Essential oils for dairy cows: how to improve ruminal fermentation and animal performance

Pietro Celi (DVM, PhD)

February 16, 2016
Presentation outline

- Overview
  - Essential oils
- Mode of Action
- Animal performances (Dairy)
  - Milk Yield
  - Frequent visits to robot milking system
- Dosage - Recommendations
**Invited Review: Essential Oils as Modifiers of Rumen Microbial Fermentation**

S. Calsamiglia, M. Busquet, P. W. Cardozo, L. Castillejos, and A. Ferret

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Table 1. Essential oils with antimicrobial activity, their main active components, and susceptible microorganisms

<table>
<thead>
<tr>
<th>Essential oil of</th>
<th>Name</th>
<th>Active components</th>
<th>Susceptible microorganisms</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allium sativum</td>
<td>Garlic</td>
<td>Allicin, diallyl sulfide</td>
<td>Enteropathogenic bacteria</td>
<td>Ross et al., 2001</td>
</tr>
<tr>
<td>Anethum graveolens</td>
<td>Dill</td>
<td>Limonene, carvone</td>
<td>Gram-positive and gram-negative bacteria</td>
<td>Deans and Ritchie, 1987</td>
</tr>
<tr>
<td>Capsicum annum</td>
<td>Paprika</td>
<td>Capsaicin</td>
<td>Gram-positive and gram-negative bacteria</td>
<td>Deans and Ritchie, 1987</td>
</tr>
<tr>
<td>Cinnamomum cassia</td>
<td>Cassia</td>
<td>Cinnamaldehyde</td>
<td>Escherichia coli, Staphylococcus aureus, Listeria monocytenes, Salmonella enteritidis</td>
<td>Ouattara et al., 1997; Mahmoud, 1994; Smith-Palmer et al., 1998</td>
</tr>
<tr>
<td>Juniperus oxycedrus</td>
<td>Juniper</td>
<td>Cadinene, pinene</td>
<td>Aeromonas sobria, Enterococcus fecalis, Staph. aureus</td>
<td>Hammer et al., 1999</td>
</tr>
<tr>
<td>Melaleuca alternifolia</td>
<td>Tea tree</td>
<td>Terpinen-4-ol</td>
<td>Staph. aureus, E. coli, gram-positive and gram-negative bacteria</td>
<td>Chao and Young, 2000; Cox et al., 2001</td>
</tr>
<tr>
<td>Origanum vulgare</td>
<td>Oregano</td>
<td>Carvacrol, thymol</td>
<td>Gram-positive and gram-negative bacteria</td>
<td>Syrropoulou et al., 1996; Dorman and Deans, 2000</td>
</tr>
<tr>
<td>Pimpinella anisum</td>
<td>Anise</td>
<td>Anethol</td>
<td>Aeromonas hydrophila, Brevibacterium linens, Brochothrix thermosphaeta</td>
<td>Deans and Ritchie, 1987</td>
</tr>
<tr>
<td>Rosmarinus officinalis</td>
<td>Rosemary</td>
<td>1,8-Cineole</td>
<td>Staph. aureus, L. monocytogenes, Campylobacter jejuni</td>
<td>Ouattara et al., 1997; Smith-Palmer et al., 1998</td>
</tr>
<tr>
<td>Syzygium aromaticum</td>
<td>Clove</td>
<td>Eugenol</td>
<td>E. coli, Staph. aureus, L. monocytogenes, S. enteritidis, C. jejuni</td>
<td>Ouattara et al., 1997; Smith-Palmer et al., 1998</td>
</tr>
<tr>
<td>Thymus vulgaris</td>
<td>Thyme</td>
<td>Thymol, carvacrol</td>
<td>Salmonella typhimurium, Staph. aureus, Aspergillus flavus</td>
<td>Juven et al., 1994; Ouattara et al., 1997; Mahmoud, 1994</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Ginger</td>
<td>Zingiberene, zingerone</td>
<td>Gram-positive and gram-negative bacteria</td>
<td>Chao and Young, 2000</td>
</tr>
</tbody>
</table>
Thymol: Reduction of N-ammonia, inhibited microboal population ...

Eugenol: Less acetate and more propionate in the rumen .... improved feed- and energy-efficiency ....

Vanillin, Limonene ......... Cinnamonaldehyde, Capsaicin ...
CRINA® Ruminants - a unique blend of essential oil compounds

Essential oils are

- Steam volatile secondary plant compounds
- Natural flavours and fragrances
- Natural plant defense systems
- Terpenes or phenols
- Antibacterial, antifungal, antimicrobial
- No fats, neither oils, nor indispensible!
Botanical: What’s the difference?

- **Plant extracts**
  - Different and numerous molecules interacting (also including carbohydrates, fibre, protein,...)

