf-life commodities (butter, cheddar cheese, milk powders)

- Up to 1000 % self-sufficient in some products - 85 % of products exported
  - (worth ~ €6.8 billion in 2022)
  - 40 % of all milk in Ireland used for cheese

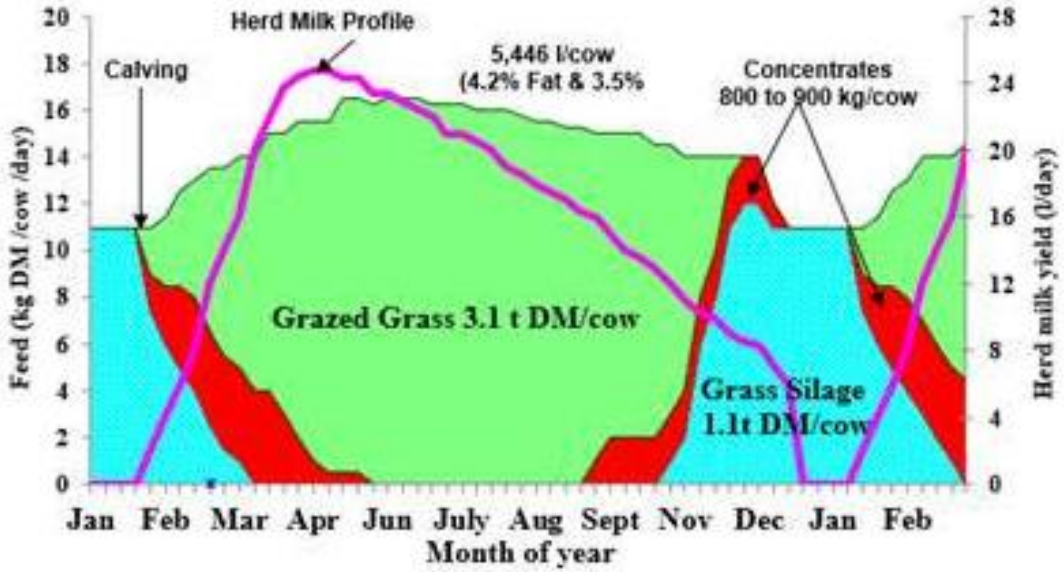
On a national level, pasture-based system is fundamental to the majority of milk production and export product portfolio

Images: Strathroy Dairies/Ornua

“Grass-fed” marketing

Grass-fed standard

- Cows averaging 95% grass fed on a fresh weight basis.
- Most Irish cows grazing for average of 240 days per year, often up to 300 days grazing (mid Feb to mid Nov)
- Contrast: organic production – 120 days, Netherlands - 120 days, but premium applied.



Manufacturing milk pool - Seasonality

- Changes due to combination of lactational and dietary effects
- Milk less suitable for processing & lower volumes available
- Can pose challenges for processor in early/late lactation

%	Total solids	Protein	Fat	Lactose	Ash	Casein	Whey
Early-lactation	13.6	3.33	4.56	4.98	0.73	2.66	0.48
Mid-lactation	13.56	3.51	4.46	4.92	0.67	2.78	0.54
Late-lactation	14.58	3.89	4.9	4.75	1.04	3.31	0.65
Yearly average	13.95	3.65	4.65	4.87	0.78	2.95	0.56

Data from seasonality study conducted on Moorepark herd

Recent studies on effects of pasture diets on milk quality:

Grass, Grass/clover & total mixed ration

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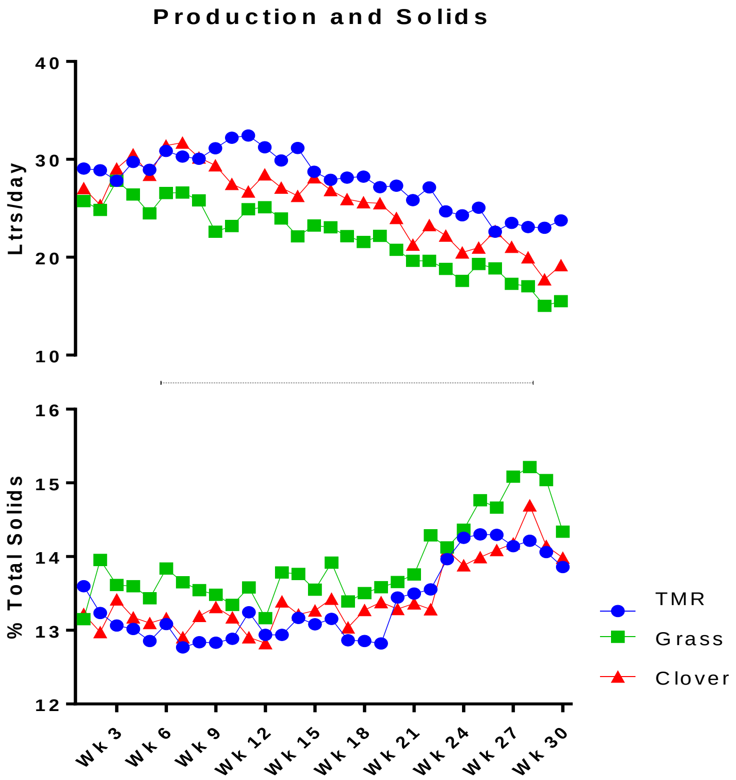
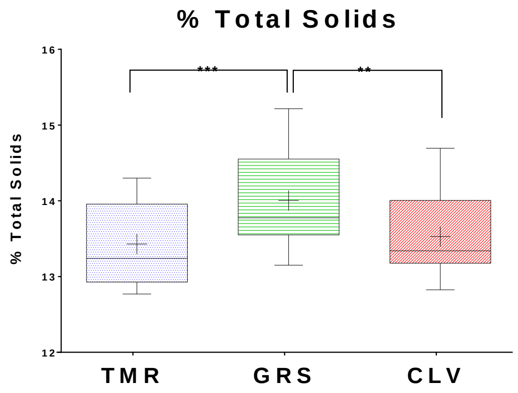
Perennial ryegrass pasture
– 18 kg DM per day
(GRS)

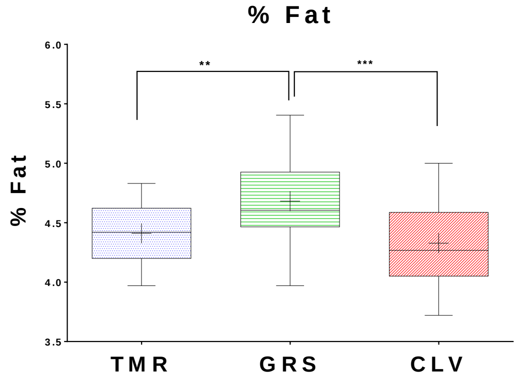
Perennial ryegrass / 20 % white clover pasture
– 18 kg DM per day
(CLV)

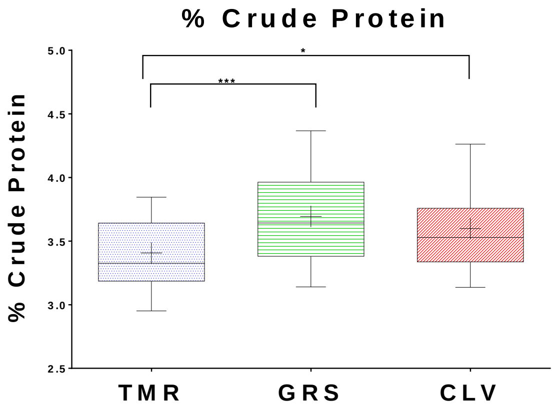
Indoor total mixed ration
– 7.15 kg grass silage, 7.15 kg maize silage + 8.3 kg concentrate per day
(TMR)

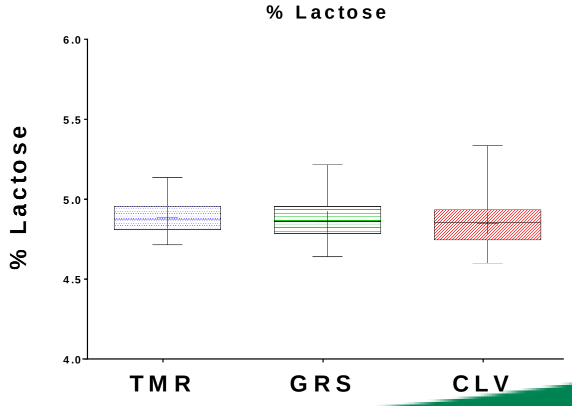
Diet impacts milk yield & constituents throughout lactation











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Pasture based feeding has a beneficial effect on milk fatty acid profile

In total 26 Fatty Acids were analyzed from bulk milks weekly from March to October

	Yearly Average			Significance:	
	TMR	GRASS	CLOVER	*P*	η^2
Butyric Acid (C4:0)	3.93 ± 0.71	3.69 ± 0.63	3.66 ± 0.45	0.257	-
Caproic Acid (C6:0)	2.01 ± 0.37	1.90 ± 0.35	1.90 ± 0.24	0.410	-
Caprylic Acid (C8:0)	1.08 ± 0.19	1.05 ± 0.20	1.06 ± 0.14	0.872	-
Capric Acid (C10:0)	2.34 ± 0.46	2.35 ± 0.55	2.42 ± 0.38	0.567	-
Undecanoic Acid (C11:0)	0.04 ± 0.02	0.06 ± 0.03	0.05 ± 0.02	0.001	0.769
Lauric Acid (C12:0)	2.65 ± 0.53	2.68 ± 0.64	2.76 ± 0.43	0.678	-
Tridecanoic Acid (C13:0)	0.07 ± 0.02	0.08 ± 0.03	0.08 ± 0.02	0.009	0.650
Myristic Acid (C14:0)	8.29 ± 1.67	8.17 ± 1.53	8.22 ± 0.92	0.943	-
Myristoleic Acid (C14:1)	0.72 ± 0.21	0.76 ± 0.22	0.71 ± 0.17	0.459	-
Pentadecanoic Acid (C15:0)	0.78 ± 0.16	0.95 ± 0.21	0.92 ± 0.12	0.003	0.732
Palmitic Acid (C16:0)	24.39 ± 4.60	20.73 ± 3.21	19.98 ± 2.26	0.002	0.743
Palmitoleic Acid (C16:1)	1.22 ± 0.25	1.19 ± 0.21	1.10 ± 0.16	0.094	-
Heptadecanoic Acid (C17:0)	0.43 ± 0.08	0.49 ± 0.09	0.47 ± 0.07	0.009	0.646
Stearic Acid (C18:0)	7.25 ± 1.42	7.06 ± 1.32	6.90 ± 1.09	0.280	-
Oleic Acid (C18:1n9c)	14.59 ± 2.83	13.99 ± 3.02	13.23 ± 2.46	0.064	-
Linolelaidic Acid (C18:2n6t)	0.15 ± 0.07	0.33 ± 0.06	0.36 ± 0.04	0.001	0.968
Linoleic Acid (C18:2n6c) (LA)	1.31 ± 0.28	0.55 ± 0.21	0.64 ± 0.17	0.001	0.972
α-Linolenic Acid (C18:3n3) (ALA)	0.27 ± 0.10	0.53 ± 0.09	0.68 ± 0.10	0.001	0.973
CLA (c9t11)	0.58 ± 0.15	1.44 ± 0.37	1.32 ± 0.25	0.001	0.956
CLA (c12t10)	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.021	0.578
Eicosenoic Acid (C20:1)	0.05 ± 0.02	0.08 ± 0.03	0.09 ± 0.03	0.001	0.842
Eicosatrienoic Acid (C20:3n6)	0.10 ± 0.06	0.00 ± 0.01	0.01 ± 0.02	0.001	0.979
Behenic Acid (C22:0)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.685	-
Erucic Acid (C22:1n9)	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.001	0.888
Tricosanoic Acid (C23:0)	0.00 ± 0.01	0.04 ± 0.03	0.05 ± 0.03	0.001	0.876
Arachidonic Acid (C20:4n6)	0.00 ± 0.01	0.05 ± 0.03	0.06 ± 0.02	0.001	0.970

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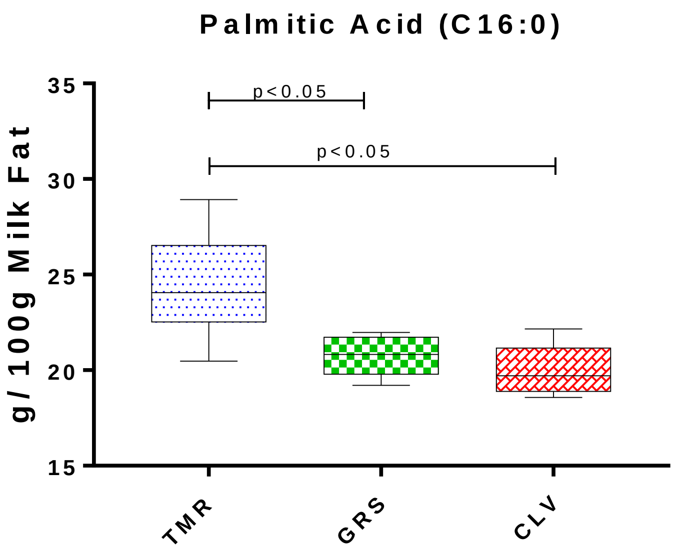

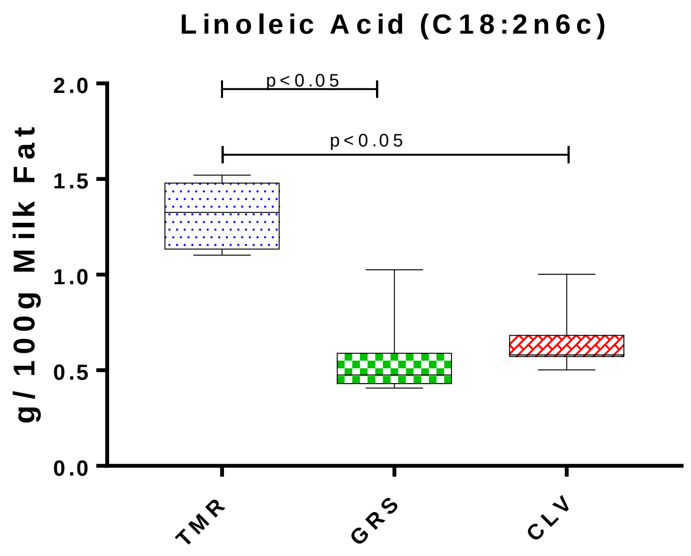

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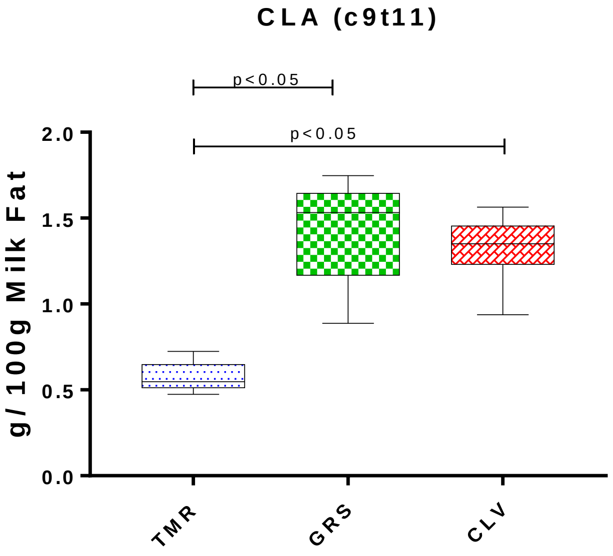

