Antioxidant nutrition in dairy ruminants

A. Baldi & L. Pinotti

[luciano.pinotti@unimi.it]
Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, 20133, Milano, Italy
PRO-OXIDANT AND ANTIOXIDANT BALANCE

- **Reactive oxygen species (ROS)**
  - Radicals
    - Superoxide, $\cdot O_2^-$
    - Hydroxyl, $\cdot OH$
    - Hydroperoxyl, $H_2O\cdot_2$
    - Alkoxyl, $RO\cdot_2$

- **Reactive nitrogen species (RNS)**
  - Radicals
    - Nitric oxide, NO
    - Nitrogen dioxide, $NO_2$

- **Reactive chlorine species**

- **Preventive and scavenging**
  - Metalloproteins Fe: Ferritin, transferrin; Cu: Caeruloplasmin; Zn: Metallothionein
  - Superoxide dismutase, Glutathione peroxidase, Glutathione reductase, Catalase
  - Vitamin E, vitamin C, vitamin A, uric acid
  - carotenoids, flavonoids

- **Chain breaking**
  - Vit. C, vit. E, carotenoids, flavonoids, ubiquinol, albumin, bilirubin, uric acid
  - Superoxide dismutase
  - Glutathione peroxidase
  - Glutathione reductase
Antioxidant 3 major groups

• 1° group comprises enzymatic antioxidants (mainly intracellular) > glutathione peroxidase, SOD

• 2° group includes non-enzymatic protein antioxidants that are primarily found in plasma > SH-albumin

• 3° group includes low-molecular weight antioxidants, and it is found mainly in plasma but also in other extracellular and intracellular fluids > glutathione, α-tocopherol, β-carotene.....

Chauhan et al., 2014
Oxidative stress (OS) & related disorders in dairy ruminants

Retained foetal membrane (RFM)

IMI and mastitis

(Baldi & Pinotti, 2007, Politis 2012)
Peripartum serum vitamin concentrations in cows with and without RFM

- A survey of 44 studies reports that vitamin E supplementation (up to 3000 IU) reduce the risk of RFM >fertility (Bourne et al., 2007)
- For every 1mg/l increase of vitamin E, the risk of RFM decreases by 21% (LeBlanc et al., 2004)
Peripartum serum antioxidants concentrations in cows with and without mastitis before 30 DIM

Antioxidants supplementation (i.e. vitamin E) is generally associated with a decreased risk of mastitis (Poltis 2012).
There is a significant correlation between antioxidant supplementation and decreased incidence of mastitis (Sordillo & Aitken, 2008)
Antioxidants and mammary gland health & SCC:

<table>
<thead>
<tr>
<th>α-tocopherol serum content</th>
<th>DIM</th>
<th>7 DIM</th>
<th>14 DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>dry off</td>
<td>30</td>
<td>1.125</td>
<td>0.923</td>
</tr>
<tr>
<td>&gt;3 μg/mL</td>
<td>60</td>
<td>0.923</td>
<td>1.669</td>
</tr>
<tr>
<td>&lt;3 μg/mL</td>
<td>90</td>
<td>1.669</td>
<td>1.484</td>
</tr>
<tr>
<td>P-value</td>
<td>0.18</td>
<td>0.07</td>
<td>0.68</td>
</tr>
</tbody>
</table>

(Vitamin E supplementation can reduce SCC, if Se is adequate)

(Vitamin E status at dry off is correlated with SCC in fresh cows)

- Results are equivocal

(Savoini et al., 2015)

(Baldi et al., 2000)
Vitamin E (& Se): mechanisms of action

• Vit.-E plays a central role as antioxidant by scavenging ROS with consequent reduction of cellular damage.

• In addition Vit.-E exerts also non-antioxidant cellular activities suggesting alternative molecular pathways for disease prevention.

• “Ref. Values”: Based on health and immune function in cows, plasma α-TC should be above 3 µg/ml in plasma [Adequate Selenium status: Whole blood >0.20 µg/ml]
In all dairy ruminants, the transition period is risky.

**Cows** (Hogan, 1993)

**Goats** (Pinotti & Baldi, 2009)

**Buffaloes** (Panda et al., 2005)

Effect of mastectomy on plasma $\alpha$-TC in cows (Goff et al., 2002)
Route of administration: Injection vs feed

Plasma $\alpha$-TC in beef cows

Feed supplementation long term effect
Injection can be used for an immediate response
## Forms and formulations