- **Essential Oils (EOs)**
  - Mixtures of compounds obtained from plants (e.g. EOs of thyme)

- **Essential Oil Compounds**
  - Pure active compounds either extracted from plants or synthesized (nature identical) (e.g. Thymol)
Essential Oils (EOs)

- Natural vegetable products extracted by steam and / or water distillation
- Currently some 2,600 known EOs
- Mixtures of different chemical compounds (e.g. alcohol, esters, aldehydes, ketones) and small amounts of non-volatile residues (paraffins, waxes)
- Each compound having its own positive or negative properties
- Fluctuating compositions influenced by many factors e.g.:
  - Species
  - Soil
  - Climate
  - Harvesting
  - Storage
Influence of geographical origin on EOs profile

Rosemary from Spain

Rosemary from Tunisia

Important Essential Oils Compounds

- **Eugenol**
  - Molecular formula: $\text{C}_{10}\text{H}_{12}\text{O}_2$
  - Chemical structure:
  - Characteristics/effects: analgesic, anaesthetic, antibacterial, antiherpetic, candicide, nematicide

- **2-Methoxyphenol (Guaiacol)**
  - Molecular formula: $\text{C}_7\text{H}_8\text{O}_2$
  - Chemical structure:
  - Natural occurrence: aromatic oil is derived from guaiacum or wood creosote (i.e. beech wood)
  - Characteristics/effects: expectorant, antiseptic, and local anaesthetic

- **3-Methylphenol**
  - Molecular formula: $\text{C}_7\text{H}_8\text{O}$
  - Chemical structure:
  - Natural occurrence: widely occurring in nature
  - Characteristics/effects: antiseptic
Important Essential Oils Compounds

• **Piperine**
  - Molecular formula: C$_{17}$H$_{19}$NO$_3$
  - Chemical structure:
  - Natural occurrence: ex. Piper nigrum (total content in black pepper: about 5% piperine)
  - Characteristics/effects: enhancing intestinal absorption, anti-inflammatory, anti-malarial, thermo genesis increasing

• **Thymol**
  - Molecular formula: C$_{10}$H$_{14}$O
  - Chemical structure:
  - Natural occurrence: Monarda didyma, L. (31% Thymol in essential oil)
  - Characteristics/effects: acaricide, anthelmintic, antibacterial, antiherpetic, antispasmodic, fungicide
Essential Oils Compounds Properties

- Many essential oil compounds have **antimicrobial activities**
- Thymol, eugenol, 2-methoxyphenol (guaiacol), 3-methylphenol,... develop their action against bacteria through interacting with the **cell membrane**
- This interaction causes conformational changes in the membrane structure, leading to the leakage of ions across the cell membrane. Bacteria can usually counterbalance these effects, but bacterial growth is slowed down.
- The spectrum of activity varies between compounds, with a main activity on Gram + bacteria, but some compounds have a wider spectrum and act as well on Gram - (i.e. thymol)
- Some essential oils **stimulate the secretion of enzymes** from the digestive glands (pancreas, salivary glands,...) and the intestinal wall
- Piperine is a good example
Main Components of CRINA® Ruminants

All compounds are: food grade listed by the F.E.M.A. / G.R.A.S. all appear on EU Register of Feed Additive, (Reg.1831/2003)
Advantages of Nature Identical Compounds

- Selected and defined composition of essential oil compounds
- High potency and efficacy
- Standardized well defined product free of antagonistic substances
CRINA® products
Research & Development process

- *in vitro* evaluation
- single compounds or in combination

*in vivo* validation
research stations

*in vivo* validation
commercial conditions

CRINA®
Ruminants
# Similarity between Ionophores and CRINA® Ruminants

<table>
<thead>
<tr>
<th></th>
<th>What is it</th>
<th>Hydrophobic</th>
<th>Attachment to bacterial cell membrane</th>
<th>Disturbs flow of nutrients</th>
<th>Effect mainly on gram positive bacteria</th>
<th>Effects in the rumen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C2/C3</td>
</tr>
<tr>
<td>Ionophores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboxylic Polyethers</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Steam volatile plant compounds</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CRINA® Ruminants</th>
<th>Rumensin®</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2 %</td>
<td>50.9</td>
<td>50.6</td>
<td>50.1</td>
</tr>
<tr>
<td>C3 %</td>
<td>28.5</td>
<td>33.8</td>
<td>32.8</td>
</tr>
<tr>
<td>C2/C3</td>
<td>1.8</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NH₃ (mmol/l)</th>
<th>Control</th>
<th>Eugenol</th>
<th>Control</th>
<th>Monensin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15.7</td>
<td>12.0</td>
<td>12.4</td>
<td>8.7</td>
</tr>
</tbody>
</table>

* Meyer et al. 2009

* Castillejos et al 2006

* Yang and Russell, 1993
Presentation outline

- Overview
  - Essential oils
- Mode of Action
- Animal performances (Dairy)
  - Milk Yield
  - Frequent visits to robot milking system
- Dosage - Recommendations
Mode of action of CRINA® Ruminants

- **CRINA® Ruminants** Reduces the deamination process
- **CRINA® Ruminants** Reduces the rate of degradation of some protein sources
CRINA® Ruminants affect rumen metabolism

1. Essential oil compounds increase membrane permeability.

2. The H⁺ ions of the rumen fluid rapidly invade the intra-bacterial region.

3. Bacteria metabolism is disrupted, and multiplication of bacteria is altered.

Effect of CRINA® Ruminants
- DEGRADATION OF FEED
  - Reduces the attachment of bacteria to feed particles.

