Organic Acids and Essential Oils

A viable alternative to antibiotic growth promoters in poultry production

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Jefo
Summary

• We better understand the mode of action of organic acids and essential oils on bacteria

• There is a strong synergy between these two compounds

• In vitro and in vivo experiments were conducted showing microencapsulation can substantially reduce the dose rate while improving performance
Introduction

• In late 80’s and early 90’s, antibiotic growth promoters (AGP’s) were being banned in the European Union market

• The last ones were removed January 1996

• Many different compounds have been tried as replacements

• A consensus developed among scientific community (Rosen 2005)
  - Organic acids and ‘botanicals’ or essential oils are topping the list as viable alternatives
Organic Acids

- Organic acids have been used successfully in pig production for over 30 years
- They are efficacious in poultry provided they are adapted to the physiology of poultry
- It was thought their mode of action was pH reduction of the GIT
- Research has proven differently
- The mode of action of organic acids on bacteria is that non-dissociated organic acids can penetrate the bacteria cell wall and disrupt their normal physiology
- Examples of pH sensitive bacteria are *E. coli*, *Salmonella sp.*, *C. perfringens*, *Listeria monocytogenes*, *Campylobacter sp.*
Organic Acids

- Upon diffusion of the Organic Acid into the bacteria, where pH is near neutral, the acids dissociate and lower the internal pH

- This triggers mechanisms that will impair or stop bacteria growth

- The anionic portion of the acid, after dissociation, accumulates in the bacteria, increases osmotic pressure, impairing the bacteria

- It is important that the acids are in their non-dissociated form, as it requires 10-100 times less dosage than a dissociated acid (Presser et al, 1997)
The antimicrobial properties of organic acids

Bactericidal effect:
- Energy exhaustion
- Cellular death

Bacteriostatic effect:
- Accumulation of the carboxyl group
- Toxic effect

Adapted from R.J. LAMBERT and M. STRATFORD, Journal of Applied Microbiology 86, 157-154, 1999
Undissociated and dissociated organic acids have different Minimum Inhibitory Concentration (MIC)

Example of sorbic acid (Eklund in Thuault, 1995)

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>MIC (ppm) undissociated</th>
<th>MIC (ppm) dissociated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis ATCC 6633</td>
<td>2.8</td>
<td>190</td>
</tr>
<tr>
<td>Bacillus subtilis W 23</td>
<td>2.6</td>
<td>190</td>
</tr>
<tr>
<td>Bacillus cereus JSM 103</td>
<td>1.2</td>
<td>110</td>
</tr>
<tr>
<td>Escherichia coli ML 308-225</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 9027-9</td>
<td>10</td>
<td>130</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>0.6</td>
<td>400</td>
</tr>
<tr>
<td>Candida albicans JSM 101</td>
<td>2.0</td>
<td>20</td>
</tr>
</tbody>
</table>
Organic Acids

• Most organic acids are efficacious at low ph of 3.0 - 3.5

• This does not reflect the GIT of poultry

• If acids are unprotected, they will dissociate through the crop and intestine

• Protected acids deliver the non-dissociated acids to GIT where the bulk of bacteria is located, thereby reducing some pathogenic bacteria (ex. *C. perfringens*)

• They also control the population of bacteria that compete with the bird for nutrients
pH and mean duration of transit time of mash feed in different compartments of the broiler gut after ad libitum feeding for 6 weeks

<table>
<thead>
<tr>
<th>GIT compartment</th>
<th>Duration of transit time (min.)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop</td>
<td>50</td>
<td>5.5</td>
</tr>
<tr>
<td>Proventriculus &amp; gizzard</td>
<td>90</td>
<td>2.5 - 3.5</td>
</tr>
<tr>
<td>Duodenum</td>
<td>5-8</td>
<td>5 - 6</td>
</tr>
<tr>
<td>Jejenum</td>
<td>20-30</td>
<td>6.5 - 7</td>
</tr>
<tr>
<td>Ileum</td>
<td>50-70</td>
<td>7 - 7.5</td>
</tr>
<tr>
<td>Rectum</td>
<td>25</td>
<td>8</td>
</tr>
</tbody>
</table>

Simons & Versteeg, 1989 in Vanbelle M.
Essential Oils

- A class of volatile oils extracted from plants used primarily in flavors and pharmaceuticals
- They can be used as appetite or saliva stimulants and aroma enhancers
- These factors are not important for poultry
- Most essential oils are GRAS (generally recognized as safe), but they can be toxic and their odor and taste may contribute to feed refusal (Lis-Balchem 2003, Lambert et al. 2001)
- They are volatile leading to variation in concentration in the final feed
- Encapsulation of essential oils could solve the problem (Lis-Balchem 2003)
Encapsulated Organic acids & Essential Oils

- To protect the organic acids and essential oils from the chemical environment of the digestive tract, a patented vegetable hydrogenated triglyceride matrix-type protection is used.
Synergistic Effect

• The cytoplasmic membrane of bacteria has two principal functions (Ultee et al. 1999); a barrier function and energy transduction, which allow the membrane to form ion gradients that can be used to drive various processes and the formation of a matrix for membrane-embedded proteins influencing the ATP-synthase complex.