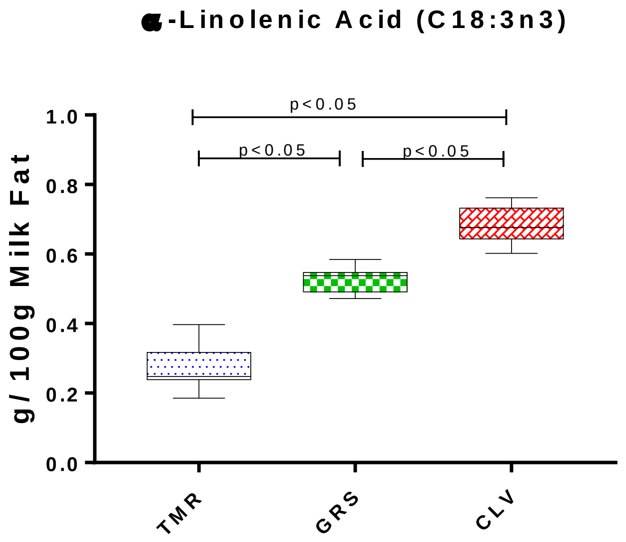

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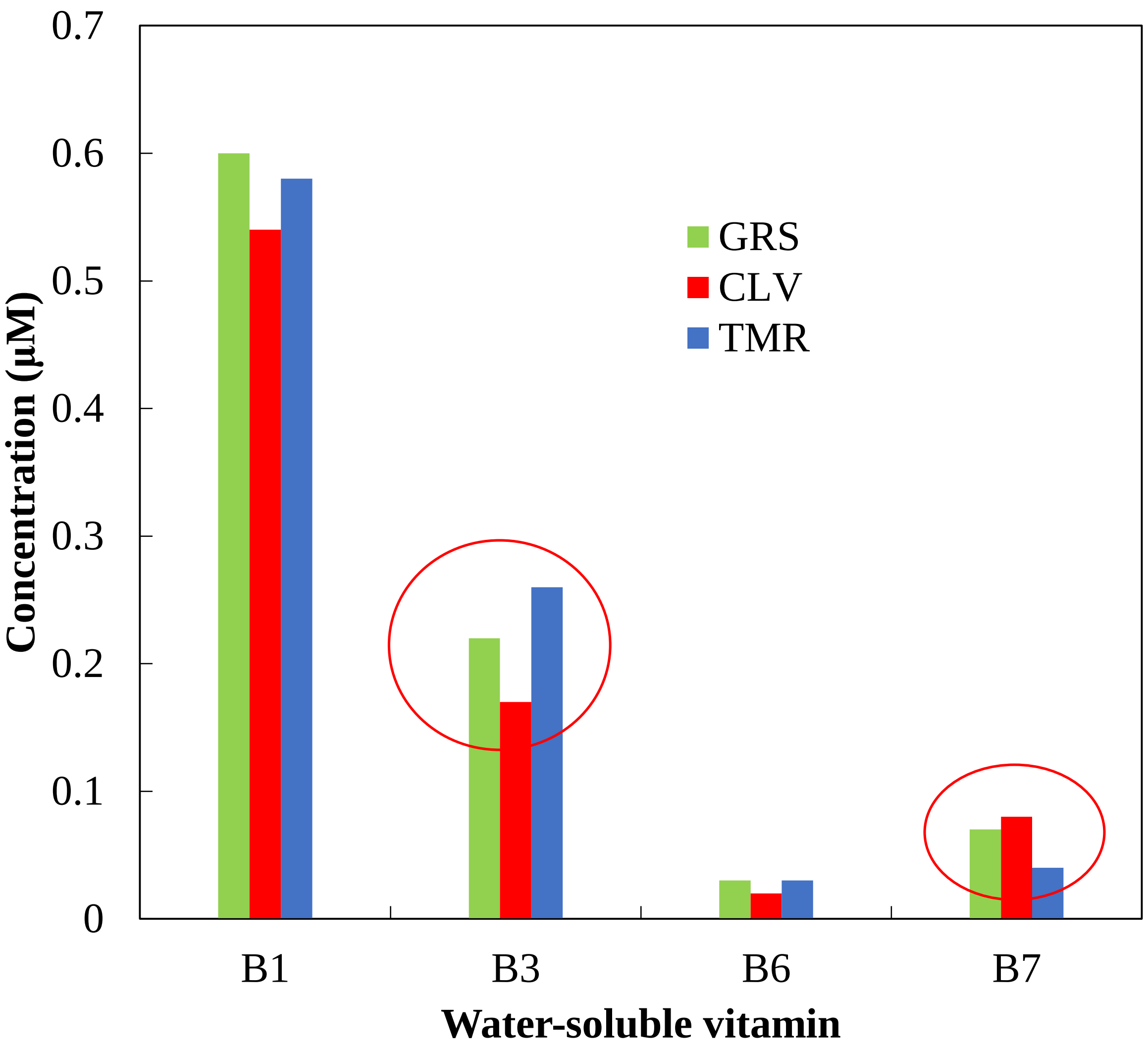
Milk mineral content

	Mineral	GRS	CLV	TMR
Major elements (mg per 100 g)	**Calcium**	**142.2**	133.7	131.8
	Phosphorus	**104**	98.9	101
	Sodium	**49.1**	47.0	46.7
	Magnesium	13.4	13.0	13.0
Trace elements (µg per kg)	**Zinc**	4589	4417	**4822**
	Iron	542	504	331
	Copper	60.3	47.2	**76.9**
	Molybdenum	45.9	43.4	46.4
	Manganese	**42.5**	40.7	29.2
	Selenium	15.7	13.9	**27.1**
	Cobalt	0.80	0.75	0.82
	Iodine	117	79.3	**407**

Vitamin B Profile of skim milk and whey



Values within each series cluster not sharing a common letter differed significantly.

- Significantly higher vitamin B2 (riboflavin) in both GRS and CLV samples than in TMR sample.
- Significantly higher vitamin B3-amide (niacinamide) in TMR sample than in GRS sample.

- Significantly higher vitamin B3 (niacin) in TMR sample than in CLV sample.
- Significantly higher vitamin B7 (biotin) in in both GRS and CLV samples than in TMR sample.

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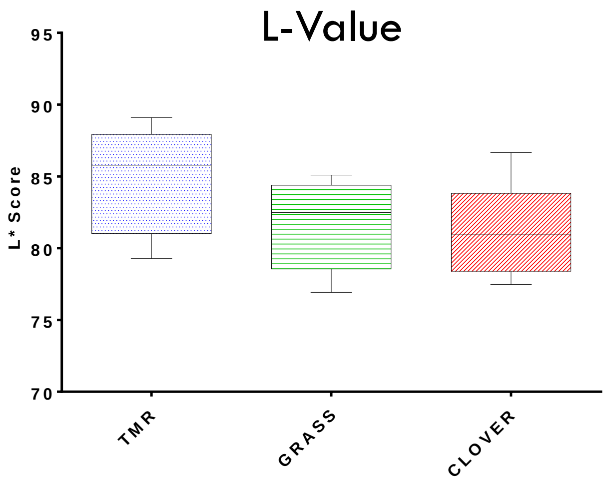
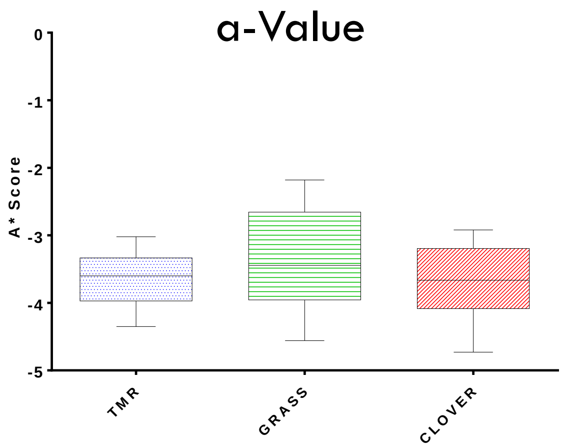
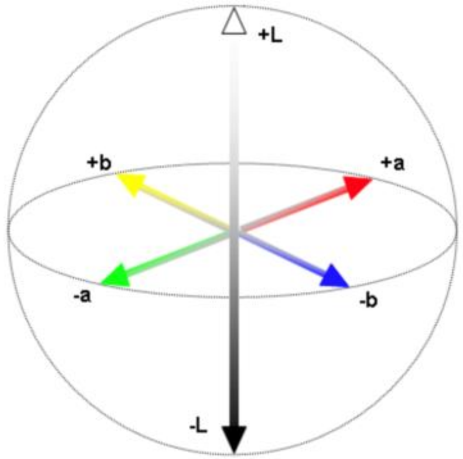
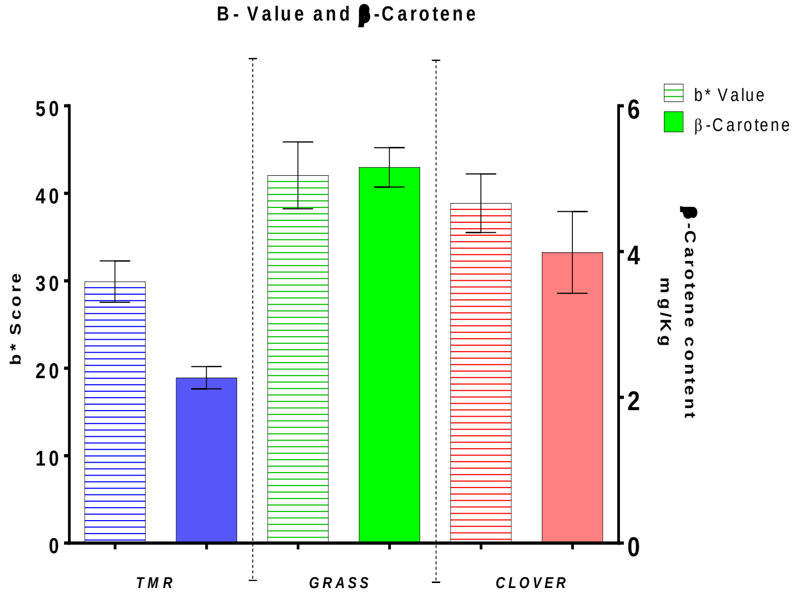
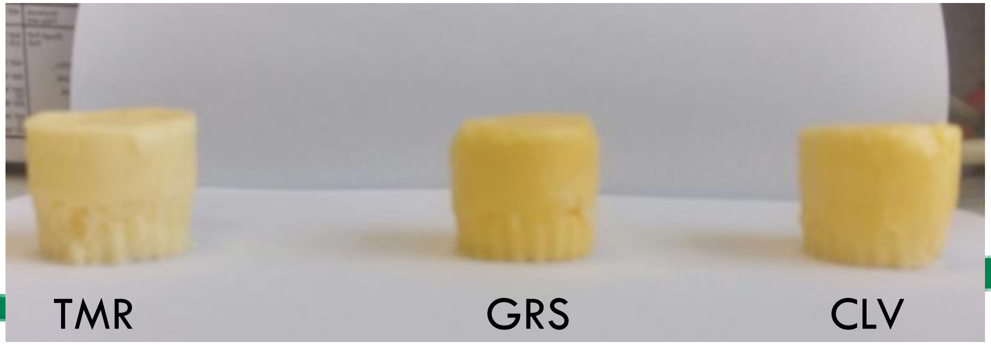
Pasture derived butter is more yellow in colour