Effect of CRINA® Ruminants
- DEVELOPMENT OF RUMEN MICROFLORA
  - Favours the production of VFA and microbial proteins.

The essential oil compounds in CRINA® Ruminants modulate the rumen microflora. CRINA® Ruminants supports the synthesis of VFA and increases the production of microbial protein.
<table>
<thead>
<tr>
<th>Bacterial Species</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; Ino. (ppm) CRINA Ruminants (McIntosch et al., 2003)</th>
<th>Ruminal niche (Russell et al., 2001)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium sticklandii</em></td>
<td>36.0</td>
<td>AA</td>
</tr>
<tr>
<td><em>Peptostreptococcus anaerobius</em></td>
<td>42.5</td>
<td>AA</td>
</tr>
<tr>
<td><em>Selenomonas ruminantium</em></td>
<td>57.0</td>
<td>ST, DX, SU, L, S</td>
</tr>
<tr>
<td><em>Ruminococcus flavefaciens</em></td>
<td>60.0</td>
<td>CU, HC</td>
</tr>
<tr>
<td><em>Prevotella brevis</em></td>
<td>57.5</td>
<td>ST, PC, XY, SU</td>
</tr>
<tr>
<td><em>Prevotella albensis</em></td>
<td>50.0</td>
<td>ST, PC, XY, SU</td>
</tr>
<tr>
<td><em>Eubacterium ruminantium</em></td>
<td>70.0</td>
<td>HC, DX, SU</td>
</tr>
<tr>
<td><em>Anaerovibrio lipolytica</em></td>
<td>73.8</td>
<td>GL, SU</td>
</tr>
<tr>
<td><em>Veillonella parvula</em></td>
<td>88.0</td>
<td>ST, PC, XY, SU</td>
</tr>
<tr>
<td><em>Prevotella ruminicola</em></td>
<td>33.8</td>
<td>CU</td>
</tr>
<tr>
<td><em>Fibrobacter succinogenes</em></td>
<td>95.0</td>
<td>ST, CU, HC, PC, SU</td>
</tr>
<tr>
<td><em>Butyrivibrio fibrisolvens</em></td>
<td>56.2</td>
<td>ST, CU, HC, PC, SU</td>
</tr>
</tbody>
</table>

*IC<sub>50</sub> is the concentration of CRINA® Ruminants that led to a 50% in cell density at 24 h of incubation.

AA, amino acids
CU, cellulose
HC, hemicellulose
DX, dextrins
SU, sugars
St, starch
PC, pectin
XY, xylans
L, lactate
S, succinate
GL, glycerol
ST, starch
involved in starch fermentation (Nagaraja, 2007)
CRINA® Ruminants
Effect on bacteria degrading starch or amino acids (2)

<table>
<thead>
<tr>
<th>effect on growth of pure culture of rumen bacteria</th>
<th>IC_{50} Ino. (PPM) CRINA Ruminants (McIntosch et al., 2003)</th>
<th>Ruminal niche (Russell et al., 2001)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prevotella bryantii</strong></td>
<td>54.0</td>
<td>ST, PC, XY, SU</td>
</tr>
<tr>
<td><strong>Lachnospira multipara</strong></td>
<td>112.5</td>
<td>PC, SU</td>
</tr>
<tr>
<td><strong>Ruminococcus albus</strong></td>
<td>49.0</td>
<td>CU, HC</td>
</tr>
<tr>
<td><strong>Ruminobacter amylophilus</strong></td>
<td>42.6</td>
<td>ST</td>
</tr>
<tr>
<td><strong>Mitsuokella multiacidas</strong></td>
<td>113.5</td>
<td>ST</td>
</tr>
<tr>
<td><strong>Megasphaera elsdii</strong></td>
<td>113.0</td>
<td>L, SU</td>
</tr>
<tr>
<td><strong>Lactobacillus casei</strong></td>
<td>56.2</td>
<td>ST, SU</td>
</tr>
<tr>
<td><strong>Streptococcus bovis</strong></td>
<td>127.5</td>
<td>AA</td>
</tr>
<tr>
<td><strong>Clostridium aminophilum</strong></td>
<td>94.2</td>
<td>ST, SU</td>
</tr>
</tbody>
</table>

involved in starch fermentation (Nagaraja, 2007)

CU, cellulose  
HC, hemicellulose  
DX, dextrans  
SU, sugars  
St, starch  
PC, pectin  
XY, xylans  
L, lactate  
S, succinate  
GL, glycerol  
AA, amino acids  

^IC_{50} is the concentration of CRINA® Ruminants that led to a 50% in cell density at 24 h of incubation.
CRINA® Ruminants

Effect on the microbial attachment and colonisation

CRINA Ruminants reduces the attachment of rumen microbes on feed particles and their colonisation
**CRINA® Ruminants**

*reduced protease activity on some protein sources*

Effect of CRINA® Ruminants on the microbial attachment and colonisation Impact on the attached protease activity on different protein sources

- **Soya bean**
- **Pea**
- **Fish meal**
- **Sunflower**
- **Rape seed**

Attached protease activity (mg casein/g wet weight/hr)

