Influence of pasture-feeding on milk and meat product quality

Stanton, C., Mills, S., Ryan, A., Di Gioia, D. and Ross, R.P.

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Talk is in two parts

1. The differences between pasture-fed and indoor fed beef and dairy products

2. The implications of these differences to human Health.
Consumers’ attitudes to food

- Increase in risk factors and chronic disease
- Greater consumer awareness
- Drivers of the food and pharma industries
- Desire for personalized products
- Aging populations
- Greater emphasis on preventative measures
A Scientific basis for

We set out to

1. Develop metabolite biomarkers associated with pasture-feeding
2. Characterize the metabolome of pasture derived milk
3. Describe the metabolome of pasture-based dairy products
4. Characterize specific metabolites in terms of nutrition
5. Determine the effect of specific metabolites on human health
Pasture-based dairy and beef products

• In United States, the Middle East, Asia and parts of Europe, cows and cattle are fed indoors on grass/maize silages supplemented with concentrates - total mixed ration (TMR).

• In Ireland and New Zealand, they are typically fed outdoors on fresh pasture.

• Pasture-based dairy and beef products are positively associated with good animal welfare and more natural and healthier products (Verkerk, 2003).

• Specific feeding regimes and herd management practices such as pasture feeding are linked to superior organoleptic and nutritional quality attributes of milk, dairy and meat products (Haug et al., 2007; Mann et al., 2003).
Beef Choice

• In a survey of 676 beef consumers in Germany, participants exhibited a high preference for beef labelled as 'organic' and 'pasture-based' (Risius & Hamm (2017) Meat Sci.).

• Additionally, when informed about the conditions of 'extensive suckler cow husbandry', consumers were most likely to be motivated by the label 'extensive suckler cow husbandry'.

• Informing consumers about suckler cow husbandry results in a change of their preferences from 'organic' to 'extensive suckler cow husbandry'. This holds great potential for extensively produced beef.
Intra-muscular beef fatty acid composition is improved by inclusion of grass in the diet. (French et al. 1999)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Treatment*</th>
<th>SC</th>
<th>CO</th>
<th>CG</th>
<th>GC</th>
<th>GO</th>
<th>SE</th>
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<td>.12</td>
<td>.27</td>
<td>.27</td>
<td>.23</td>
<td>.023</td>
<td>*</td>
</tr>
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<td>SFA*</td>
<td></td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>.029</td>
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<td>MUFA*</td>
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<td>47.72</td>
<td>48.07</td>
<td>45.71</td>
<td>44.86</td>
<td>42.82</td>
<td>.415</td>
<td>***</td>
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<tr>
<td>PUFA*</td>
<td></td>
<td>41.83</td>
<td>41.48</td>
<td>40.90</td>
<td>42.31</td>
<td>43.07</td>
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<td>n-6 fatty acids</td>
<td></td>
<td>7.96</td>
<td>8.28</td>
<td>8.46</td>
<td>8.60</td>
<td>9.23</td>
<td>.15</td>
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</tr>
<tr>
<td>n-6 : n-3 ratio</td>
<td></td>
<td>.91</td>
<td>.84</td>
<td>1.13</td>
<td>1.25</td>
<td>1.36</td>
<td>.042</td>
<td>***</td>
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<tr>
<td>PUFA : SFA</td>
<td></td>
<td>3.61</td>
<td>4.15</td>
<td>2.86</td>
<td>2.47</td>
<td>2.33</td>
<td>.197</td>
<td>**</td>
</tr>
</tbody>
</table>

*The treatments were grass silage for ad-libitum intake plus 4 kg concentrate (SC), 8 kg concentrate plus 1 kg hay (CO), 6 kg grass DM plus 5 kg concentrate (CG), 12 kg grass DM plus 2.5 kg concentrate (GC) and 22 kg grass DM (GO).

Means within rows with common superscripts are not significantly (P<0.05) different.

SFA = total saturated fatty acids, MUFA = total monounsaturated fatty acids, PUFA = total polyunsaturated fatty acids.
Pasture versus TMR feeding system on rumen and milk metabolome

**Experimental Design**

**Study Design:**
54 Friesian Cows

- **Herd 1**
  - Perennial-Rye Grass
  - ~18 kg DM/day
  - n=18

- **Herd 2**
  - Perennial-Rye/20% White Clover Pasture
  - ~18 kg DM/day
  - n=18

- **Herd 3**
  - Total Mixed Ration
  - 7.15 kg of grass silage, 7.15 kg of maize silage and 8.3 kg concentrates/day
  - n=18

The Irish Agriculture and Food Development Authority
Impact of pasture versus TMR feeding systems on the bovine rumen and milk composition