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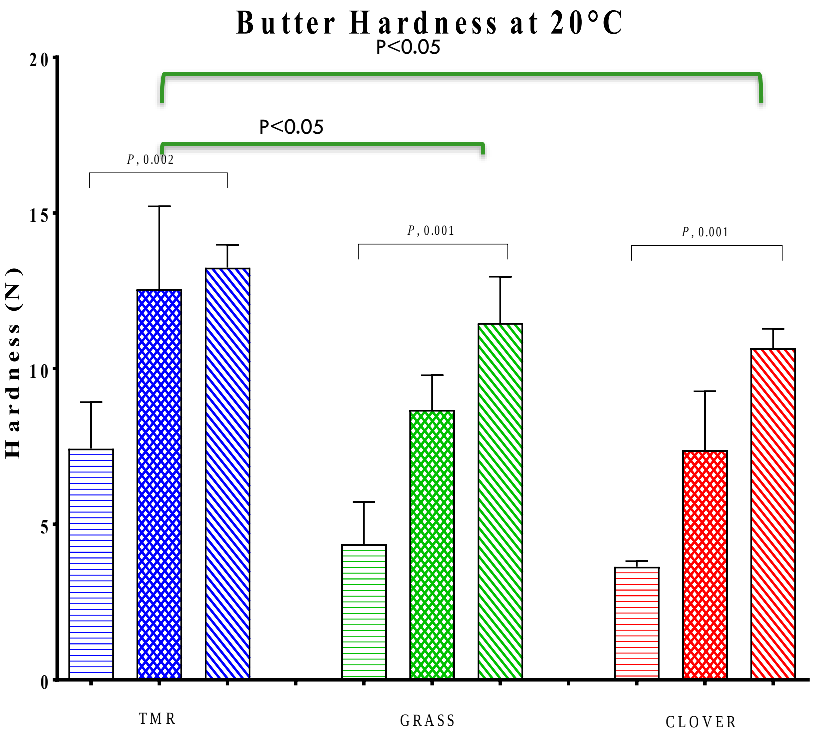
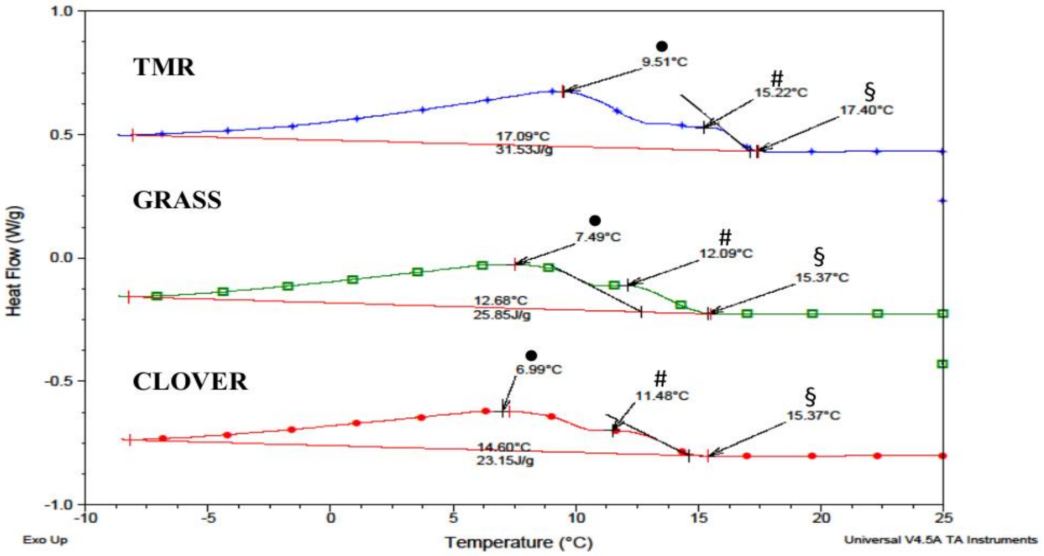
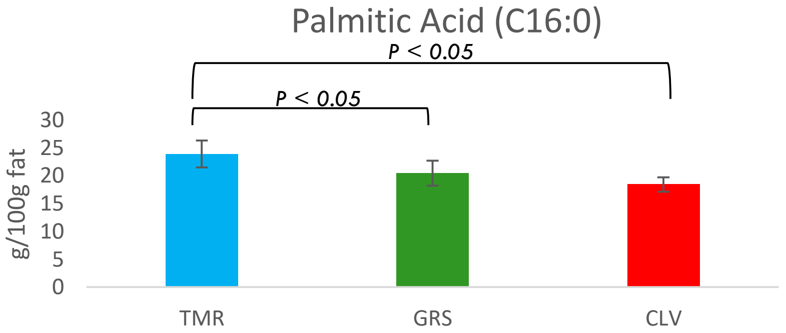
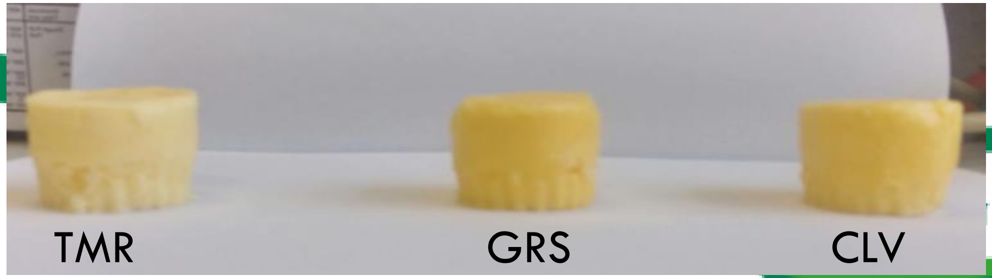
Cow diet has a significant effect on butter texture









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Recent studies on effects of pasture diets on milk quality:

Grass, partial mixed ration & total mixed ration

GRS

- 95% grazed pasture annually
- 5% supplemented concentrates

PMR

- GRS diet at day
- TMR diet at night

TMR

- 40% maize silage
- 40% concentrates
- 20% grass silage

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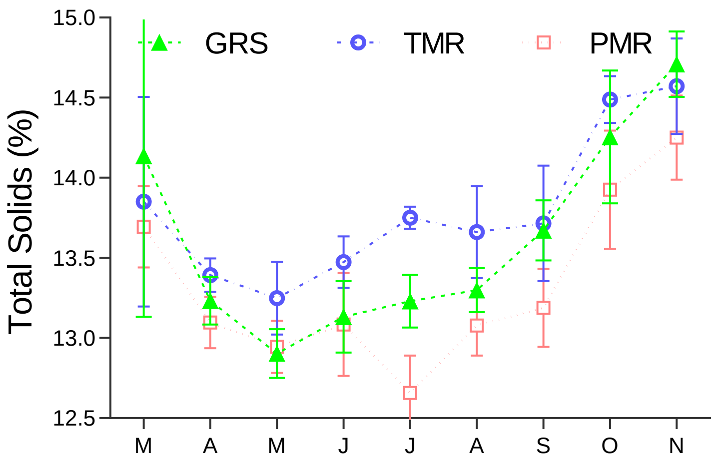
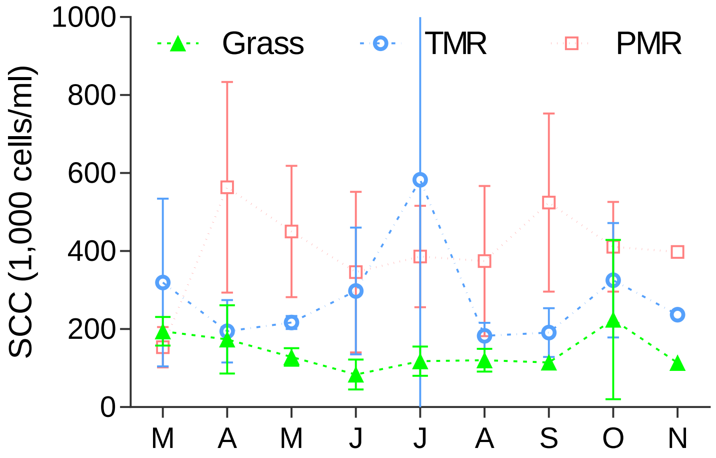
Diet impacts milk quality throughout lactation



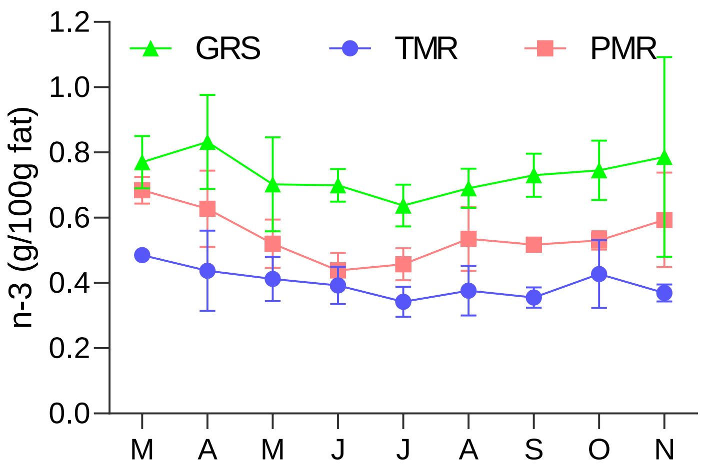


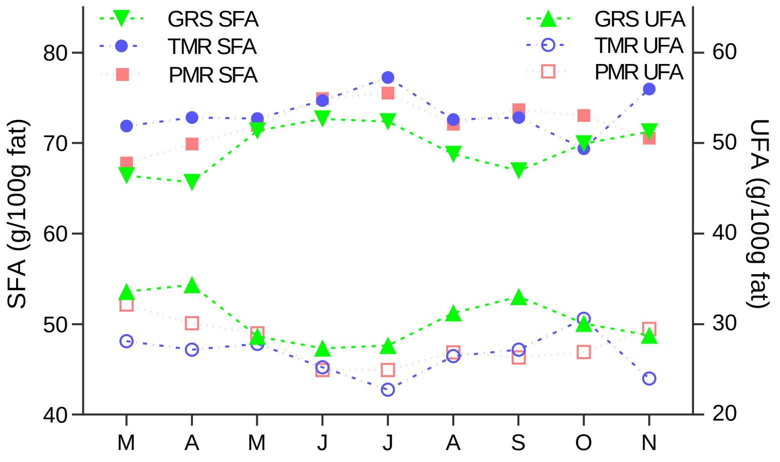
- **Contrast to previous study (different years):** higher solids in TMR milk throughout mid-lactation than GRS and PMR

- GRS cows with significantly better udder health than more conventional diets

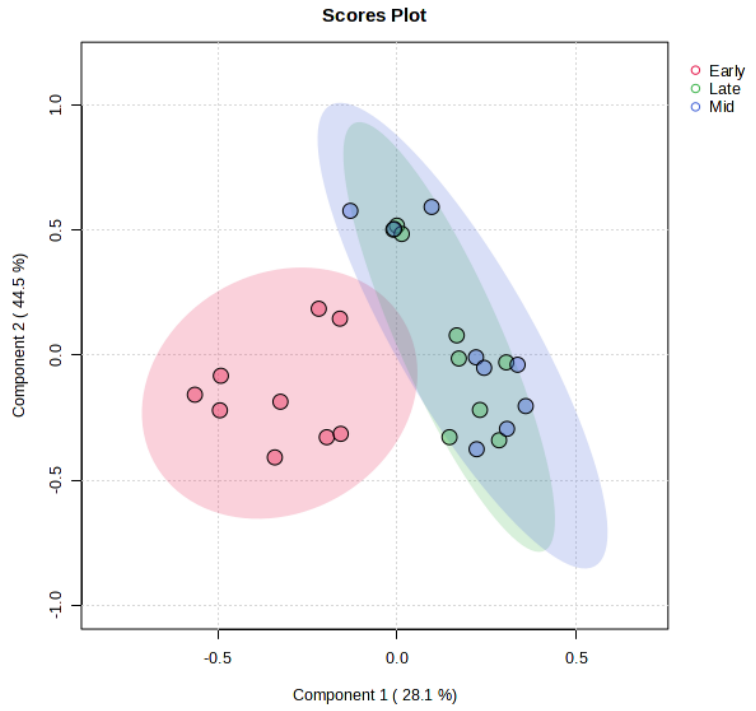
- Under 200,000 cells/mL for most of the season

Pasture based feeding has a beneficial effect on milk fatty acid profile





Nutritionally Beneficial FA



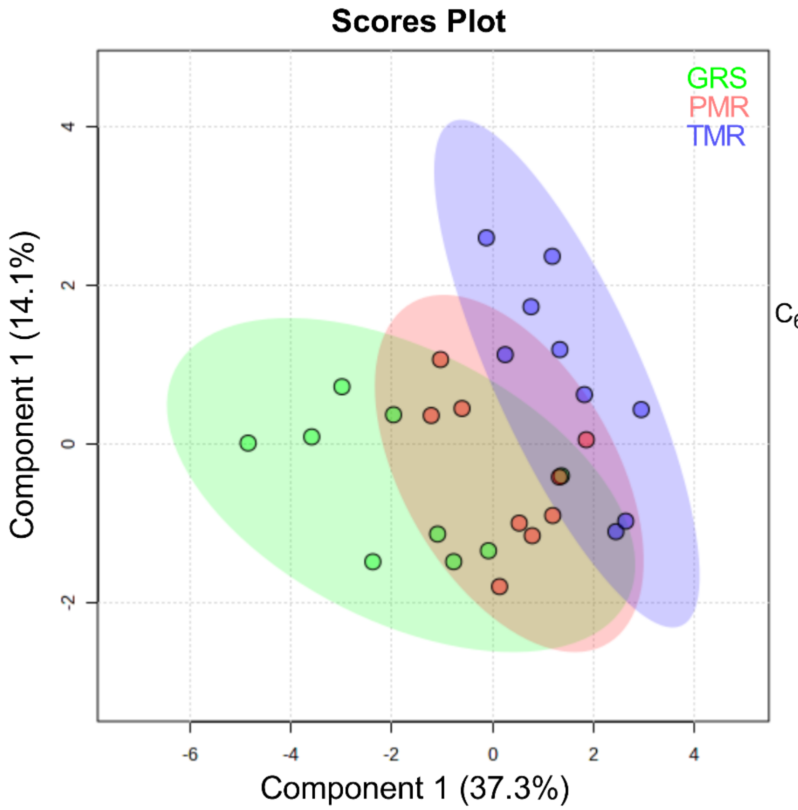
Increasing with increasing proportions of pasture (GRS > PMR > TMR) including:

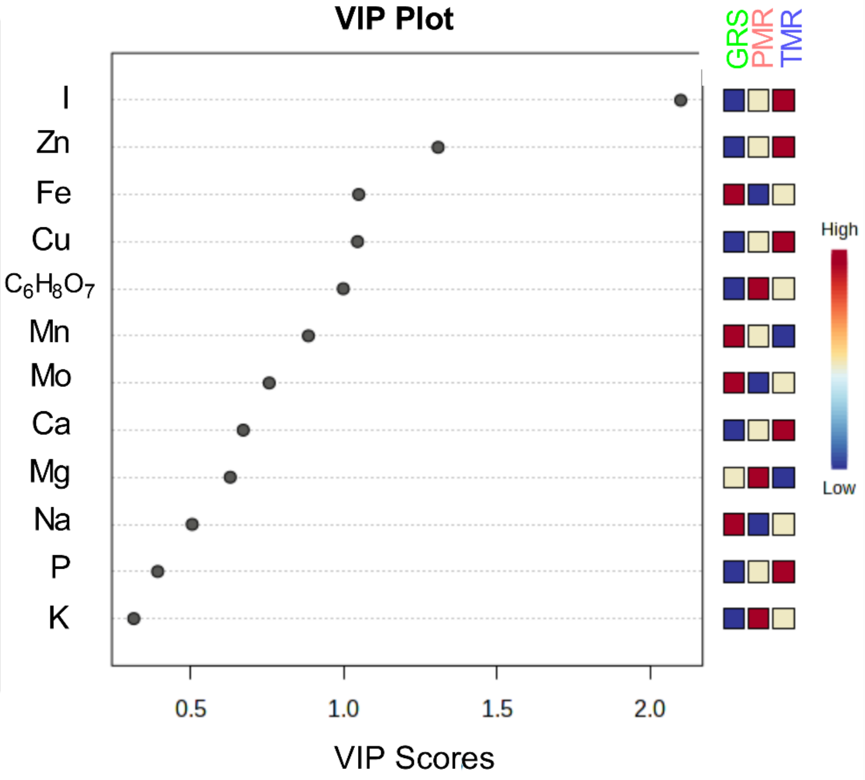
- 141% increase in conjugated linoleic acid
- 83% increase in n-3 FA
- Unsaturated FA

- Negative energy balance of the animals in early lactation the main reason for a stage of lactation effect on FA profile

Trend of increased beneficial fatty acids in pasture-derived products seen across milk, butter, cheddar cheese and whole milk powder

Milk mineral content





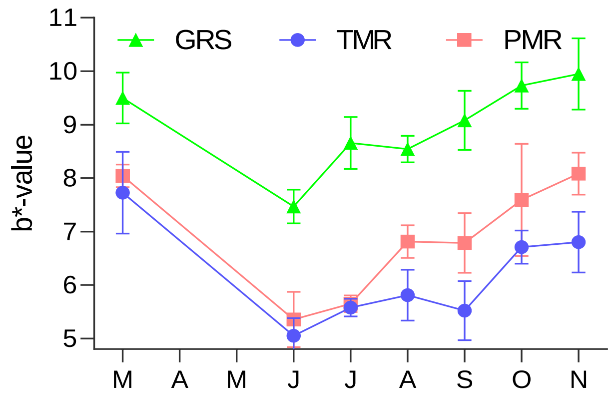
Ca	GRS < TMR & PMR
Fe	GRS > TMR & PMR
K	GRS < PMR
Cu	GRS < TMR & PMR
Zn	GRS < TMR & PMR
$C_6H_8O_7$	GRS < TMR & PMR
I	GRS < PMR < TMR
Na	GRS > PMR
Mo	GRS > PMR

Quality and consumer acceptance

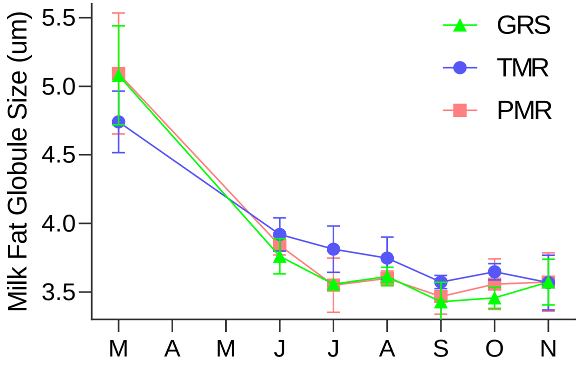
Do dietary differences impact the final product?