• Combining essential oils and organic acids is proving efficacious (van Wesel et al. 2004) because there appears to be a synergy between the two concepts (van Kol 2005, van Dam et al. 2005)

• Essential oils
  - increase the permeability of cell membrane allowing organic acids to diffuse easily
  - influence the ATPase H+ pump
The antimicrobial properties of organic acids and essential oils

EO increase the membrane permeability:
- Facilitation of the entry of the organic acids
- Cellular losses of components

Loss of components: cellular death

Essential oils also act:
- By disturbing the enzymatic system of the bacteria
- By inactivating the DNA aggregate
In vitro synergy between acids and essential oils

Trial conducted at a pH of 6.5
Protection Efficacy

- Concerns with Organic Acids = corrosion, worker safety, vitamin stability, etc.

- Protecting Organic acids and Essential Oils in a triglyceride matrix can dramatically reduce the dose required for maximum performance

- Without protection, organic acids are readily dissociated in the first part of the GIT and rendered useless (Dibner et al. 2002)

- Essential oils are rapidly absorbed in the duodenum and cannot interact with the microflora (Lee et al. 2004)

- The active ingredients can be delivered to the intestine (Piva et al. 2004)
GI slow-release and microbial studies in broilers

CERZOO (Italy) Oct. 20, 2005 - Dec. 02, 2005

Experimental Trial

- 240 male broilers, 20 birds/pen, 6 pens/treatment
  - TREATMENT 1: Control
  - TREATMENT 2: Protected Organic Acids & Essential Oils
- Initial weight (hybrid ROSS 508) 37 g
- Three-phase diet was fed as follows: 1-10, 11-21 and 22-42 days
- In the 3-phase diet, a coccidiostat (Elancoban) was included.
- At the end of the trial 12 broilers (6 animals from each treatment) were sacrificed and the contents of gizzard, small intestine, and ceca were collected. Each sample was analyzed by HPLC-MS for organic acids and essential oils.
GI slow-release and microbial studies in broilers

Slow-release study: SORBIC ACID along the GIT

![Graph showing concentration (mmol/L) for Gizzard, Small intestine, and Ceca for Control and Gallinat+™ groups.](image-url)
GI slow-release and microbial studies in broilers

Slow-release study: essential oils along the GIT

![Graph showing concentration of essential oils in different parts of the GIT]
GI slow-release and microbial studies in broilers

Microbial study: summary

**Coliforms**

- Gizzard Coliforms
- Small intestine Coliforms

- CTR
- Gallinat+™

- $n = 6$

**Lactobacilli**

- Gizzard LAB
- Small intestine LAB

- CTR
- Gallinat+™
Necrotic enteritis challenge model
Coccidiosis lesions, a predisposing factor in necrotic enteritis
Necrotic enteritis lesions
Necrotic enteritis challenge trial

- Table 1. Effect of essential oils and essential oils-organic acid combination on production parameters, no challenge farm trial, broiler chickens, 28 days of age, P<0.05 (Jefo Nutrition internal data).

<table>
<thead>
<tr>
<th></th>
<th># chickens</th>
<th>BW (g)</th>
<th>ADG (g)</th>
<th>FCR</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>1198</td>
<td>1.406 a</td>
<td>48.70 a</td>
<td>1.605 a</td>
<td>3.83</td>
</tr>
<tr>
<td>Essential Oils</td>
<td>1200</td>
<td>1.367 b</td>
<td>47.31 b</td>
<td>1.621 a</td>
<td>3.00</td>
</tr>
<tr>
<td>OA/EO 1</td>
<td>1198</td>
<td>1.436 c</td>
<td>49.79 c</td>
<td>1.557 b</td>
<td>2.59</td>
</tr>
<tr>
<td>OA/EO 2</td>
<td>1200</td>
<td>1.467 d</td>
<td>50.90 d</td>
<td>1.560 b</td>
<td>3.67</td>
</tr>
</tbody>
</table>
Conclusion - A viable alternative

• Protected organic acids have shown consistent results with over 90% of trials showing positive response for weight gain and feed conversion.

• They can act as growth promoters.

• They play a role in reducing necrotic enteritis.

• They help reduce intestinal *Salmonella sp.*

• Encapsulation appears to be key to viable efficacy at low dose levels.
Questions?

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Thank you.