- Nine cannulated cows were rotated through each of the three feeding systems in early, mid and late lactation.
- Rumen samples were collected and separated into rumen fluid and rumen solid portions.
- DNA was extracted from samples and subjected to 16s Miseq sequencing.
- Milk samples from 10 cows on each feeding system were collected in triplicate during mid-lactation.
- Rumen fluid samples and milks were analysed using 1H-NMR at The Metabolomics Innovation Center, University of Alberta, Edmonton.
NMR metabolomics distinguish between rumen metabolome from different feeding systems

- Phenylpropionate and phenylacetate compounds have been identified previously as important aromatic acids in ruminal fluid [12].
- They are generated through the hydrogenation of plant phenolic compounds such as p-coumaric, ferulic, and caffeic acid by the ruminal microorganisms, with subsequent dehydroxylation [27].
- Nicotinate was attributed to the ability of several bacterial species identified in rumen contents to synthesize nicotinate.
- Dimethyl sulphone in the rumen is produced by the catabolism of sulfur amino acids, in particular methionine, which is hydrolysed to dimethyl sulphide [25] and subsequently oxidised to dimethyl sulfone.
- Methionine has been previously reported in highest concentrations in pastures as opposed to silage and hay diets [26].
- Increased levels of dimethyl sulfone from pastures could be related to increased levels of digestible protein and is highest in CLV as a result of its increased sward digestibility.
Diet has a significant effect on the rumen metabolome.

**Dimethyl sulfone**

<table>
<thead>
<tr>
<th></th>
<th>TMR</th>
<th>GRS</th>
<th>CLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>µM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
</tbody>
</table>

**Choline**

<table>
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<th>TMR</th>
<th>GRS</th>
<th>CLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>µM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>a</td>
<td>b</td>
<td>a</td>
</tr>
</tbody>
</table>

**Isopropanol**

<table>
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<th></th>
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<th>GRS</th>
<th>CLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>µM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>a</td>
<td>a</td>
<td>b</td>
</tr>
</tbody>
</table>

---

**Dimethyl sulfone**

- TMR
- GRS
- CLV

**Choline**

- TMR
- GRS
- CLV

**Isopropanol**

- TMR
- GRS
- CLV

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**Text**

The figure illustrates the significant effect of diet on the rumen metabolome, showcasing the levels of dimethyl sulfone, choline, and isopropanol across different diets (TMR, GRS, CLV). The data indicates a clear variation in metabolite concentrations, highlighting the importance of diet on rumen metabolism.
Diet has a significant effect on the milk metabolome
MILK

Effect of pasture versus indoor feeding systems on raw milk composition and quality over an entire lactation

Tom F. O’Callaghan,*†† Deirdre Hennessy,§ Stephen McAuliffe,§# Kieran N. Kilcawley,‡ Michael O’Donovan,§ Pat Dillon,§ R. Paul Ross,*† and Catherine Stanton*‡

BUTTER

Quality characteristics, chemical composition, and sensory properties of butter from cows on pasture versus indoor feeding systems

Tom F. O’Callaghan,*†† Hope Faulkner,† Stephen McAuliffe,§# Maurice G. O’Sullivan,‖ Deirdre Hennessy,¶ Pat Dillon,¶ Kieran N. Kilcawley,‡ Catherine Stanton,*‡ and R. Paul Ross*¶¡

CHEESE

Effect of pasture versus indoor feeding systems on quality characteristics, nutritional composition, and sensory and volatile properties of full-fat Cheddar cheese

Cows on TMR has a Greater Milk Yield

<table>
<thead>
<tr>
<th>Item</th>
<th>TMR</th>
<th>Grass</th>
<th>Clover</th>
<th>SE</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Milk yield (L/d)</td>
<td>27.71</td>
<td>20.98</td>
<td>24.59</td>
<td>0.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Milk solids (kg/d)</td>
<td>2.24</td>
<td>1.78</td>
<td>1.99</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein (kg/d)</td>
<td>0.94</td>
<td>0.76</td>
<td>0.87</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat (kg/d)</td>
<td>1.31</td>
<td>1.02</td>
<td>1.12</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lactose (kg/d)</td>
<td>1.32</td>
<td>1.01</td>
<td>1.18</td>
<td>0.01</td>
<td>0.716</td>
</tr>
<tr>
<td>Live weight (kg)</td>
<td>591.51</td>
<td>532.11</td>
<td>550.45</td>
<td>13.15</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Milk composition throughout lactation
Chapter 2

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

Palmitic Acid

\[ \text{g/100g of fat} \]

\[ \text{TMR} \quad \text{GRS} \quad \text{CLV} \]

\[ P < 0.05 \]