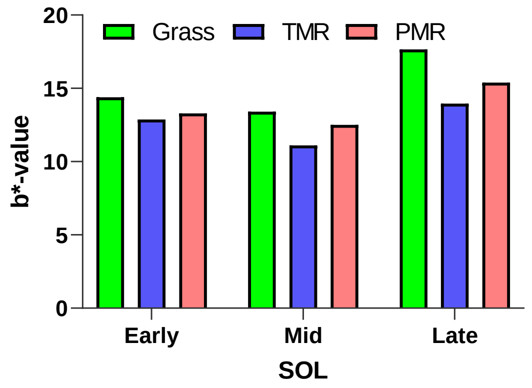
Can differences be perceived between groups?

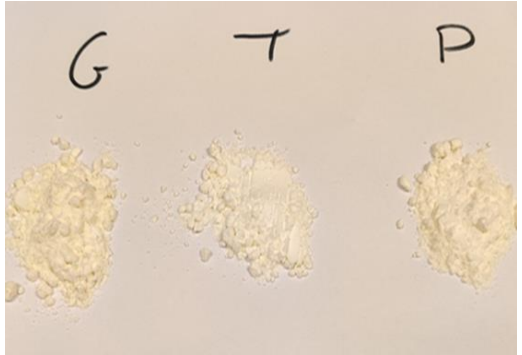
Grass feeding and colour

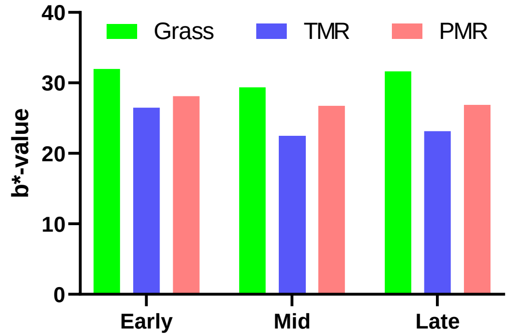


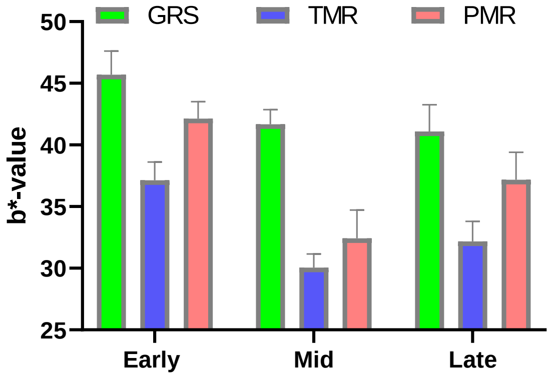
- Significant differences in the yellow colour of milk
- Similar impact of diet and SOL on the colour of whole milk powder, cheese and butter
- Size of milk fat globules impacted by SOL



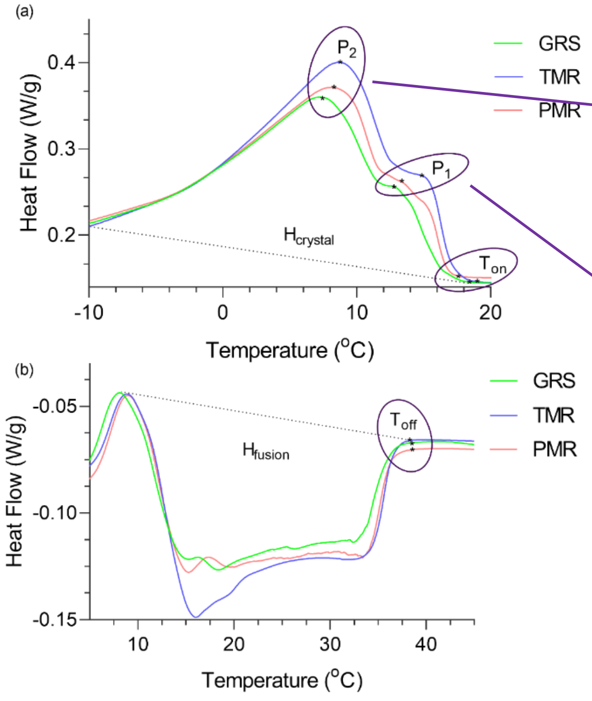


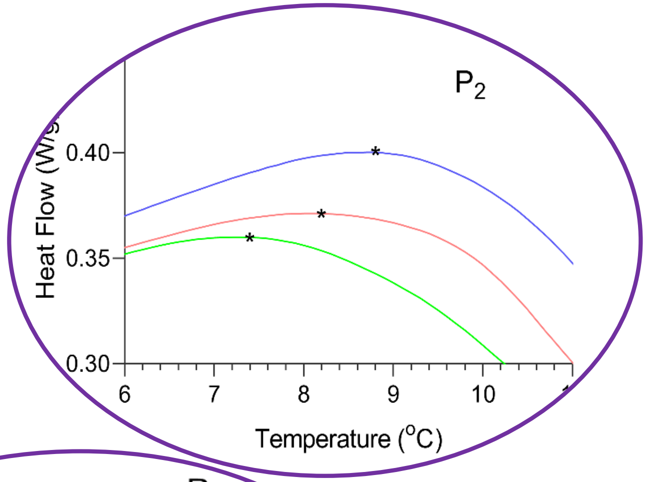


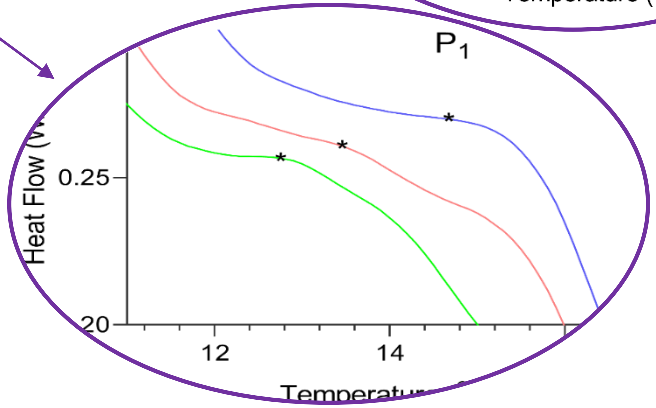


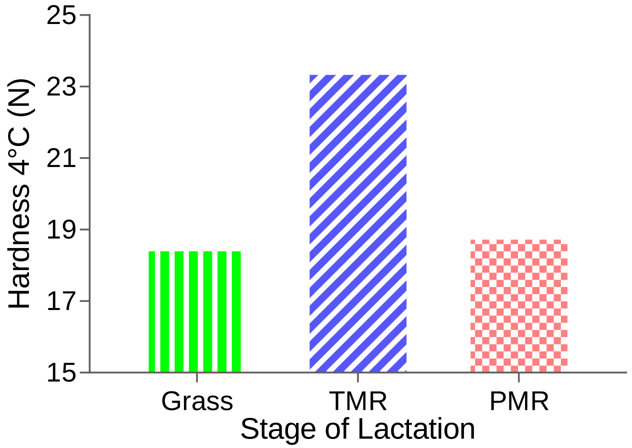


Melting profile of butter





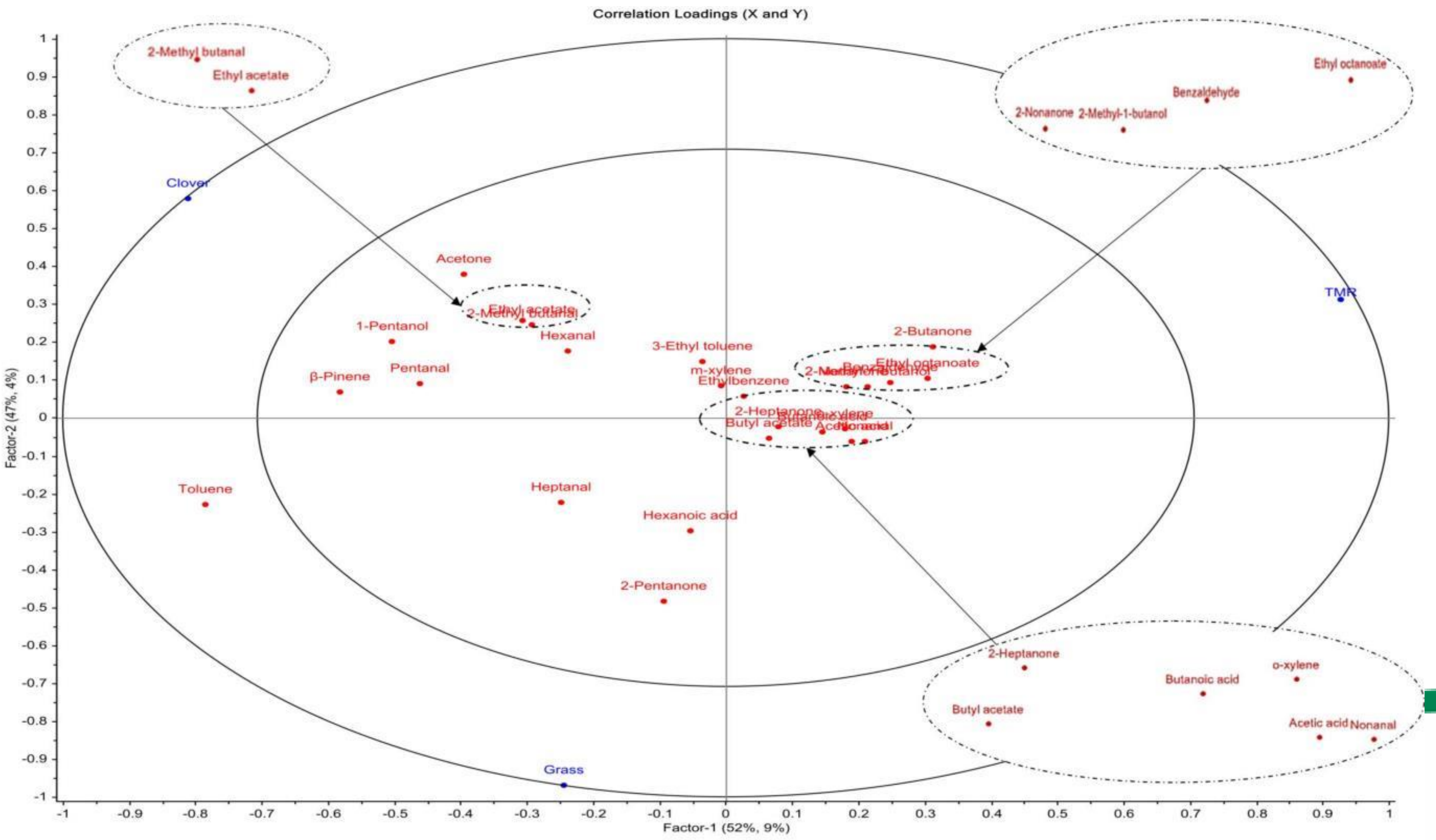




Difference in saturated:unsaturated fat ratio impacting the crystallising/melting behaviour of butter

