Nutritional impact on lameness in dairy cows

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Lameness: Longstanding dairy cattle disease (Greenough, 1997): Contemporary husbandry (Particularly weeks 0-20 post partum)

Major disease incidence per 100 cows eg:

FERTILITY > MASTITIS >

*24.0 - LOCOMOTORY LAMENESS (... 20-60%) ( + lying, getting up etc...)

Cause?
In the foot: “claw horn disorder” and/or non- infectious inflammatory “laminitis”?

Symptoms, typically painful, include sole bruising, haemorrhage, ulceration

Note also: Infectious dermatitis: (Treponemal spirochaetes) (Cheli and Mortellaro, 1974)
How? - associated with suspension of bodyweight: issues for susceptibility

- Location:
  - claws of hind (mainly) and front feet
  - Weight pressing on soft tissues
  - Vertical impact and/or torsional stress

Susceptibility and risk factors; Resilience. Complex.

Extrinsic: (external); floor surface (hard concrete vs rubber mats...), cubicles, time standing...cow-cow interactions. (Claw trimming). DIET

Intrinsic: (cow biology), genetics, pregnancy, parturition, lactation stage... Interactions...
Foot disease: focus on diet and nutrition: Factors to consider

Foot anatomy
- Identify structure and composition
- at tissue, cell and molecular levels
- consider effect of failure of function of tissue components

- Nutrition?

- Role in growth and maintenance of foot tissues
- Meeting compositional requirements
- Avoiding digestive upset - eg ruminal acidosis with systemic impact

- Being aware of partitioning of nutrients
- competition between body tissues, eg growth (heifers) foetus, lactation...... ( lactation curve; feed intake capacity)..

What nutrients are needed for functional integrity?

Depends on structure/composition of the claw...
Functional foot anatomy: importance of epidermal horn; dermal connective tissue -

Body weight suspension -
Bone attaches to wall horn via
*Internal laminae and connective tissue (collagens, elastins)

Failure in suspension:
PIII bone presses on solear soft tissue .. Causing damage...

Note:
*Wall and sole horn (protects soft tissue)
Digital cushion (Shock absorption- force dispersion)
Lameness prevention? Healthy laminae: provide effective body weight suspension

- Wall laminar suspension involves:
  - Connective tissue; **collagens/elastin** (Synthesis/ break down)
- Horn: **keratins** (intermediate filaments and associated proteins)

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**Healthy laminae**

**Damaged laminae**

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What regulates maintenance of integrity of collagens and other CT molecules? Zn-metalloproteinases

Test for collagenase - breaks down collagen
Typical of **claw horn disorder** or laminitis

....... Lameness......
Sole region: Vascularised dermal tissue with papillae and epidermal sole horn

- Anatomy showing dermis with connective tissue (Collagens; elastin) and blood vessels (Blood leakage and damage)
- and epidermis horn (Tubular horn - from tip of papillae...)

Histological section

- Dermis; connective tissue
- Papilla
- Blood vessel
- Corium dermis
- Sole horn
- Epidermal basal keratinocytes nucleated (new cells)
- Keratinocytes; Enucleated (Cornified hard horn)
Dermis, basement membrane, hypodermis

DERMIS
• Cells are mesenchymal fibroblasts (Vimentin, not keratins in IFs)
• Low cell population
• High extracellular matrix, collagens etc in connective tissue ...
• Vascular: supplies nutrients and growth factors
• Enervated: vaso-effects, pain perception

BASEMENT MEMBRANE
• Macromolecules. connect dermis to epidermis
• Regulates keratin gene expression
• Transport of nutrients, mitogens and morphogens

BOTH
• degraded by (Zn) metalloproteinases
• Growth-factor stimulated synthesis of macromolecules

HYPODERMIS
• Collagenous tissue with fat cells forms the Digital cushion - shock absorbers
Composition of epidermis and horn

Made up of: Ectodermal (epithelial) keratinocytes (cells)