Linoleic Acid

\[ \text{g/100g of fat} \]

\[ \text{TMR} \quad \text{GRS} \quad \text{CLV} \]

\[ P < 0.05 \]

\[ P < 0.05 \]

\[ \alpha\text{-Linolenic Acid} \]

\[ \text{g/100g of fat} \]

\[ \text{TMR} \quad \text{GRS} \quad \text{CLV} \]

\[ P < 0.05 \]

\[ P < 0.05 \]

\[ CLA (c9t11) \]

\[ \text{g/100g of fat} \]

\[ \text{TMR} \quad \text{GRS} \quad \text{CLV} \]

\[ P < 0.05 \]

\[ P < 0.05 \]
Pasture based feeding has a beneficial effect on milk fatty acid profile

- Essential fatty acids.
- Precursors to eicosanoids roles in inflammation
  - n3 derived eicosanoids possess anti-inflammatory
  - n6 derived eicosanoids possess pro-inflammatory properties
    (Patterson et al., 2012)
- Western diet has resulted in ↑ n6 fatty acid (Molendi-Coste et al., 2010)
- Concomitant increases in chronic inflammatory diseases (Patterson et al., 2012)
  - non-alcoholic fatty liver disease, cardiovascular disease,
  - obesity,
  - inflammatory bowel disease, rheumatoid arthritis and Alzheimer’s disease
- Foods rich in n3 FA could be beneficial in reducing risk of such diseases
  (Benbrook et al., 2013)
Is milk from grass-fed cows more heart-healthy?

Conclusion: 9c,11t-CLA, which is present in meaningful amounts in the milk of pasture-grazed cows, might offset the adverse effect of the saturated fat content of dairy products. Am J Clin Nutr 2010;92:34–40. Liesbeth A Smit, Ana Baylin, and Hannia Campos

(Reuters Health) - If milk does the heart good, it might do the heart better if it comes from dairy cows grazed on grass instead of on feedlots, according to a new study.
Chapter 2

FA acid profiling of milk for verification of pasture feeding
Ireland's yellow butter:

Increased Beta-Carotene from fresh pasture gives Irish butter its characteristic yellow colour.
TMR diet produces harder butter

![Graph showing butter hardness at different temperatures for TMR, Grass, and Clover diets, with significant differences indicated by asterisks and hash marks.](image)
Cheddar Cheese Manufacture

- Milk standardized to P/F ratio of 0.95
- Cheddar cheese manufactured in 500L Vat
- Ripened for 9 months at 8°C
- Analysed for composition, texture and sensory.
# Cheese Composition

<table>
<thead>
<tr>
<th></th>
<th>TMR</th>
<th>GRS</th>
<th>CLV</th>
<th>Treatment P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein (% wt/wt)</strong></td>
<td>$26.70 \pm 0.10$</td>
<td>$26.65 \pm 0.39$</td>
<td>$26.64 \pm 0.37$</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Fat (% wt/wt)</strong></td>
<td>$30.95 \pm 0.20$</td>
<td>$30.54 \pm 0.25$</td>
<td>$31.28 \pm 0.16$</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Moisture (% wt/wt)</strong></td>
<td>$35.81 \pm 0.39$</td>
<td>$36.21 \pm 0.60$</td>
<td>$35.93 \pm 0.70$</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>F/DM (% wt/wt)</strong></td>
<td>$48.21 \pm 0.40$</td>
<td>$47.88 \pm 0.07$</td>
<td>$48.84 \pm 0.78$</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>MNFS (% wt/wt)</strong></td>
<td>$51.85 \pm 0.57$</td>
<td>$52.13 \pm 0.67$</td>
<td>$52.29 \pm 1.13$</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>Salt (% wt/wt)</strong></td>
<td>$1.87 \pm 0.07$</td>
<td>$1.83 \pm 0.06$</td>
<td>$1.80 \pm 0.07$</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>S/M (% wt/wt)</strong></td>
<td>$5.23 \pm 0.25$</td>
<td>$5.05 \pm 0.24$</td>
<td>$5.00 \pm 0.18$</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Ash (% wt/wt)</strong></td>
<td>$3.95 \pm 0.18$</td>
<td>$4.01 \pm 0.08$</td>
<td>$3.82 \pm 0.05$</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>Calcium (% wt/wt)</strong></td>
<td>$0.84 \pm 0.01$</td>
<td>$0.82 \pm 0.02$</td>
<td>$0.81 \pm 0.02$</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>β-Carotene (mg/Kg of Cheese)</strong></td>
<td>$0.57 \pm 0.05$</td>
<td>$1.40 \pm 0.06$</td>
<td>$1.21 \pm 0.03$</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

1Fat in dry matter, 2Moisture in non-fat substances, 3salt in moisture
Cheddar Cheese Colour Over Ripening