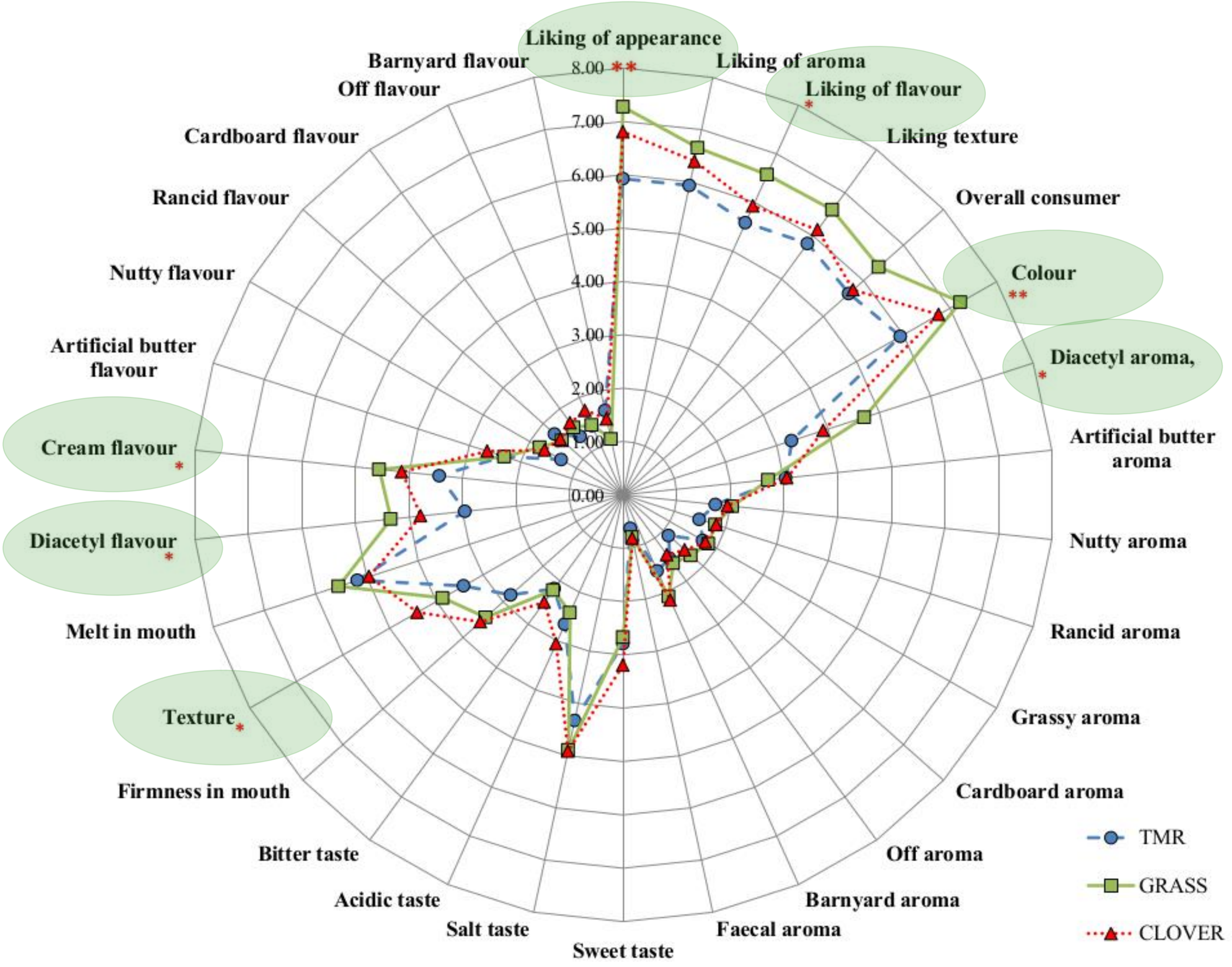
- Result of differences in structure (e.g. palmitic acid)
- Altering the liquid:solid fat ratio

Volatiles: Compounds responsible for flavour



- Effect of feed on rumen microorganisms – generating volatiles
- Acetone associated with CLV butter samples – higher than TMR and GRS.
- 2-Butanone was present at higher intensity in TMR butters than GRS butters.
- Toluene associated with pasture derived butters, more than TMR, highest in CLV.
- Para-cresol – compound responsible for “barnyard flavour” in CLV samples – unfavourable in tasting panels

Cow diet has a significant effect on butter sensory properties



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Recent studies on effects of pasture diets on milk quality:

Feed and breed

Breed 1
Holstein Friesian (**HF**)

Feed 1:
Perennial ryegrass (**PRG**)

PRG-HF

Feed 2:
Multispecies sward (**MSS**)
- 40% perennial ryegrass
- 15% other grasses
- 30% legumes
- 15% herbs

MSS-HF

Breed 2
Jersey x Holstein Friesian cross (**JHF**)

Feed 1:
Perennial ryegrass (**PRG**)

PRG-JHF

Feed 2:
Multispecies sward (**MSS**)
- 40% perennial ryegrass
- 15% other grasses
- 30% legumes
- 15% herbs

MSS-JHF

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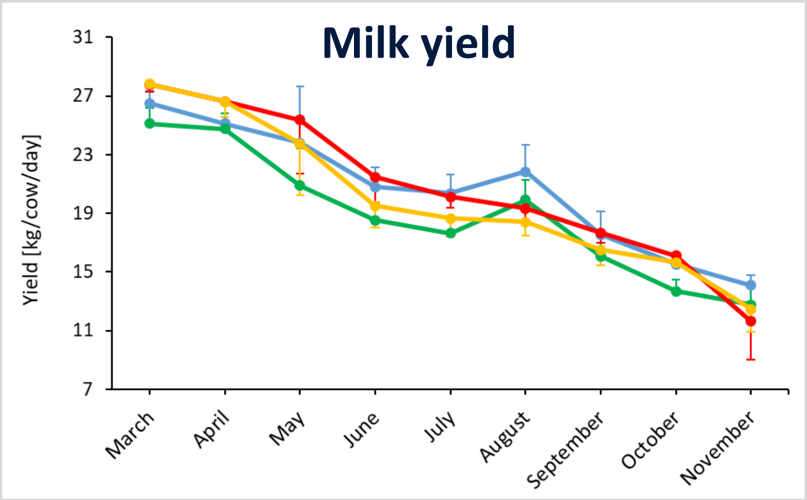
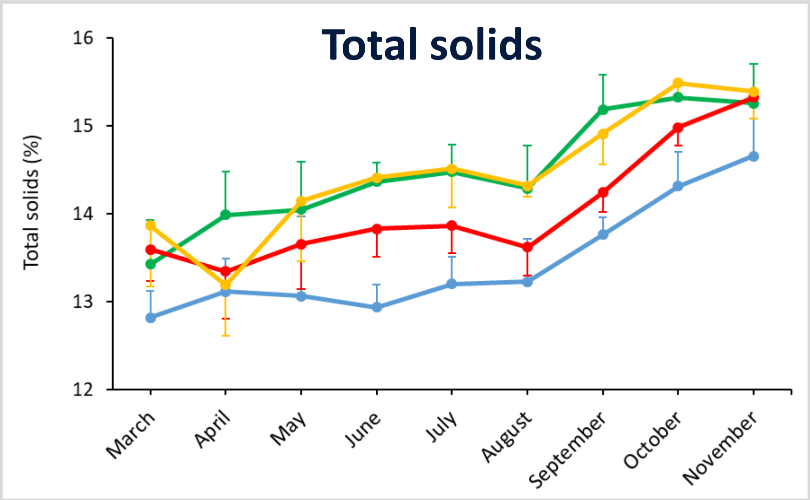
Diet and breed impact milk yield & constituents throughout lactation





Seasonal effect:

- Yield ⇩
- Lactose ⇩
- Protein & fat ⇧
- Total solids ⇧

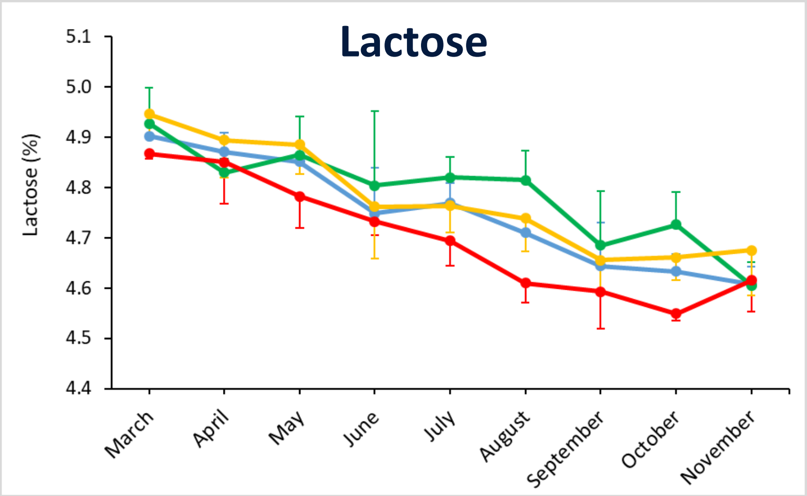
Breed effect

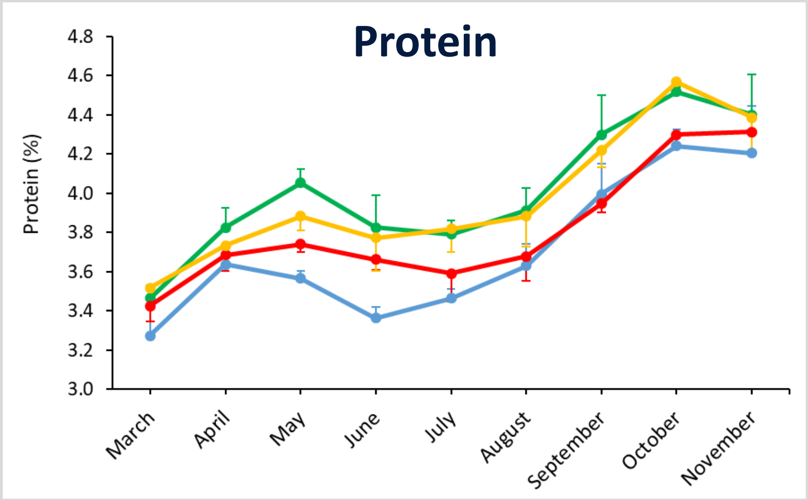
- JHF - lower yield, greater total solids (lactose, protein & fat) than HF

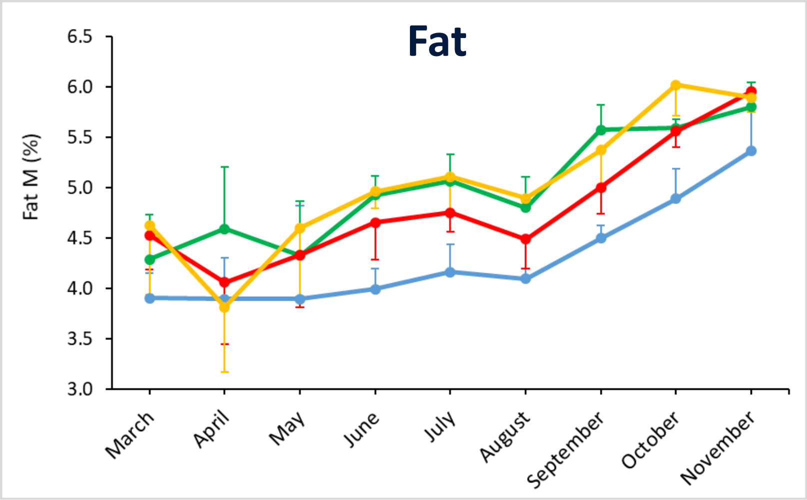
Feed effect

- MSS showed higher fat (& solids) content than PRG for HF

PRG_HF PRG_JHF MSS_HF MSS_JHF







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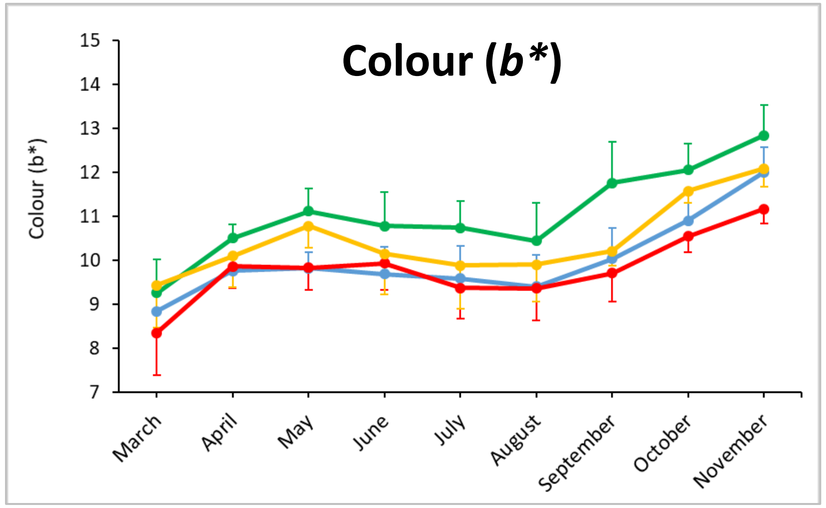
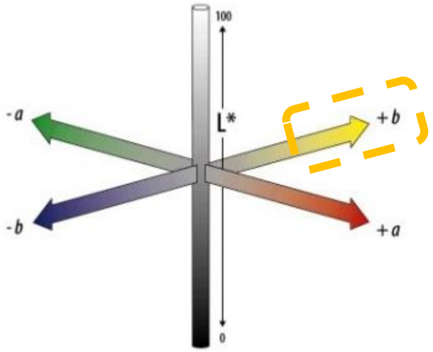
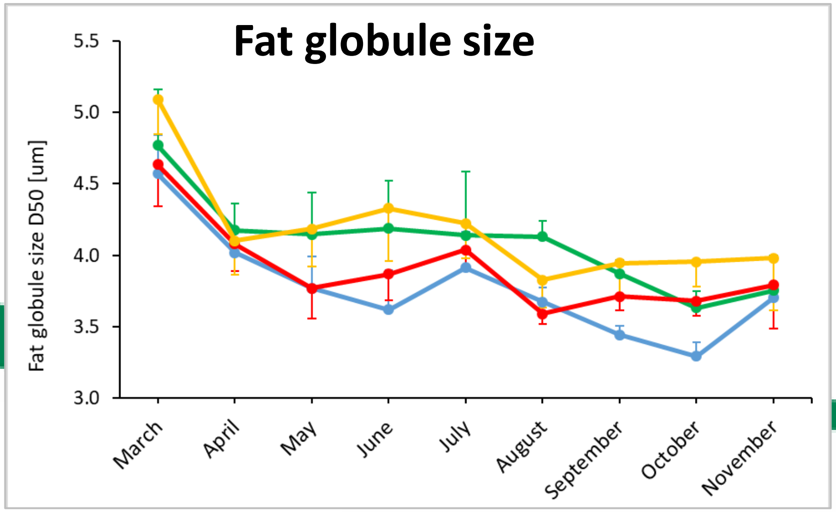
Fat, fat globules and colour