Ca 100 Keratin proteins:
- form polymers in intermediate filaments (IFs) (low cysteine: 4-7%)
- Combine with (IF Associated proteins) (higher cysteine- up to 30%)
- Form cytoskeleton in cells
- Bonds: intra-and inter-molecular disulphide
- (cysteine) -C- SH + SH- C - = - S - S -
- Proteins: Important in production of good quality horn...
**Function; Composition of claw horn and impression hardness; Met/cys concentrations (g/kg. Galbraith et al 2006)**

<table>
<thead>
<tr>
<th>*Claw Site</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>#SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness values</td>
<td>55.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>55.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.9&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>36.9&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>32.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>44.8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>47.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>42.9&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>8.20</td>
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</table>

**Amino Acid**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>S.D.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine</td>
<td>6.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.87</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Cysteine</td>
<td>65.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.5</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

**Wall; hard, higher cysteine: Heel softer flexible, lower cysteine**
Amino acid composition of horn and feed sources (g/16gN): Supply, partition.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Wall horn</th>
<th>Sole horn</th>
<th>Muscle</th>
<th>Rumen microbial protein</th>
<th>Extracted soyabean meal</th>
<th>White fishmeal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>5.2</td>
<td>4.8</td>
<td>3.9</td>
<td>5.2</td>
<td>4.2</td>
<td>4.2</td>
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<tr>
<td>Leucine</td>
<td>8.1</td>
<td>8.9</td>
<td>5.8</td>
<td>7.4</td>
<td>8.2</td>
<td>6.7</td>
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<tr>
<td>Phenylalanine</td>
<td>2.3</td>
<td>1.4</td>
<td>3.1</td>
<td>5.5</td>
<td>5.5</td>
<td>3.9</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.1</td>
<td>1.4</td>
<td>5.9</td>
<td>8.1</td>
<td>6.8</td>
<td>5.7</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.70</td>
<td>1.04</td>
<td>1.8</td>
<td>2.5</td>
<td>1.4</td>
<td>3.0</td>
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<tr>
<td>Cyst(e)ine</td>
<td>6.51</td>
<td>4.05</td>
<td>1.1</td>
<td>1.0</td>
<td>1.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Amino acids in claw horn, competing tissues and in feed sources (Galbraith et al, 2006).
Importance of cysteine supply

• Cysteine is disproportionately present in claw horn

• Why? Keratins, IFAPS....

• Solution 1? Supplement diet with protected cysteine?

• NO. Problem: usually unstable - oxidises ....

Solution 2:
Utilise methionine and post-absorptive transulphuration (with serine) to cysteine. YES. (In proteins or "protected") ...or synthesised - give S in diet)

• How much is needed for cysteine synthesis + meeting specific requirement for methionine? (10-20g/day??)

• How interacts directly with claw tissue: uptake + incorporation?
Claw tissue-interactions: L-Methionine (\(^{35}\)S) incorporation into protein in claw tissue explants

Effects of L-methionine concentration (\(\mu\text{mol/L}\)) on protein synthesis in sole explants (21h incubation, then incubation 3 h with 6.0 \(\mu\text{Ci/ml}\) L-[\(^{35}\)S]-methionine. (Hepburn et al, 2008)

In practice supply good quality protein (or protected methionine) in the diet.
Importance of protein/amino acid supply
Major constituent is protein

Nutritional supply needed in:

**EPIDERMIS**;
Good quality horn - Keratinocytes, IFs and IFAPs, cell envelope, actin, enzymes, adhesion proteins (intercellular cementing proteins)...