Source: Rowett and Crina, 2001
CRINA® Ruminants

*Effect on the degradability of soybean meal*

![Graph showing the effect of CRINA® on soybean meal degradation over time.](image-url)

- **DM digestion (%)**
- **Incubation time (h)**
- **Control**
- **Crina Ruminants**
## CRINA® Ruminants

**Influence on proteolysis to ammonia (NH₃)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CRINA® Ruminants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>0.46</td>
<td>0.49</td>
</tr>
<tr>
<td>Oligopeptide</td>
<td>1.03</td>
<td>1.22</td>
</tr>
<tr>
<td>Dipeptide</td>
<td>0.60</td>
<td>0.69</td>
</tr>
<tr>
<td>Amino acids</td>
<td>410</td>
<td>372*</td>
</tr>
<tr>
<td>Ammonia (NH₃)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(nmol NH₃ / mg protein / min)</td>
<td></td>
</tr>
</tbody>
</table>

* P<0.05

Data from 4 dairy cows receiving concentrate: maize silage
CRINA Ruminants dosage at 1 g/d

Source: adapted from McIntosh F.M. et al., 2003
Getting more out of the feed

• By inhibiting hyperammonia-producing bacteria CRINA® Ruminants has a direct and beneficial effect on protein digestion by:
  - Slowing down the rate of protein digestion
  - Reducing protein degradability
  - Increasing the proportion of by-pass protein and boosting the value of lower grade protein sources
  - Reducing the amount of amino acids that are degraded to ammonia
CRINA® Ruminants Improves Propionate Concentration

### In vitro (dairy cows)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CRINA® Ruminants</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCFA mmol/l</td>
<td>76.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetate %</td>
<td>67.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Propionate %</td>
<td>22.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Butyrate %</td>
<td>5.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Source: Kung et al., 2008, J. Dairy Sci, 91, 4793-4800

### In vivo (beef cattle)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CRINA® Ruminants</th>
<th>Rumensin&lt;sup&gt;®&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCFA mmol/l</td>
<td>109.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>125.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>104.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetate %</td>
<td>50.9</td>
<td>50.6</td>
<td>50.1</td>
</tr>
<tr>
<td>Propionate %</td>
<td>28.5</td>
<td>33.8</td>
<td>32.8</td>
</tr>
<tr>
<td>Butyrate %</td>
<td>12.9</td>
<td>9.9</td>
<td>11.2</td>
</tr>
</tbody>
</table>

(Means without a common superscript are significantly different (P<0.05)

Source: Meyer et al., 2009, J. Anim. Science

More available energy for milk production and weight gain
Nutritional Benefits of CRINA® Ruminants

Rumen - degradation of

Protein ↓ → + 5 % more bypass protein!

Starch ↓ → + 3 % more bypass starch!

NDF ↑ → + 2 % more digestible!

Rumen - volatile fatty acids

Propionate concentration ↑ → more available energy
Presentation outline

• Overview
  - Essential oils
• Mode of Action
• Animal performances (Dairy)
  - Milk Yield
  - Frequent visits to robot milking system
• Dosage - Recommendations
Effect of CRINA® Ruminants on milk yield in a dairy farm over 1 year

Species: Dairy Cows  
Country: Germany

Objective
• To study the effects of CRINA® Ruminants on milk production in dairy cows for 12 months

Trial details
• Location: Malchin Germany, 2010
• 400 Holstein cows, 650 kg, 1 - 200 d in milk
• Milk yield: 9900 kg/cow/year
• Trial design: 3 weeks adaptation during dry period
• 1 DIM - 200 DIM trial period
• Treatments fed as total mixed ration (TMR) main components - corn silage, grass silage and wet corn.
  - Control: TMR, no supplementation
  - CRINA®Ruminants: Same TMR + CRINA® Ruminants 1 g/cow/day
• Parameters measured:
  - Milk yield
  - Milk composition

Source: Kung et al. (2008), J. Dairy Sci, 91, 4793-4800
Effect of CRINA® Ruminants on milk yield in a dairy farm over 1 year

Species: Dairy Cows  
Country: Germany

Conclusions & Benefits

- CRINA® Ruminants increased;
  - milk yield by 1.5 kg / cow per day
  - stable milk fat and protein content
- CRINA® Ruminants as a top dress increased fat corrected milk (FCM) yield by 1.5 kg/cow/day
- ROI = 16:1 (Based on improved milk production)

Source: Kung et al. (2008), J. Dairy Sci, 91, 4793-4800
Effect of CRINA® Ruminants in dairy cows

Species: Dairy Cows
Country: USA, Delaware

Objective
- The purpose of this trial was to compare the effects of monensin and of CRINA® Ruminants on milk production and feed efficiency in dairy cows.