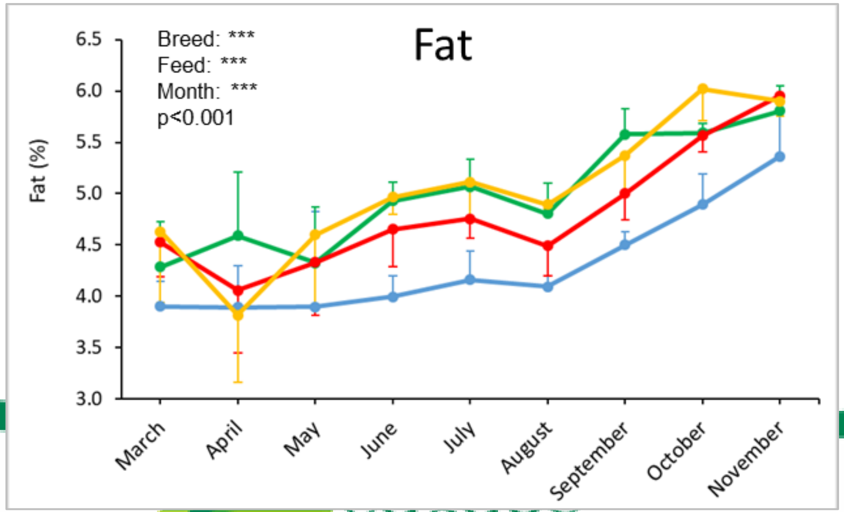




- Samples were increasingly yellow throughout lactation
- Highest for JHF

PRG_HF PRG_JHF MSS_HF MSS_JHF





- Fat globule size decreased throughout the season
- Again, highest for JHF

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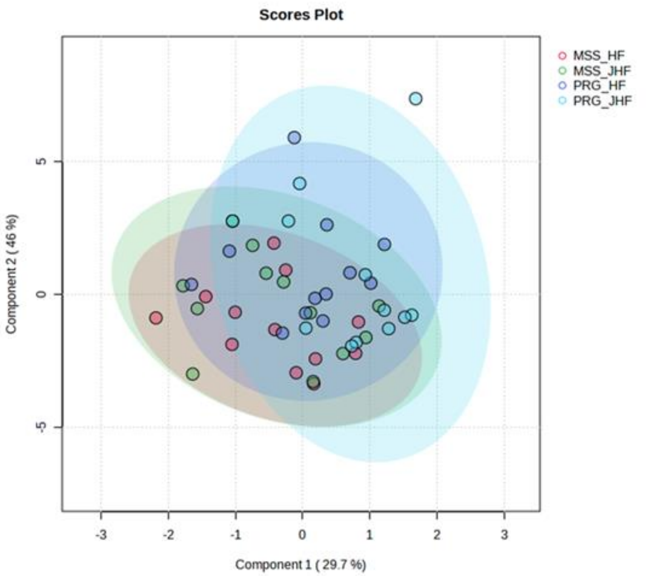
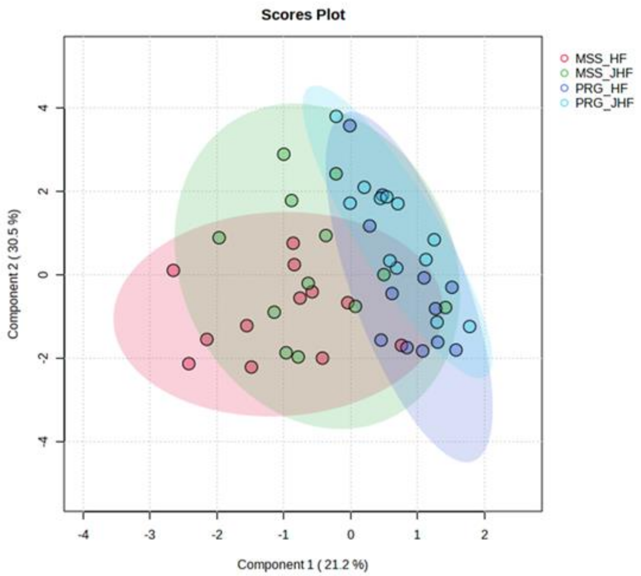
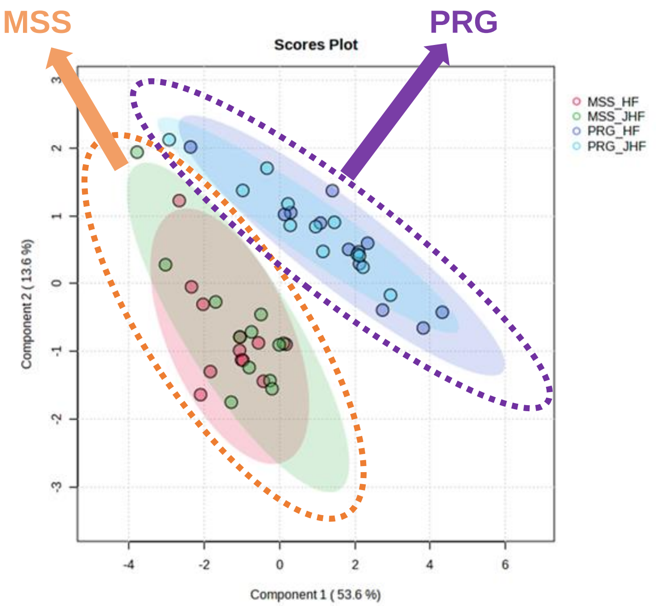
Fatty acid profile throughout the season



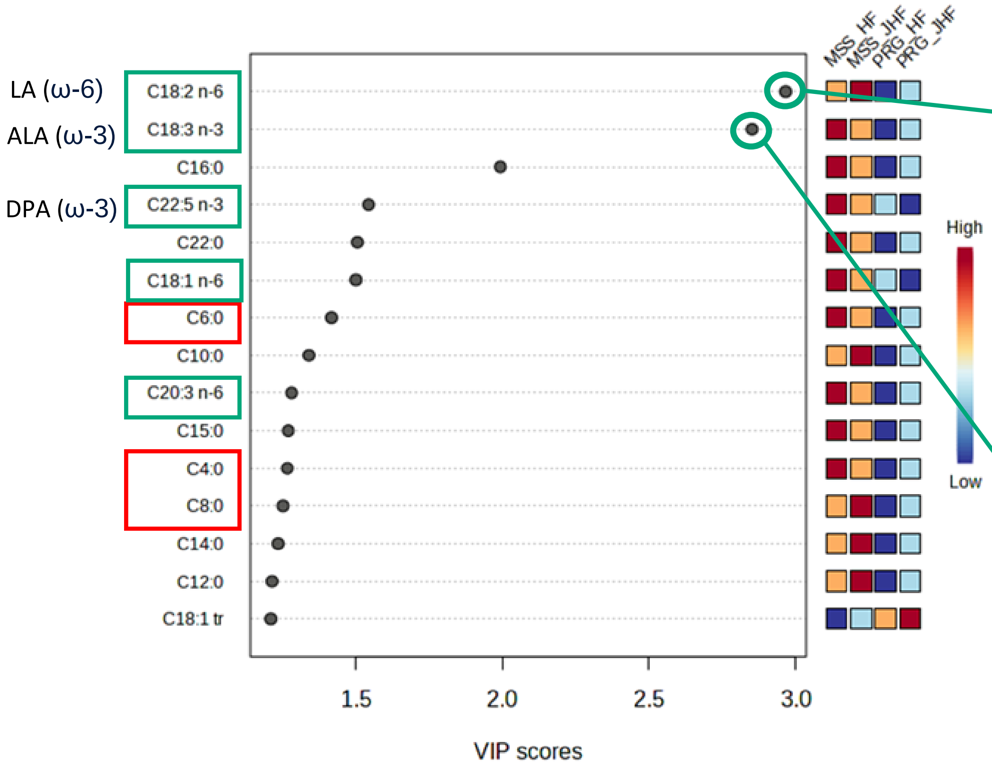


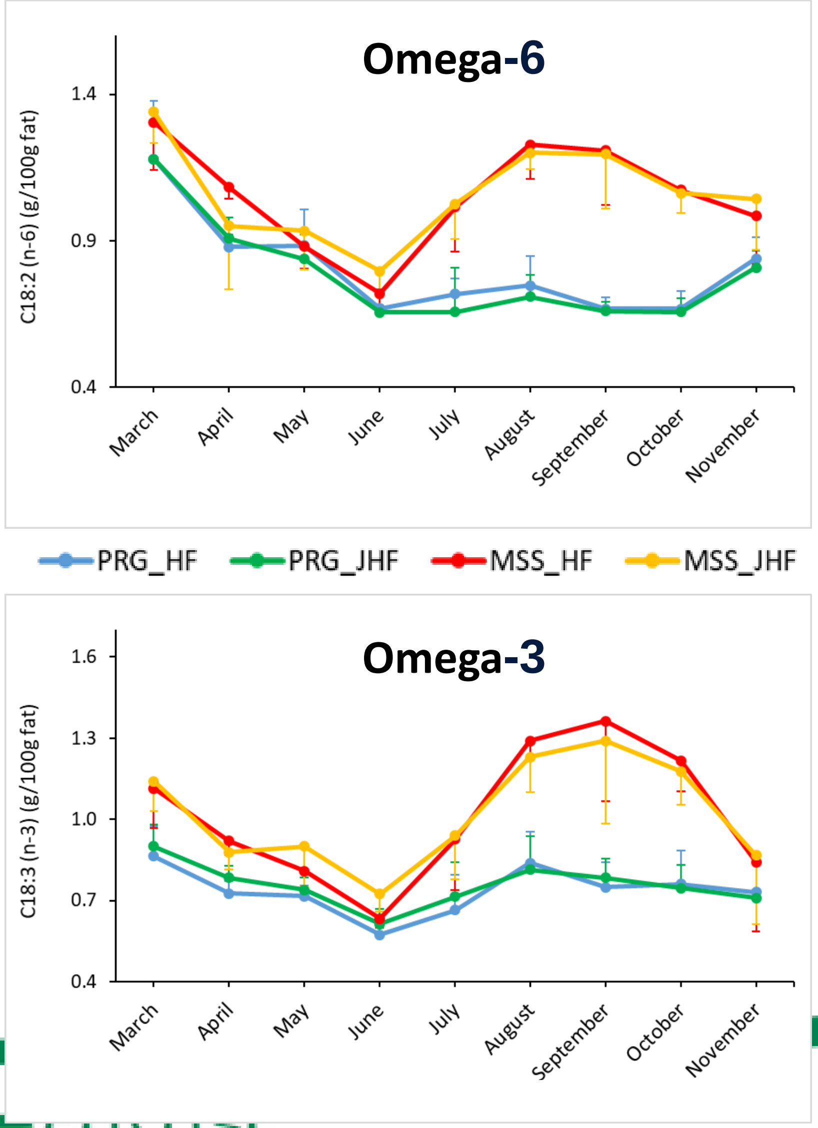


Mar-May | Jun-Aug | Sep-Nov

Clearer separation of FA profiles when progressing throughout lactation depending on <u>feed</u>.

Fatty acids: feed effect





- Major change in omega-3/omega-6 balance for MSS from June onwards
- The proportion of omega-6 and omega-3 was higher in MSS, especially in mid- and late-lactation
- Higher short chain fatty acids (rumen derived) from MSS.
- No breed effect

Late lactation

- MSS milk has higher **PUFA** content
- MSS contributes to higher **SCFA** content

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Take home points

Nutritional Quality

- Pasture feeding increased concentrations of Omega 3, CLA, VA
- TMR Feeding increased concentrations of Omega 6 fatty acids, palmitic acid
- Pasture feeding increased vitamin B1, B2 and B7 content.
- TMR feeding increased vitamin B3 and B3-amide content.

Sensory

- Feeding system significant effect on sensory profile of milk and products
- Different sensory perceptions in different cultures

Feed Biomarkers

Several compounds have been identified as being significantly higher in pasture derived products and show potential to be biomarkers, including; CLA, β-carotene (characteristic yellow colour), toluene, dimethyl sulfone and hippuric acid.

Breed and feed

- MSS increase fat, total solids in HF
- MSS increase both omega-3 and omega-6 FA
- Jersey cross: Increased solids and fat globule size, decreased yield and DPA (omega-3)
- FA profile changes from mid-lactation onwards

Product Characteristics

Diet induced alterations to milk has a significant effect on product textural, volatile and colour properties

The protein fraction in milk is genetically determined

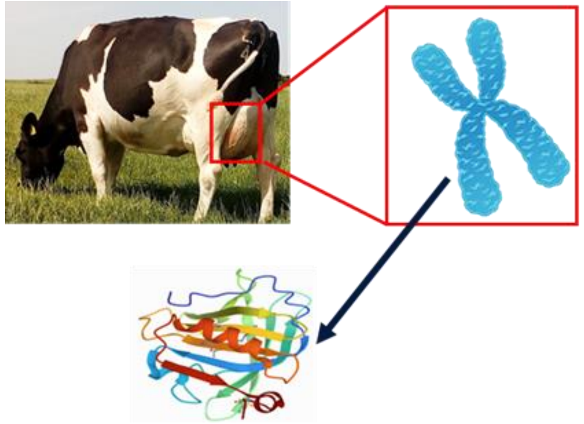
- Nitrogen content of milk is distributed among the caseins, whey proteins and non-protein nitrogen.

- Each of the 6 primary proteins (2 whey, 4 caseins) has an effect on the nutritional value of milk.

- Genetic factors have a major influence on the protein profiles of milk, which in turn influence milk yield, composition, and milk processability.

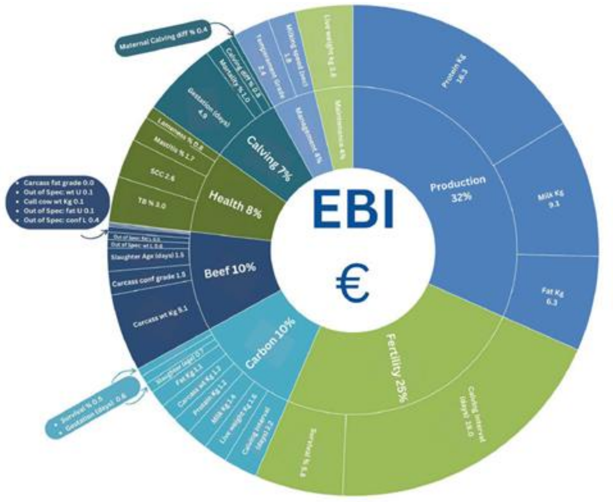
Our interests:

- ➢ Investigate natural variations in the nitrogen composition and protein profile of individual cows.
- ➢ Determine potential correlations between specific protein variants and milk nitrogen fractions.
- ➢ Determine the potential for tailoring milk for specific protein dominant product classes.