**DERMIS**;
- Fibroblasts, vascular and neural cells. Healthy - connective tissue - collagens, elastins, fibronectins, glycosaminoglycans...
- Resistance to mechanical forces on foot..
Structure:function;
Importance of molecular architecture: α-keratins in horn by X-ray diffraction (XRD)

XRD pattern showing arcs on equatorial axis typical of hard α-keratin (Interfibril spacings)

Diffraction angles show alignment of fibrils with importance in load-bearing in horn (Browne et al, 2007)
Digital cushion pads: “Shock absorbers”
Important role in lameness prevention

• **Sole ulcers and white line** diseases negatively associated with thickness of the digital cushion pads
• **Body condition scores** positively associated with digital cushion thickness
• Digital cushion thickness **decreased** from the first month of lactation
• Recent results (Newsome et al. 2017)- suggested that thin solar soft tissue predisposed to occurrence of sole ulcer or sole haemorrhage in dairy cows

**Composition: lipids**

• Lipid content in the pads was significantly **higher** in cows than in the heifers
• The lipids in all pads contained >77% monounsaturated fatty acids (**MUFA**),
• Among the polyunsaturated fatty acids (**PUFA**) a significantly higher proportion of **arachidonic acid** (**AA**) was found in heifer pads than in those of the cows

• **Nutritional supply** - need to provide substrate for lipids and fibrous proteins
Healthy horn cells: Adhesion from inter-cellular cementing substance (eg. Mülling et al. 2006)

**Composition:**
Glycoproteins and lipids between horn cells

Lipids in horn: eg. cholesterol: FFA, TAG, cholesterol sulphate, ceramides

Total lipid: 0.015 for wall; 0.03 for heel

**Composition: lame vs non-lame-**
- Lame cow horn - more linoleic (C18:3n-6); linolenic (C18:3n-3) and arachidonic acid (C20:4n-6) than the claws of sound cows. (Offer, et al., 2000)

- Important lipohilic barrier function

- Feeding fatty acids (fish oil) changed lipid composition - more PUFA
Nutrition/ regulation: considerations in pregnancy and development

- What has been noted is that:
  - For cows: poor pre-partum (white line) claw health of heifers predisposes to poor quality horn post-partum (Kempson and Logue, 1993).
  - Related to poor IFAP formation and intercellular adhesion
  - Increased susceptibility to solar lesion development post partum

What about pre-natal development of claw tissues of the heifer calf?
- Impact of nutrition in utero?
- Limited information
In addition: changes in physiological state post-partum from...

- Combinations of changes in eg. animal growth, pregnancy, parturition, lactation influencing
- Systemic homeostatic/homeorhthetic signalling
- Spillover to produce direct effects on claw tissues?

- Metabolic hormones affecting synthesis and breakdown of protein (eg collagens...)
- and lipid (digital cushions...)? (decreases after calving); ...
- fat mobilisation..... Feed intake vs milk output...

- Also indirect effects on partition of nutrients to claw and affecting functional integrity?
- (Low efficiency of utilisation of protein for integumental tissues eg wool... 0.26 (AFRC, 1993)...)
Evidence for endocrine-related aetiology: reproduction and lactation

- Comparison of laminar region composition and physical properties. Pregnant vs maiden heifers (Tarlton et al, 2002)

- Increased laxity of connective tissue around parturition and into lactation not associated with nutritional acidosis.

- Expanded and distorted laminae with more active metalloproteinases

- Site-specific bio-mechanical properties relating to composition

- Question of association with endocrine/systemic signalling for pregnancy and lactation:
  - Hormones: relaxin: oestriadiol-17β....??

Note: different (non-reproductive) mechanisms responsible for claw horn lesions in male dairy or beef cattle.
Evidence for nutritional/inflammatory aetiology. SARA. Ruminal pH effects?

- Nutritional imbalances produce acute/subacute ruminal acidosis (SARA) causing inflammation? (Socek, 1997). (Ruminal pH < 5.3...)
- Diet-induced SARA produced ruminal LPS endotoxin release and systemic inflammatory response in rumen-fistulated steers (Gozho et al, 2005)

- Model system with oligofructose overload of cows produced lameness, and ruminal acidosis (Thoefner et al, 2004, 2005)
- Reports of Danscher et al. 2009; 2013
  - Acute clinical laminitis by 48h and histologically expanded dermal laminae and changed basement membrane

- Similarity to the equine model - more clearly nutritional??
- In practice, how record SARA in cows?? pH boluses..
  - UK BBSRC-funded projects
Potential role of the hindgut in SARA? (Adapted from: **McCartney et al. 2014) http://old.eaap.org/Previous_Annual_Meetings/2014Copenhagen/Papers/Published/S07_09.pdf

**“Visible inflammation of the rumen wall correlates with caecal lipopolysaccharide concentrations”**

**LPS from caecum translocates into bloodstream?**

**“Translocated LPS during SARA may aggravate ruminal acidosis” (Jing et al., 2014)”**

Question: Affecting inflammation in other tissues also?
Nutrition-related SARA Issues; pH effects....