Trial details
- Breed: 28 HF cows weighing 687 kg, 72 d in milk
- Milk yield: 41 kg/head/day (hd/d)
- Trial design: Latin Square, 2008
- Treatments:
  - Control TMR
  - 1.2 g CRINA Ruminants / hd/d
  - 350 mg monensin / hd/d
  - 1.2 g CRINA Ruminants + 350 mg monensin / hd/d
- Parameters measured:
  - Dry matter intake (DMI)
  - Milk yield and composition: fat, crude protein (CP), milk urea nitrogen (MUN)
  - Somatic cell count (SCC)

Results

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Monensin (M)</th>
<th>CRINA (CR)</th>
<th>M + CR</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>26.9</td>
<td>26.8</td>
<td>26.3</td>
<td>27.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>41.9b</td>
<td>43.2 ab</td>
<td>44.1a</td>
<td>42.3b</td>
<td>1.6</td>
</tr>
<tr>
<td>3.5% FCM, kg/d</td>
<td>41.3b</td>
<td>42.9ab</td>
<td>44.9a</td>
<td>41.9b</td>
<td>1.6</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.44</td>
<td>3.50</td>
<td>3.60</td>
<td>3.40</td>
<td>0.12</td>
</tr>
<tr>
<td>Fat, kg/d</td>
<td>1.43b</td>
<td>1.50ab</td>
<td>1.59a</td>
<td>1.44b</td>
<td>0.07</td>
</tr>
<tr>
<td>CP, %</td>
<td>2.93</td>
<td>2.97</td>
<td>2.94</td>
<td>2.93</td>
<td>0.04</td>
</tr>
<tr>
<td>CP, kg/d</td>
<td>1.22b</td>
<td>1.28ab</td>
<td>1.30a</td>
<td>1.24ab</td>
<td>0.04</td>
</tr>
<tr>
<td>MUN mg/dl</td>
<td>14.0</td>
<td>14.2</td>
<td>14.2</td>
<td>13.9</td>
<td>0.4</td>
</tr>
<tr>
<td>SCC × 1000/ml</td>
<td>231</td>
<td>186</td>
<td>171</td>
<td>235</td>
<td>60</td>
</tr>
<tr>
<td>Feed efficiency, 3.5% FCM/DMI</td>
<td>1.56b</td>
<td>1.59ab</td>
<td>1.72a</td>
<td>1.54b</td>
<td>0.07</td>
</tr>
</tbody>
</table>

(Means without a common superscript are significantly different (P<0.05)

Conclusions & Benefits
- Monensin had no effect on milk yield, composition or feed efficiency.
- No effects of the combination of monensin with CRINA® Ruminants suggest that both additives are not compatible with each other.
- CRINA® Ruminants as a top dress increased FCM yield by 1 kg/hd/d and milk protein yield by 8%/hd/d.
- CRINA® Ruminants improved feed efficiency by 10%.

Source: Kung et al, 2009 internal report, unpublished
Effect of CRINA® Ruminants on milk production in dairy cows

Species: Dairy Cows
Country: Germany

Objective

- To demonstrate the effect of CRINA® Ruminants on milk production and milk components in a commercial dairy herd

Trial details

- 55 Holstein dairy cows
- Commercial dairy herd November 2009 - January 2010
- Ø 10,136 kg milk/cow/year
- Ø 153 days in milk
- 3 kg supplementary feed, 8 kg corn silage, 7 kg grass silage, 0.5 kg straw.
- Treatments:
  - Control
  - CRINA® Ruminants, 1g/cow/day

Results

- Ø 2.4 kg/cow/day more milk with CRINA® Ruminants

Conclusion & Benefits

- The inclusion of CRINA® Ruminants at 1g/cow/day results in:
  - 7.0% improvement in milk production.
  - Return on investment: > 10:1

Source: HF Dairy Cows I, Germany, 2010
Effect of CRINA® Ruminants on milk production in dairy cows

Species: Dairy Cows
Country: Germany

Objective
• To demonstrate the effect of CRINA® Ruminants on milk production, milk components, somatic cell counts and milk urea in a commercial dairy herd

Trial details
• Commercial dairy herd, in
• 86 Simmental cows
• ø 32 kg milk/cow/day
• ø 153 days in milk
• 43 % concentrates, 57 % silage (grass, corn)
• Treatments:
  - Control (fed to 214 cows)
  - CRINA® Ruminants: 1 g/cow/day (fed to 235 cows)

Results

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CRINA® Ruminants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield (kg/cow/day)</td>
<td>32.2</td>
<td>34.1</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>4.05</td>
<td>3.96</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.56</td>
<td>3.50</td>
</tr>
<tr>
<td>FCM 4% (kg/cow/day)</td>
<td>32.4</td>
<td>33.9</td>
</tr>
<tr>
<td>SCC in `000</td>
<td>177</td>
<td>158</td>
</tr>
<tr>
<td>Milk urea (mg/dL)</td>
<td>26.3</td>
<td>24.7</td>
</tr>
</tbody>
</table>

Conclusions & Benefits
The inclusion of CRINA® Ruminants at 1g/cow/day resulted in:
• 6.2% improvement in milk production
• Return on investment > 10:1

Source: Fleckvieh trial, Germany 2010
Effect of CRINA® Ruminants on milk yield in dairy cows

Species: Dairy Cows
Country: Germany

Objective
• To demonstrate the effect of CRINA® Ruminants on milk production, milk components and somatic cell counts in a commercial dairy herd