<table>
<thead>
<tr>
<th>Vitamin E</th>
<th>Notes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feed technology</strong></td>
<td><img src="image" alt="Microincapsulation" /></td>
<td>Baldi et al., 1997; Bontempo et al., 1998</td>
</tr>
<tr>
<td><strong>Forms-bioavailability</strong></td>
<td>![Natural (RRR-form)] <img src="image" alt="Synthetic" /></td>
<td>Weiss et al., 2009; Meglia et al., 2006; Baldiet al., 2011; Politis, 2012</td>
</tr>
<tr>
<td><strong>Interactions with other nutrients (fats)</strong></td>
<td><img src="image" alt="Plasma α-TC increased by 1.9 times when fats were fed" /> <img src="image" alt="Limited effect in transition" /></td>
<td>Weiss et al., 2003; Baldi et al., 2000, 2011</td>
</tr>
<tr>
<td><strong>Route of administration</strong></td>
<td><img src="image" alt="Feed suppl. Long term effects" /> <img src="image" alt="Injection immediate effects" /></td>
<td>Dell’Orto, pers. Com. Politis, 2012.</td>
</tr>
</tbody>
</table>
Vitamin E: summary

• Bioavailability, bioponcy, feed tecnology & nutrients inteactions need further investigations in all dairy ruminants

• Minimum response around parturition
  • Irrespective of vitamin E intake, plasma α-TC drops at parturition (over 30%), suggesting that an extra dose of the vitamin is useful at this time

• Vitamin E supplemetation (in transition period)
  • Cows/buffaloes: 1000-2000 IU/day of vitamin E
  • Higher amounts for specific purpose (3000 IU/day for SOF in milk, herds with high inc. mastitis)
  • Ewes/goats: 100-200 IU/day vitamin E

(Baldi 2005; Baldi et al., 2000; 2007; Pinotti et al., 2008; Politis, 2012)
Selenium: in brief

Selenium effect

• Se can ameliorate OS and reduce the severity of several economically important diseases in dairy cattle including mastitis and metritis

• Many of the health benefits of Se can be attributed to the antioxidant functions of selenoproteins

Selenium forms

• Se yeast results in up to 20% higher GSH-Px activity, and Se blood concentrations compared with similar amounts of Se-selenite
  • i.e. bioavailability of organic Seis higher than inorganic Se
  • No differences in neutrophils function and health/productive traits

• Selenium supplementation:
  • Cows 0.30 mg/kg DM
  • Small ruminants 1-1.2 mg/kg DM
Vitamin E & Se are the antioxidants commonly investigated

- Traditionally, vitamin E & Se have been the antioxidants most commonly investigated in dairy ruminants
- **Both** act synergistically to prevent OS, and *supplementation* of one appears to be less effective when the other is limiting
- Vit.-E & Se can mitigate OS and reduce the severity of several economically important diseases in dairy cattle including mastitis, RFM and metritis (other dairy rum??)
- Many of the health benefits of Vitamin E & Se can be attributed to their antioxidant functions (other under invst.)

Chauhan *et al.*, 2014; Sordillo, 2013
Production: High-producing dairy cows and OS

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Milk yield (Kg/d)</th>
<th>Milk energy (MJ/d)</th>
<th>Lipohydroperoxide (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>34.2</td>
<td>122.2</td>
<td>3.6</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>51.7</td>
<td>164.8</td>
<td>6.5</td>
</tr>
<tr>
<td>$P$</td>
<td></td>
<td>0.001</td>
<td>0.021</td>
<td>0.015</td>
</tr>
</tbody>
</table>

High milk yield can be associated with OS indicated by oxidative modifications of circulating lipids

Marked increase in superoxide formation

(Lohrke et al., 2005)
Feeding regime

• Feeding regime has an indirect effect on the level of OS in lactating dairy cows particularly 2 weeks post-partum.
• Cows in severe NEB during early lactation had increased OS,
  • possibly due to the reduced availability of antioxidants rather than to changes in ROMs production.

(Pedernera et al., 2010; Chauhan et al., 2014)
Environmental hyperthermia

• Sheep study:
• Heat stress has been implicated in promoting OS either through:
  • excessive reactive oxygen species (ROS) production or
  • decreased antioxidant defenses

Chauhan et al., 2014
Conclusions

• A robust antioxidant network is recommended for improving the health and performance of dairy ruminants in several crucial phases & circumstances (transition, NEB, high dietary fat and heat stress)

• Nutrition can have a major influence on OS occurrence, since several antioxidant system components are micronutrients or are dependent on dietary micronutrients.

• Vitamin E & Se are known to be effective dietary antioxidants in ruminants, while several others are under investigation and cannot yet be recommended as routine dietary supplements

Baldi et al., 2007; Chauhan et al., 2014; Pinotti & Baldi 2015
Future directions in all dairy ruminants

- Ideal OS biomarker does not exist
- Lack of reference values for existing markers (e.g. ROMs, ROS, BAP)
- No single biomarker can adequately characterize OS status: «panel of measurements» the gold standard in ruminants
- **2° group non-enzymatic protein antioxidants**: i.e. sulfhydryl groups (SH) of albumin and are considered a significant element of extra-cellular antioxidant defense system> liver function> methyl group metabolism

COST ACTION FA1308

Celi, 2010; Loor et al., 2015; Pinotti 2015