- Rapid carbohydrate fermentation decreases rumen pH
- Sugars (AHDB - https://dairy.ahdb.org.uk/)
- Polymers: Starch fermented faster than cellulose
- Starch - particle size regulating acid VFA and lactate production...

Buffering saliva (~100L/day) stimulated by forage/fibre (also suggestion that long forage decreases feed intake allowing more saliva/dry matter intake; Beauchemin et al., 2008)

- Evidence of lameness induced by nutritional SARA in practice?? - not clear...
Recent case study; Lameness in dairy farms differing in nutritional input (Yells, 2014)

Farms:

High Risk (HR: D1): Housed; TMR; 11kg concentrate intake/head/day in TMR: 12MJ/kg. Holstein.

Low Risk (LR: D2): grazed grass; whole crop silage; mixed grain (3kg/hd/day): Holstein Friesian
Dairy rations (Farms D1: D2); issues
Starch, particle size, grass sugars, saliva: buffering

**D1(HR) TMR**

**D2 (LR) Whole crop silage + concentrate**

**D2 grass**

**TMR**
Target: 11kg conc./head/d (High Risk)

Separate grain ration (3kg/hd/d) = (13 MJ/kg) DM ME
Plus grass and silage = ad libitum.

*NB Fermentable sugars in grass (AHDB info!) (Low risk SARA?)*
Locomotion Score (LS): Higher on Farm D1(HR) than D2 (LR). (2 observations)

Increasing lameness

High concentrate; high risk

Low concentrate: low risk
Partly on grass...

Farm HR: 38% Lesion score 2; 7.6% LS3
Farm LR: 26% LS 2; 2.7% LS3
Average production data: summary for the whole lactating herd for farms D1 and D2.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Average Annual Milk Yield (kg)</th>
<th>Average butterfat (%)</th>
<th>Average milk protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D1 (HR)</strong></td>
<td>9626</td>
<td>3.60</td>
<td>3.07</td>
</tr>
<tr>
<td><strong>D2 (LR)</strong></td>
<td>5997</td>
<td>4.20</td>
<td>3.55</td>
</tr>
</tbody>
</table>

Note: **Locomotion score for Farm HR, negatively correlated with milk yield, protein and butterfat.**
Other nutrients with roles in suspension and horn production

- Major minerals: Ca, P, Mg, ... Acid/base balance
- Trace minerals: Zn (Identified: Cell proliferation...), Cu, Se, Mo...
- Water
- Vitamins A, D, E...

- Water soluble vitamins (Important question about adequacy of supply from rumen/digestive tract synthesis)

Eg. Biotin response to supplementation ...elongating fatty acids; Digital cushions; Cementing substance

Important role of nutrients in internal structure, adhesion, enzyme activity, genomic signalling, methylation, post-translational modification...
Conclusions: current evidence

- Good classical nutrition essential to support maintenance of suspensory and horn producing tissues
- In context of absorbed nutrients, competing tissues, milk/nutrient balance

- Basic biology of regulation of horn production or connective tissue metabolism not fully understood
- Although evidence that poor quality horn, pre- and post-partum, is a frequent feature of claw horn lameness
- High input intensive environment may give a greater risk than low input (SARA contribution appears variable), but good dietary fibre and buffering reduce risk?

Also; coping with adverse environments mitigated by rubber mats; reduced standing time, good cubicle design etc...
Useful source of practical information

- UK Agriculture and Horticulture Development Board (AHDB)
  
  https://dairy.ahdb.org.uk/
Grazie per la vostra attenzione

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