Trial details
• Commercial dairy herd, April-May 2010
• 32 Holstein dairy cows, Ø 36 kg milk/cow/day in early lactation
• Diet: 48 % concentrates, 52 % silage (grass, corn)
• Treatments:
  - Control
  - CRINA® Ruminants, 1 g/cow/day

Results

<table>
<thead>
<tr>
<th></th>
<th>April</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CRINA® Ruminants</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>CRINA® Ruminants</td>
</tr>
<tr>
<td>Milk yield kg/hd/d</td>
<td>36.0</td>
<td>38.3</td>
</tr>
<tr>
<td>Fat %</td>
<td>3.72</td>
<td>3.90</td>
</tr>
<tr>
<td>Protein %</td>
<td>3.56</td>
<td>3.37</td>
</tr>
</tbody>
</table>

Conclusion & Benefits
The inclusion of CRINA® Ruminants at 1 g/cow/day resulted in:
• 6% improvement in milk production
• Return on investment > 10:1

Source: HF Dairy Cows II, Germany 2010
Effect of CRINA® Ruminants on milk yield and feed efficiency in dairy cows

Species: Dairy Cows
Country: USA

Objective
• To study the effects of CRINA® Ruminants on milk production and feed efficiency in dairy cows

Trial details
• Delaware USA, 2007
• Breed: 30 Holstein cows
• 680 kg, 118 d in milk
• Milk yield: 38 kg/cow/day
• Trial design: 2 weeks adaptation, 9 weeks trial
• Treatments: fed as total mixed ration (TMR)
  - Control TMR, 25% corn silage, 15% alfalfa silage, 10% alfalfa hay and 50% concentrates
  - Same TMR + CRINA® Ruminants- 1.2g/cow/day
• Parameters measured:
  - Dry matter intake (DMI)
  - Milk yield and composition
  - Somatic cell counts (SCC)
  - Milk urea nitrogen
  - Body weight changes
  - Short chain fatty acids (SCFA) in vitro

Results

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>CRINA®Ruminants</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>26.4a</td>
<td>28.3b</td>
</tr>
<tr>
<td>3.5% FCM, kg/d</td>
<td>35.5a</td>
<td>38.2b</td>
</tr>
<tr>
<td>Fat, %</td>
<td>2.89</td>
<td>2.99</td>
</tr>
<tr>
<td>Fat, kg/d</td>
<td>1.13a</td>
<td>1.24b</td>
</tr>
<tr>
<td>CP, %</td>
<td>3.11</td>
<td>2.07</td>
</tr>
<tr>
<td>CP, kg/d</td>
<td>1.23</td>
<td>1.27</td>
</tr>
<tr>
<td>Milk Urea N mg/dl</td>
<td>11.1</td>
<td>11.6</td>
</tr>
<tr>
<td>SCC, × 1000/ml</td>
<td>242</td>
<td>243</td>
</tr>
<tr>
<td>3.5% FCM/DMI</td>
<td>1.36</td>
<td>1.35</td>
</tr>
<tr>
<td>SCFA mmol/l</td>
<td>76.5a</td>
<td>72.2b</td>
</tr>
<tr>
<td>Acetate %</td>
<td>67.3a</td>
<td>64.5b</td>
</tr>
<tr>
<td>Propionate %</td>
<td>22.9a</td>
<td>27.8b</td>
</tr>
<tr>
<td>Butyrate %</td>
<td>5.5a</td>
<td>4.9b</td>
</tr>
</tbody>
</table>

(Means without a common superscript are significantly different (P<0.05)

Conclusions & Benefits
• CRINA® Ruminants increased:
  - Feed intake, Milk yield, Propionate production in vitro, yielding more available energy for milk production
• CRINA® Ruminants as a top dress increased fat corrected milk (FCM) yield by 1.9 kg/cow/day and milk fat yield by 10%
• ROI = 16:1 (Based on improved milk production)

Effect of CRINA® Ruminants on milk production in pasture fed dairy cows

Species: Dairy Cows
Country: France

Objective
- To demonstrate the effect of CRINA® Ruminants on milk production in grazing dairy cows on a commercial farm

Trial details
- Commercial dairy farm in France
- Holstein cows < 100 days in milk
- ø 28 kg milk/cow/day
- Daily ration: 4 kg supplementary feed & 14 kg
- Basic diet (corn silage, alfalfa, rapeseed cake) & 4 kg pasture grass
- During transition: 10%, 50%, 67% less basic diet
- Treatment:
  - Control group
  - CRINA® Ruminants 1 g/cow/day

Results

Conclusion & Benefits
The inclusion of CRINA Ruminants at 1g/cow/day resulted in:
- 7% improvement in milk production
- Return on investment: > 10:1

Effect of CRINA® Ruminants on milk production in heat stressed cows

Species: Dairy Cows
Country: Tunisia

Objective
• To demonstrate the effect of CRINA® Ruminants on milk production in heat stressed dairy cows

Trial details
• Commercial dairy farm in Tunisia, Summer 2009
• 32 Holstein cows
• Ø 23 kg milk/cow/day
• Daily ration: 13 kg concentrates & forage (hay, alfalfa, ryegrass, berseem, triticale)
• Treatments:
  - Control group
  - CRINA® Ruminants: 1 g/cow/day

Results

Conclusions & Benefits
The inclusion of CRINA® Ruminants at 1g/cow/day resulted in:
• 5% improvement in milk production
• Return on investment > 10:1
• Less heat stress, more milk with CRINA® Ruminants

Source: Dairy cows trial, Tunisia 2009
Effect of CRINA® Ruminants on milk production in sheep

Species: Sheep
Country: Greece

Objective

- To demonstrate the effect of CRINA® Ruminants on milk production, milk components, somatic cell counts and milk urea in a commercial sheep flock.