Variation in protein composition among individual cows

- Nitrogen content of milk is distributed among the caseins, whey proteins and non-protein nitrogen.
- Each fraction has an effect on the nutritional value of milk.
- Genetic factors have a major influence on the protein profiles of milk, which in turn are reported to influence milk yield, composition, and milk processability



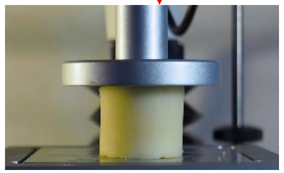
1. **Important**
2. **Genetic differences**
3. **Measurable**

Value focused on:

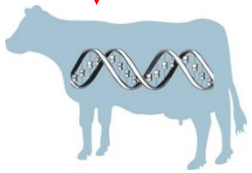
- Milk solids
- Reproductive performance
- Longevity
- Health status
- Calving

- Not just fat and protein!
- More than 100,000 components

Profile of constituent proteins



Significant for product functionality



Genetically determined

Variation in protein composition among individual cows

Selection of high casein:whey protein ratio cows				
Crude protein	True protein	Whey Protein	Casein	Casein no.
4.50	4.30	0.18	4.08	**88.00**
4.73	4.50	0.16	4.22	**87.75**
3.72	3.60	0.35	3.26	**86.80**
3.61	3.46	0.32	3.31	**86.60**
4.16	3.96	0.21	3.67	**85.85**

Selection of low casein:whey protein ratio cows				
Crude protein	True protein	Whey Protein	Casein	Casein no.
3.70	3.51	0.48	2.77	**73.85**
4.03	3.83	0.76	3.07	**75.90**
4.13	3.93	0.78	3.15	**75.30**
3.63	3.51	0.44	2.82	**77.10**
3.35	3.17	0.56	2.67	**78.10**

- Somatic cell count

→ high whey deposition in pre-mastitic cows

- Yield

→ high whey, reduced yield with infection

- Stage of lactation

→ all cows in mid-lactation

- Diet

→ all cows on perennial ryegrass-based diet

- **Strongly linked to genetic variants of individual proteins**

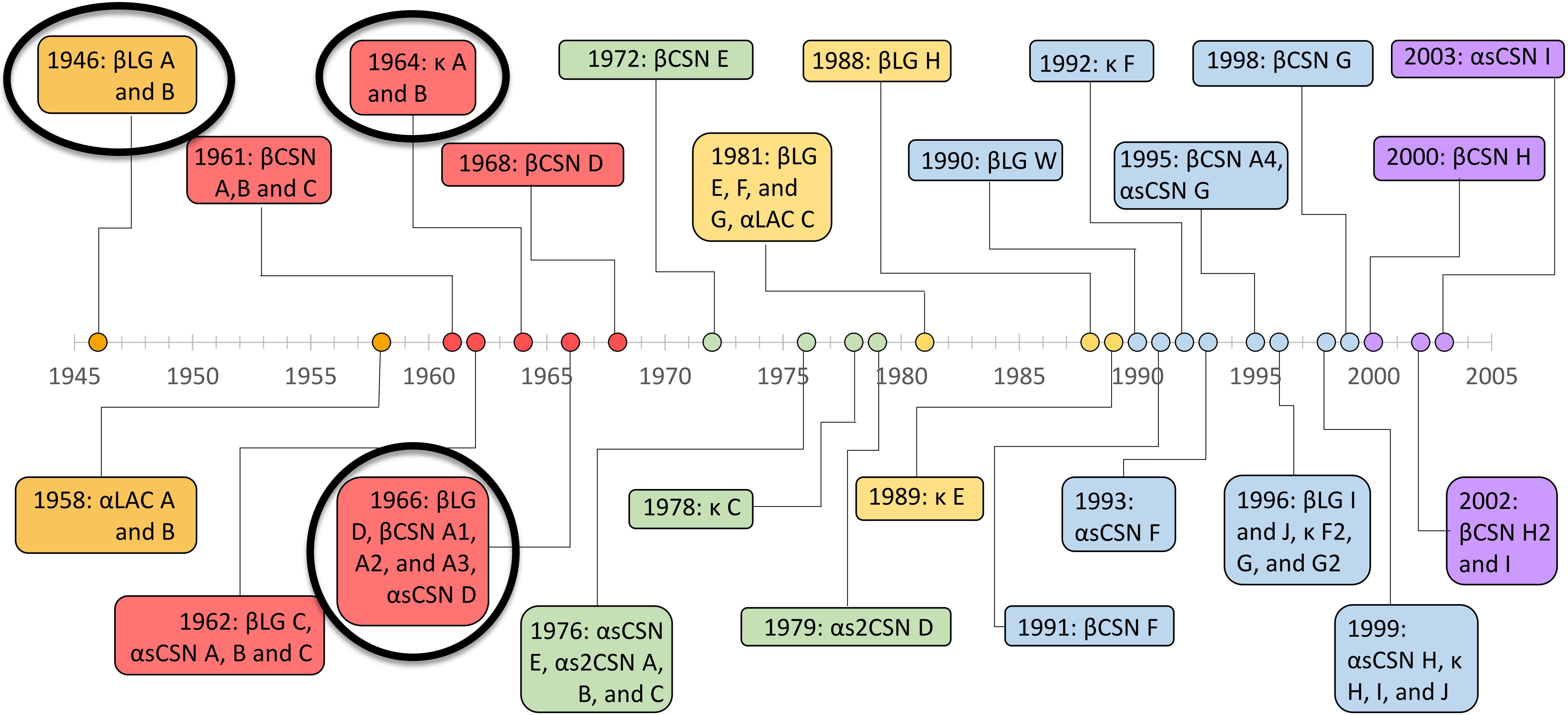
Impact of genetic variation on milk proteins

- Genetic variation is caused by a mutation within DNA resulting in the substitution of one or more amino acids within the primary structure of a protein

- There are many genetic variants of casein and whey proteins, with over 50 identified to date

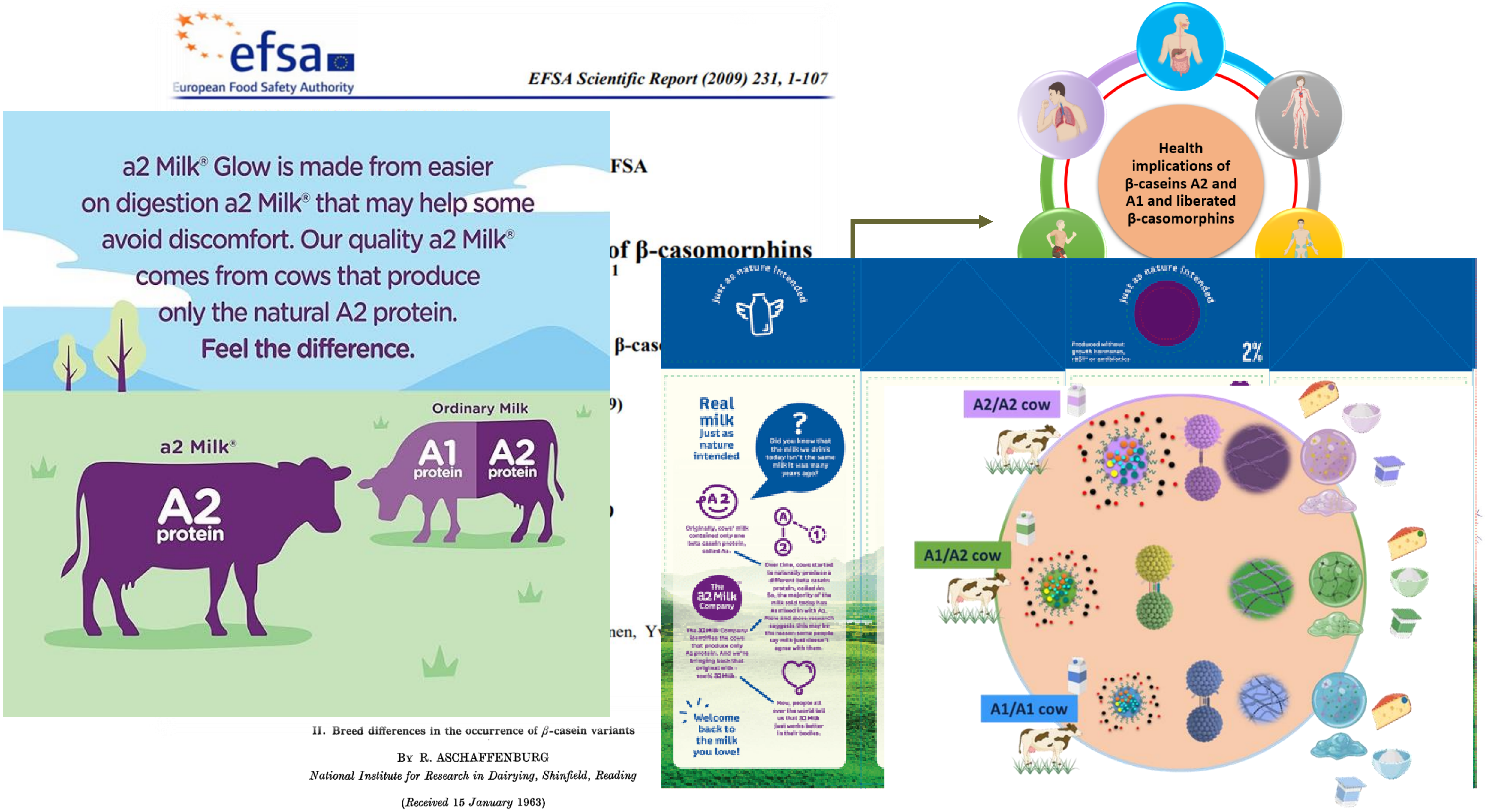
- The first of which was discovered as far back as 1946

Timeline of discovery of milk protein variants

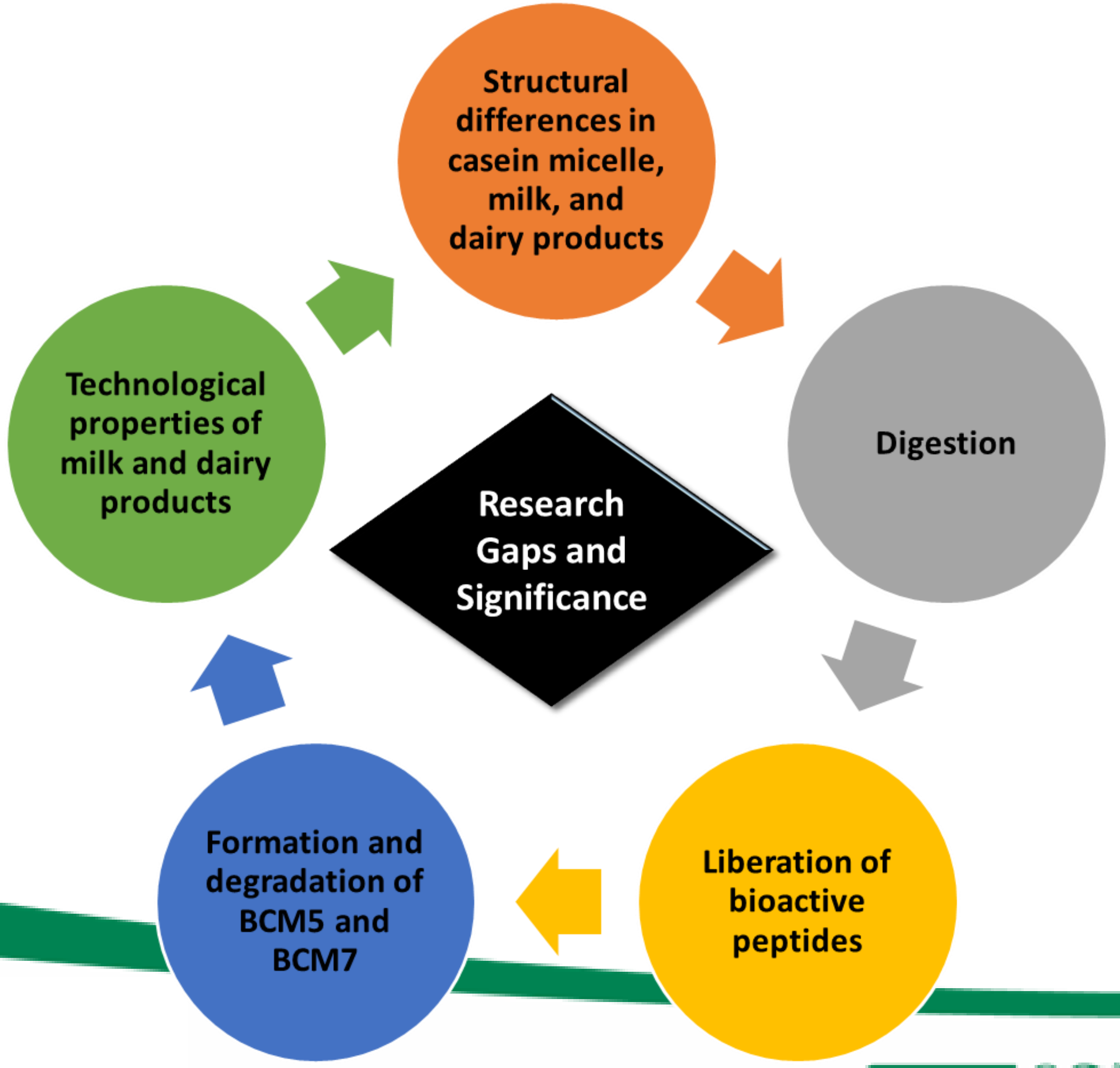


● < 1959, ● 1960-1969, ● 1970-1979, ● 1980-1989, ● 1990-1999, ● >2000

A SINGLE NUCLEOTIDE POLYMORPHISM IN THE STRUCTURE OF β-CASEIN



Bovine β-casein phenotype - structure and functionality of milk and dairy products



Current Research in Food Science 5 (2022) 1701–1712

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

What is the impact of amino acid mutations in the primary structure of caseins on the composition and functionality of milk and dairy products?

Davor Daniloski [a,b], Noel A. McCarthy [b], Thom Huppertz [a,c,d], Todor Vasiljevic [a,*]

[a] Advanced Food Systems Research Unit, Institute for Sustainable Industries and Liveable Cities and College of Health and Biomedicine, Victoria University, Melbourne, VIC, 8001, Australia
[b] Food Chemistry and Technology Department, Teagasc Food Research Centre, Moorepark, Fermoy, P61 C996, Cork, Ireland
[c] FrieslandCampina, Amersfoort, the Netherlands
[d] Wageningen University & Research, Wageningen, the Netherlands

- Raw genotype information from national herd in the form of a data dump from the Irish Cattle Breeding Federation (ICBF) which is held on the Teagasc High-Powered Computing Cluster –updated 'as needed'.