- b* values highly correlated with β-carotene content ($P < 0.001$, Pearson $r = 0.948$),
- $L^*$ values, negatively correlated with the β-carotene content ($P = 0.004; r = -0.841$)

Cheese colour highly dependent on carotenoid content
Cheese Fatty Acid Profiling

Statistical analysis by One Way ANOVA with Tukey Post Hoc Testing
Cheese Made with TMR Milk is Harder

- Palmitic acid positively correlated with hardness and chewiness attributes
- The higher CLA content of the pasture-derived cheese negatively correlated with hardness
Sensory analysis of Cheddar Cheese

Statistical analysis by Partial least Square Regression Analysis
Ripening period resulted in significant alterations to cheese volatile profiles, with increases in acid-, alcohol-, aldehyde-, ester-, and terpene-based volatile compounds.
Talk is in two parts

1. The differences between pasture-fed and indoor fed dairy products

2. The implications of these differences to human Health.
Top Health Concerns (US)

- Heart/Circulatory Health Issues: 54%
- Weight: 34%
- Cancer: 28%
- Diabetes: 16%
- Exercise: 8%
- Nutrition/Diet: 7%
- Lung/Respiratory: 7%
- Arthritis: 7%
- high quality protein
- Best Source of Calcium
- Good Source of minerals (Zn, Mg, K)
- Good Source of Vitamins (A, D, B)
- Additive and contaminant free
Health attributes of milk components

- Whey protein
- Immunoglobulins
- Calcium & Peptides
- Peptides
- Probiotics/Prebiotics/carbohydrates

Components:
- α-Lactalbumin
- β-Lactoglobulin
- Sphingolipids
- CLA
- Lactoferrin
- Lactoperoxidase
+ Caseinophosphopeptides

The Irish Agriculture and Food Development Authority
Intra-muscular beef fatty acid composition is improved by inclusion of grass in the diet. 

(French et al. 1999)
Bovine Lipids/Fat

Conjugated Linoleic Acid (CLA)

- Anticancer
- Improves heart health
- Improves immune system
- Improves body composition
- Fat regulating
- Bone formation-promoting properties

Health benefits associated with CLA:
- Anticancer, antiatherogenic, fat regulating, immune modulating & bone formation-promoting properties.
CLAs: Biosynthesis in rumen and mammary gland

- **C<sub>18:2</sub> cis-9, cis-12** (linoleic acid)
- **C<sub>18:3</sub> cis-9, cis-12, cis-15** (linolenic acid)
- **C<sub>18:3</sub> cis-9, trans-11, cis-15** (conj.octadecatrienoic)
- **C<sub>18:2</sub> trans-11, cis-15** (octadecadienoic)
- **C<sub>18:1</sub> trans-11** (trans-vaccenic acid)
- **C<sub>18:0</sub> stearic acid**

**Enzymes:**
- linoleate (cis-12, trans-11) isomerase
- Delta-9-desaturase
- 2H
Effect of Grass Allowance on Milk Fat CLA (Stanton et al., 1998)

Herds on pasture produce milk fat which is higher in CLA.
Enriching CLA in Milk

CLA–enriched Cheddar cheese manufactured for human consumption, from milk produced by supplementation with sunflower / linseed mixture

<table>
<thead>
<tr>
<th>Date</th>
<th>Low CLA cheese</th>
<th>High CLA cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb 10-20</td>
<td>~0.5 g/100g FAME</td>
<td>~2.0 g/100g FAME*</td>
</tr>
<tr>
<td>Mar 1-11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar 21-31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr 10-20</td>
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</tbody>
</table>

(*milk analysed in this case)
n-3 Fatty acids and CLA in plasma following cheese intake (50g/day) for 2 months

(n=36/group)
Conclusions

We found out that

1. We can distinguish pasture derived produce from indoor animals
2. Characterize the metabolome of pasture derived milk
3. Describe the metabolome of pasture-based dairy products
4. Characterize specific metabolites in terms of nutrition
5. Determine the effect of specific metabolites on human health
Scientific basis for Origin Green

- Milk, butter and cheese contain higher levels of CLA and less saturated fat
- Metabolome of the rumen differs from pasture versus indoor fed cows
- Project to provide scientific basis for Origin Green
- New SFI Centre
- VISTAMILK funded September 2018

O’Callaghan et al. J. Dairy Sc. 2016/17 (x 3)
Acknowledgements

Dr Pat Dillon
Dr Kieran Kilcawley
Mairead Coakley
Dr Diarmuid Sheehan
Dr Deirdre Hennessy
Mr Stephen McAuliffe
Mr David Mannion
Miss Hope Faulkner

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Funding From:
Teagasc Overseas Training Award