Trial details

- Commercial sheep farm in Greece, May 2009 - October 2009, in cooperation with the University of Thessaly
- 80 Chios sheep
- ø 1.6 kg milk/ewe/day
- 1.4 kg concentrates & 2.4 kg forage (corn silage, alfalfa, straw)/ewe/day
- Treatments:
  - Control group
  - CRINA® Ruminants
    - 50 mg/kg concentrates
    - 100 mg/kg concentrates
    - 150 mg/kg concentrates

Results

<table>
<thead>
<tr>
<th></th>
<th>CRINA® Ruminants (mg/kg concentrate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Milk yield kg/ewe/day</td>
<td></td>
</tr>
<tr>
<td>SCC (x10^3/mL)</td>
<td></td>
</tr>
<tr>
<td>Milk urea (mg/100mL)</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions & Benefits

The inclusion of CRINA® Ruminants at 50, 100, 150 mg/kg concentrates resulted in:
- 8% to 36% improvement in milk production
- Return on investment > 10:1

Source: Report Dr. Ilias Giannenas, University of Thessaly, Greece, 2010
**CRINA® Ruminants - NZ grazing (2008) - Trial design**

- A 500-cow farm was split into two farmlets:
  - one of 51 Ha
  - the second of 57 Ha

- With both farmlets stocked to give the same number of cows per ha (at 4.7 cows/ha)

- CRINA Ruminants at 1.0 g/head day was given via supplement fed twice per day

- The herd was randomly drafted 3 weeks prior to the start of calving in the second half of July, by block randomisation based on age, body weight and calving date
CRINA® Ruminants - NZ grazing (2008) - average milk parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CRINA® Ruminants</th>
<th>Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk, (l/head/day)</td>
<td>16.8</td>
<td>17.5</td>
<td>+4.2 %</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>4.70</td>
<td>4.79</td>
<td>+ 1.9 %</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.48</td>
<td>3.50</td>
<td>+ 0.6 %</td>
</tr>
<tr>
<td>Milk solid (kg/head/day)</td>
<td>1.370</td>
<td>1.448</td>
<td>+ 5.7 %</td>
</tr>
<tr>
<td>4.0 % Fat Corrected Milk</td>
<td>18.6</td>
<td>19.6</td>
<td>+ 5.4 %</td>
</tr>
<tr>
<td>3.5 % Fat Corrected Milk</td>
<td>20.1</td>
<td>21.2</td>
<td>+ 5.5 %</td>
</tr>
<tr>
<td>Energy Corrected Milk</td>
<td>19.9</td>
<td>21.0</td>
<td>+ 5.5 %</td>
</tr>
</tbody>
</table>
Presentation outline

- Overview
  - Essential oils
- Mode of Action
- Animal performances (Dairy)
  - Milk Yield
  - **Frequent visits to robot milking system**
- Dosage - Recommendations
Testimonial: Effect of CRINA® Ruminants on the palatability of a robot feed in the milking system

**TRIAL DETAILS**

- **Testimonial of a compound feed producer in Germany about:**
  Effect of CRINA® Ruminants-compound feed on the palatability and feed intake in the robot milking system.

- **OBJECTIVE**

  - Field experiences on 3 dairy farms, CRINA R, used as a top dressing in compound feed
  - 240, 350, 280 Holstein dairy cows
  - Ø 10000 kg / cow and year
  - Daily ration
    TMR: corn silage, grass silage, hay rape seed, soya, wheat, barley, DDGS
  - Robot feed: 4 kg, 5 kg compound feed

- **Treatments**
  - CRINA® Ruminants Matrix-Value 1 g/cow/day
  - Results compared with the situation before

- **RESULTS**

  **Composition of the feed**

<table>
<thead>
<tr>
<th>Content</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude ash %</td>
<td>6.00</td>
</tr>
<tr>
<td>Crude protein %</td>
<td>20.00</td>
</tr>
<tr>
<td>Crude fat %</td>
<td>4.20</td>
</tr>
<tr>
<td>Crude fiber %</td>
<td>6.00</td>
</tr>
<tr>
<td>Calcium %</td>
<td>0.79</td>
</tr>
<tr>
<td>Phosphor %</td>
<td>0.50</td>
</tr>
<tr>
<td>Energy level MJ NEL/ kg</td>
<td>7.20</td>
</tr>
<tr>
<td>nXP g / kg</td>
<td>190.00</td>
</tr>
<tr>
<td>RNB g / kg</td>
<td>1.60</td>
</tr>
</tbody>
</table>

- **Visits of robot**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CRINA Ruminants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visits</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Visits of robot</td>
<td>2.5</td>
<td>3</td>
</tr>
</tbody>
</table>

- **Palatability:** 5 to 12 % higher feed intake of compound feed in the milking robot with CRINA R.