- Approx 78,000 Holstein-Friesian cows (25 genetic variants)
  - A1A1 = 7%
  - A1A2 = 27%
  - A2A2 = 26%

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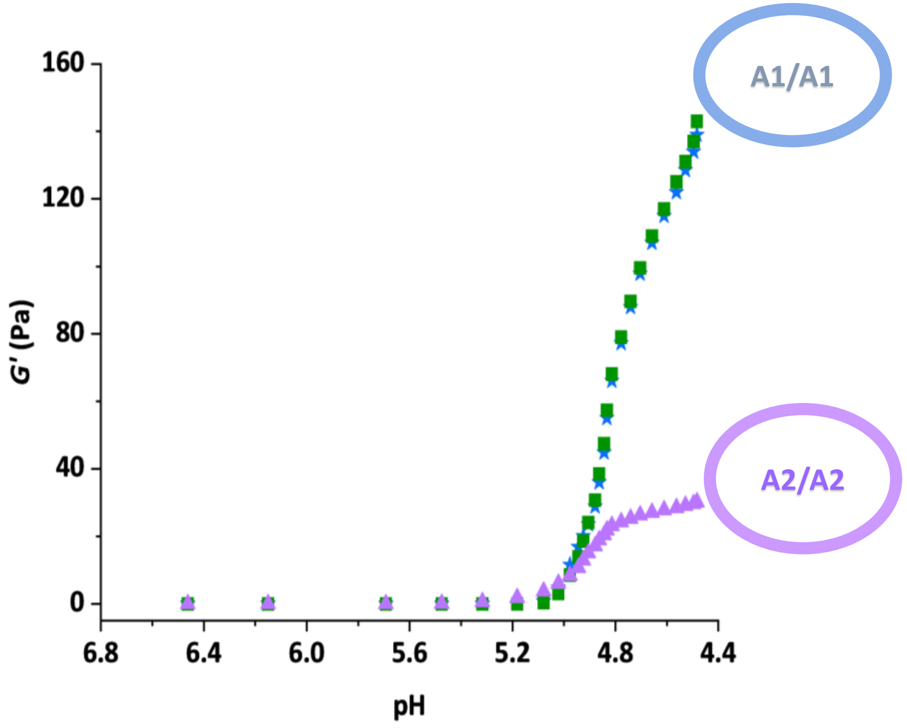
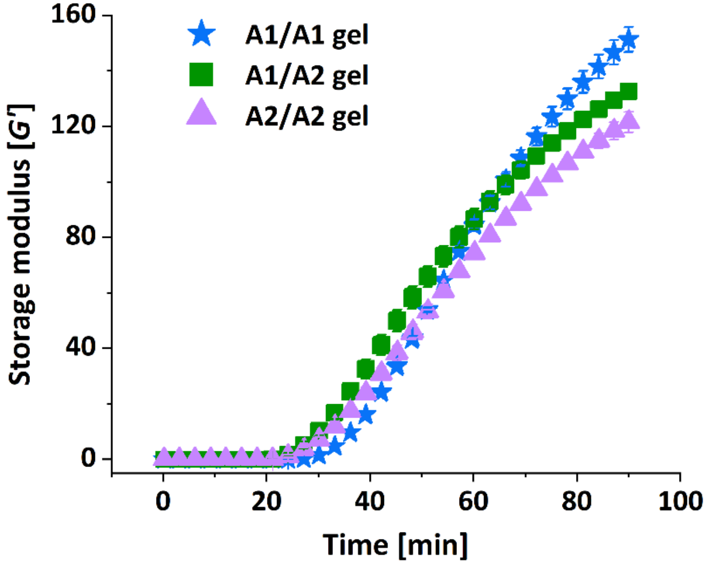
INCREASED STORAGE MODULUS IN ACID AND RENNET GELS WITH β-CASEIN A1

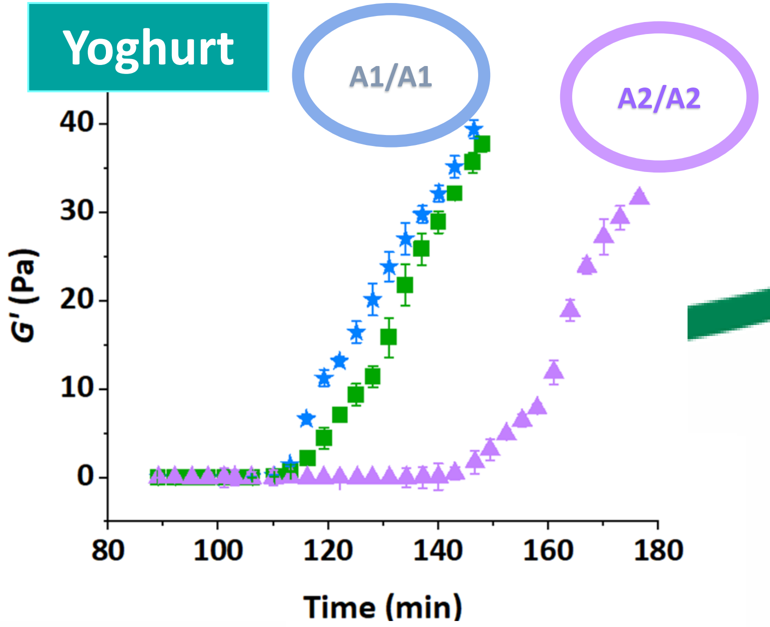
Acid gelation



Rennet coagulation



Yoghurt



Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Rheological and structural properties of acid-induced milk gels as a function of β-casein phenotype

Davor Daniloski [a,b], Noel A. McCarthy [b], Inge Gazi [c,d], Todor Vasiljevic [a,*]

[a] Victoria University, Advanced Food Systems Research Unit, Institute for Sustainable Industries and Liveable Cities and College of Health and Biomedicine, Melbourne, Victoria, 8001, Australia
[b] Teagasc Food Research Centre, Food Chemistry and Technology Department, Moorepark, Fermoy, P61 C996, Cork, Ireland
[c] Utrecht University, Biomolecular Mass Spectrometry and Proteomics, Bijvoet Centre for Biomolecular Research, Utrecht Institute for Pharmaceutical Sciences, Utrecht, the Netherlands
[d] Netherlands Proteomics Centre, Utrecht, the Netherlands

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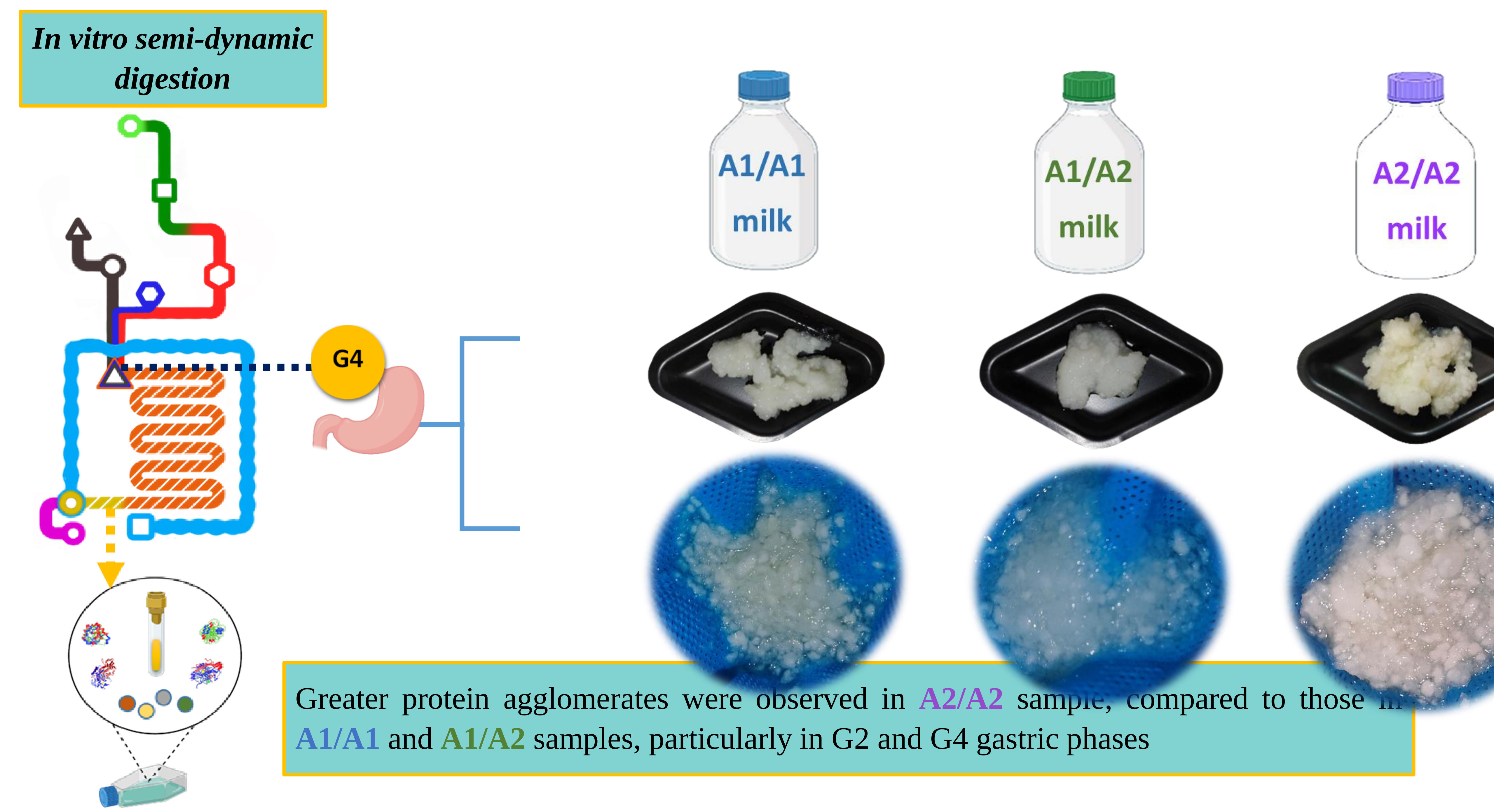

In vitro semi-dynamic digestion
G4
A1/A1 milk
A1/A2 milk
A2/A2 milk
Greater protein agglomerates were observed in A2/A2 sample, compared to those
A1/A1 and A1/A2 samples, particularly in G2 and G4 gastric phases

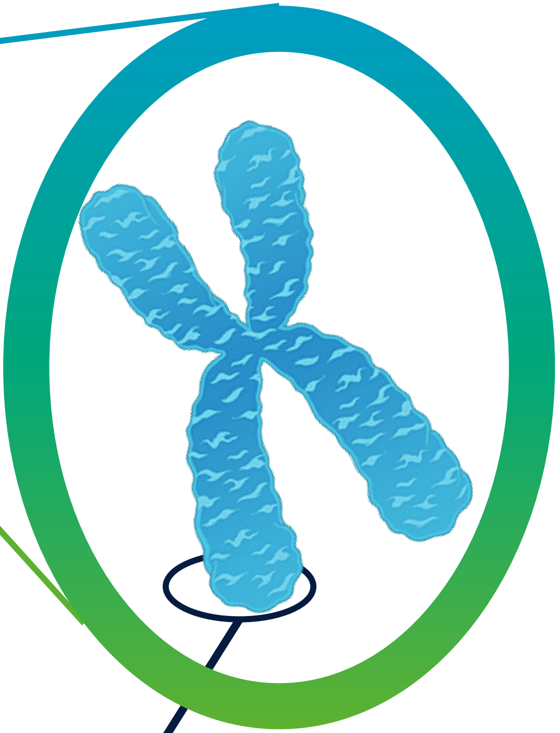
Why β-lactoglobulin?

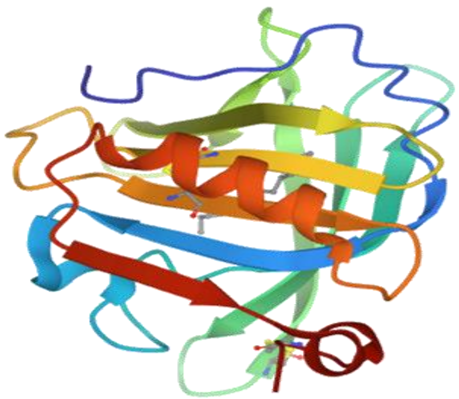
- Whey proteins can be easily isolated and purified by a combination of micro and ultra-filtration
- β-lactoglobulin is the most abundant whey protei... d in milk and is the most abundant milk protei... ...ant formula

β-lactoglo...		...ition
Aspar... Aci...		
Valine	NH_2	118

These subtle changes to the amino acid sequence have been shown to influence the pI, dimer stability, and secondary structure of β-lactoglobulin

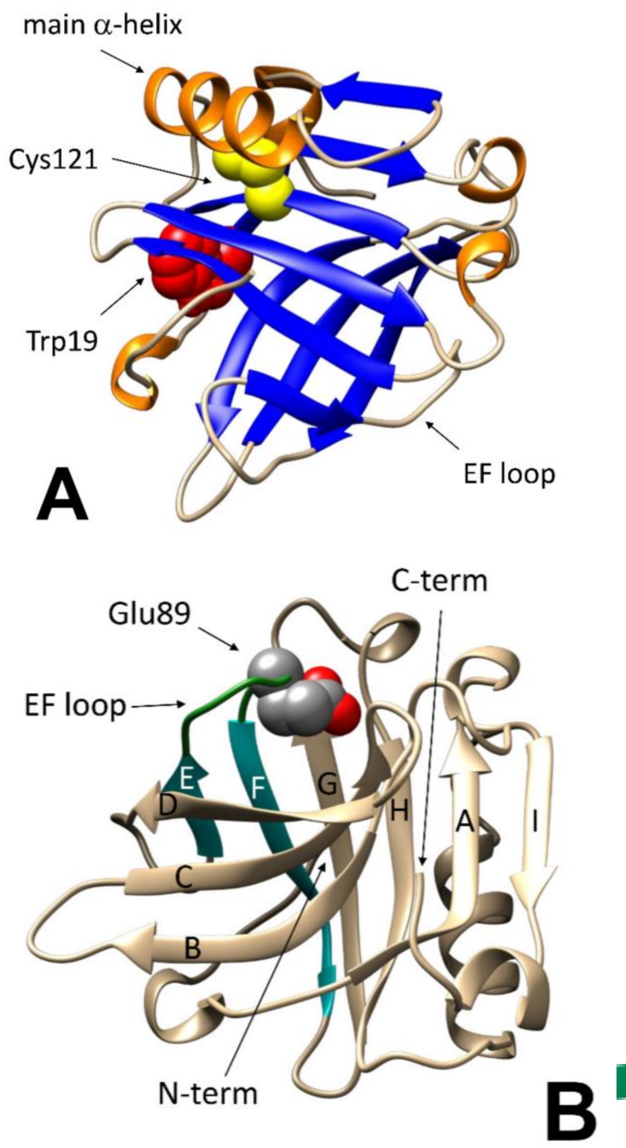
Who Cares ?





Functional indicators

- The A variant has a lower denaturation temperature than the B variant. This could be as a result of a greater proportion of β-sheets found in the B variant
- The A variant produced weaker gels between pH 6.6 and 6.8. This could be a result of the A variants greater electrostatic charge, or possibly due to the difference in mineral content between the A and B variants
- At pH 6.4 the A variant has better heat stability than the B variant
- AB variant behaves in a similar manner to the B variant during heat stability testing, but displays similar properties to the A variant during heat-induced gelation



β-lactoglobulin variants

Nutritional Properties

Whey Protein Consumption

Decreased Risk of Type 2 Diabete

Anti-
Inflammatory
Effect

Colon

↓ Colonocyte D

↑ Gut Microbial D
Composit

Do β-lactoglobulin A and B induce a different biological response?

ability

Intestinal Barrier Integrity

Gut Microbiota Modulation

Body Weight Modulation


Thank you for your attention.