- **Performance:** Higher milk yield in the CRINA Ruminants group depending on DMI 0,5 - 1,7 kg/ cow & day by tendency

- **Visits:** 10 to 20 % more visits in the milking system, depending on the DMI

**CONCLUSIONS**

The inclusion of CRINA Ruminants at 1g/ cow & day results in:

- Robot feed: ROI > 10:1
- + 10 to 20 % more visits
- + 0,5 to 1.7 kg more milk/ cow & day
CRINA® Ruminants
Belgium (2008) - Milk production

- Treatment: CRINA Ruminants was supplemented between 18/05/08 to 20/07/08 (2 months) after adaptation on grazing (14 days).
- CRINA was supplemented as dilution 50g/cow/day (corresponding to 1 g CRINA Ruminants), together with the concentrate in the milking robot (min 2 times/day).
- Amount of concentrate was given according to milk production, with a minimum of 1 kg/cow/day.
- Annual milk production: 8500-9000 kg/cow/year.

<table>
<thead>
<tr>
<th></th>
<th>Control average</th>
<th>Crina® Ruminants average</th>
</tr>
</thead>
<tbody>
<tr>
<td># cows</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>DIM (d)</td>
<td>145 ± 89</td>
<td>139 ± 81</td>
</tr>
<tr>
<td>parity</td>
<td>2,6 ± 1,4</td>
<td>2,4 ± 1,2</td>
</tr>
<tr>
<td>milk production (kg)</td>
<td>28,4 ± 6,8</td>
<td>28,5 ± 5,3</td>
</tr>
<tr>
<td># cows &lt; 50 DIM</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td># primiparous cows</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
CRINA® Ruminants
Belgium (2008) - Milk production

milk production during the trial

<table>
<thead>
<tr>
<th>Date</th>
<th>Average Milk Production (kg/d) Control</th>
<th>Average Milk Production (kg/d) Crina Ruminants®</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.05.2008</td>
<td>25.9</td>
<td>25.8</td>
</tr>
<tr>
<td>20.05.2008</td>
<td>25.8</td>
<td>25.9</td>
</tr>
<tr>
<td>20.05.2008</td>
<td>25.9</td>
<td>26.6</td>
</tr>
<tr>
<td>20.05.2008</td>
<td>26.6</td>
<td>27.3</td>
</tr>
<tr>
<td>20.05.2008</td>
<td>27.3</td>
<td>26.7</td>
</tr>
<tr>
<td>20.05.2008</td>
<td>26.7</td>
<td>26.7</td>
</tr>
<tr>
<td>20.05.2008</td>
<td>26.7</td>
<td>24.4</td>
</tr>
<tr>
<td>20.05.2008</td>
<td>24.4</td>
<td>24.1</td>
</tr>
<tr>
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<td>24.1</td>
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</tr>
<tr>
<td>20.05.2008</td>
<td>24.1</td>
<td>23.6</td>
</tr>
<tr>
<td>20.05.2008</td>
<td>23.6</td>
<td>22.9</td>
</tr>
<tr>
<td>20.05.2008</td>
<td>22.9</td>
<td></td>
</tr>
</tbody>
</table>
CRINA® Ruminants
Belgium (2008) - Milk components

**Effect of Crina Ruminants on Milk Protein Production (g/day)**

- Control: 849 g/day
- Crina Ruminants ®: 889.5 g/day

+ 40 g/d

**Effect of Crina Ruminants on Milk Fat Production (g/day)**

- Control: 982 g/day
- Crina Ruminants ®: 1036.3 g/day

+ 54 g/d
Presentation outline

- Overview
  - Essential oils
- Mode of Action
- Animal performances (Dairy)
  - Milk Yield
  - Frequent visits to robot milking system
- Animal performances (Beef)
  - Higher daily weight gain
  - Feed efficiency
- Dosage - Recommendations
## Supplementation Guidelines

<table>
<thead>
<tr>
<th>Animal Type</th>
<th>Weight (kg)</th>
<th>Supplementation (mg/hd/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy cows</td>
<td></td>
<td>1 g/hd/d</td>
</tr>
<tr>
<td>Beef cattle</td>
<td>100 kg BW</td>
<td>150 - 200 mg/hd/d</td>
</tr>
<tr>
<td></td>
<td>200 kg BW</td>
<td>300 - 400 mg/hd/d</td>
</tr>
<tr>
<td></td>
<td>400 kg BW</td>
<td>600 - 800 mg/hd/d</td>
</tr>
<tr>
<td></td>
<td>600 kg BW</td>
<td>900 - 1200 mg/hd/d</td>
</tr>
<tr>
<td>Sheep, goats</td>
<td></td>
<td>100 mg/hd/d</td>
</tr>
</tbody>
</table>

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[ DSM logo ]

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