Challenges and new tendencies in use of lipids for animal nutrition

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Main uses of lipids in animal nutrition

• Crude oils
• Refined oils
• Blends of oils
• Rendering co-products (animal origin): animal fats, fish oil

• Co-products (vegetable origin):
  acid oils / distilled fatty acids from chemical or physical refining of food grade oils - FFA 20% - 30% ….90% (PFAD)

  ❖ used in feeds as energetic supplement:
  (competitive prices – lower nutritional energetic level than crude oils)

  ❖ used to produce “high tech lipids”
Main uses of lipids in animal nutrition

Co-products (vegetable origin):
acid oils / distilled fatty acids from chemical or physical refining of food grade oils - FFA 20% - 30% ….90% (PFAD)

+Ca: fatty acid calcium soaps (monogastric; principally ruminants: enrichment of meat, milk with PUFA, Omega 3; CLA source for animal diets)

+H: Totally Hydrogenated fats (ruminants; encapsulation/protection of SCFA, organic acids, essential oils, amino-acids)

+ Glycerol:
  ➢ esterified / randomized oils (energetic supplement)
  ➢ mono-diglycerides: (emulsifying agents – antimicrobial agents)
**Esterified / Randomized oils**

**GLYCEROL**
- increasing biodiesel production
- availability of glycerol in large quantities, at stable competitive prices
- price of glycerol is now 10 times lower than 5 years ago

**ACID OILS / DISTILL. FATTY ACIDS**
- deriving from chemical / physical refining of food grade oils
- price of acid oils/distilled fatty acids are lower (up-to by 50%) than prices of crude oils

Combination of these 2 ingredients = Esterified / Randomized oils having:
- fatty acid composition, FFA, MIU = crude oils
- lower prices than crude oils
- digestibility of saturated fatty acids in randomized oils is higher than in crude oils due to the placement of palmitic and stearic acid in the Pos. 2 of the triglyceride
Digestion of the Triglycerides.

In the first intestinal tract lipases break the bonds fatty acid/glycerol in pos. 1 and 3. 2 free fatty acids and 1 monoglyceride with the fatty acid in pos. 2 are obtained.

Monoglyceride with the Fatty Acid in pos. 2 Free Fatty acids

Glycerol

Palmitic Acid

Oleic Acid

Oleic Acid

Lipases

Pancreas
Digestion of the Triglycerides.

Monoglycerides are emulsifying substances which form micelles with bile salts and unsaturated fatty acids, performing the absorption of the lipidic substance, while saturated fatty acids form with the calcium “soaps” and are expelled with the faeces.
Digestion of the Triglycerides.

If palmitic acid is in the pos. 2 of the triglyceride, it is absorbed as monoglyceride
Digestion of the Triglycerides.

Micelles

Palmitic Acid

Oleic Acid

Bile Salts

Palmitic Acid is absorbed
Advantages of randomized oils

W. Smink, Fatty acid digestion and deposition in broiler chickens fed diets containing either native or randomized palm oil. Poultry Science 87 - (2008).

Digestion of Palmitic acid, %

Digestion of Stearic acid, %
Advantages of randomized oils

Sheila M. Innis, “Formula containing randomised fats with palmitic acid (16:0) in the 2-Position increases in the 2-position of plasma and chylomicron triglyceride in Formula-fed piglets to levels approaching those of piglets fed Sow’s milk”– Journal of Nutrition 1997

- Sows milk fatty acids contain 20-30% palmitic acid
- approxim. 70% is bound to the sn-2 position of the milk triacylglycerol
- randomized oils with palmitic acid in pos.2 (33%) in piglets can significantly improve the weight gain per litre of ingested feed if compared with non-randomized oils

Growth performance results at 18 days of piglet fed randomised or non randomised lipid supplement from birth

<table>
<thead>
<tr>
<th>Co-randomized palm olein and canola oil 50</th>
<th>Native canola + palm oil 50/50</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain per each litre of ingested feed / kg</td>
<td>0.214</td>
<td>0.192</td>
</tr>
</tbody>
</table>
Energetic values of crude and randomized oils

Calculated according to Wiseman Equation and considering that 33% of saturated FA are placed in pos. 2 in randomized oils

<table>
<thead>
<tr>
<th>OIL</th>
<th>AME Kcal/kg Broiler &gt; 21 d</th>
<th>OIL</th>
<th>AME Kcal/kg Broiler &gt; 21 d</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized Soybean oil</td>
<td>9,237</td>
<td>Soybean oil</td>
<td>9,069</td>
<td>1,8%</td>
</tr>
<tr>
<td>Randomized Coconut oil</td>
<td>8,840</td>
<td>Coconut oil</td>
<td>8,469</td>
<td>4,4%</td>
</tr>
<tr>
<td>Randomized Palm oil</td>
<td>8,405</td>
<td>Palm oil</td>
<td>7,978</td>
<td>5,35%</td>
</tr>
</tbody>
</table>
Monoglycerides enhancing lipids digestibility

GLYCEROL
- increasing biodiesel production
- availability of glycerol in large quantities, at stable competitive prices
- price of glycerol is now 10 times lower than 5 years ago

ACID OILS / DISTILL. FATTY ACIDS
- deriving from chemical / physical refining of food grade oils
- price of acid oils/distilled fatty acids are lower (up-to by 50%) than prices of crude oils

Combination of these 2 ingredients = monoglycerides of fatty acids
- Medium Chain and Long Chain fatty acid Monoglycerides are highly digestible, also by young animals
- Unsaturated Long Chain Monoglycerides (of oleic, linoleic, linolenic acid) significantly enhance the digestibility and utilization of saturated saturated fatty acids such as palmitic and stearic acids

The efficacy of oleic acid in enhancing palmitic acid absorption has been compared with that of monoglycerides of oleic or linoleic acid.

<table>
<thead>
<tr>
<th>Lipid mixture</th>
<th>Unsaturated lipid</th>
<th>Palmitic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (P)</td>
<td></td>
<td>- 3 +/- 3,6</td>
</tr>
<tr>
<td>Oleic acid + P (1)</td>
<td>(2) 67 +/- 0,7</td>
<td>11 +/- 0,9</td>
</tr>
<tr>
<td>1-Monoglyc. of oleic acid + P (1)</td>
<td>(2) 99 +/- 2,5</td>
<td>28 +/- 3,1</td>
</tr>
<tr>
<td>2- Monoglyc. of oleic acid + P (1)</td>
<td>(2)100 +/- 1,2</td>
<td>24 +/- 0,7</td>
</tr>
<tr>
<td>1-Monoglyc. of linoleic acid + P (1)</td>
<td>(2) 96 +/- 0,7</td>
<td>24 +/- 1,0</td>
</tr>
</tbody>
</table>

(1) Molar ratio of unsaturated lipids to palmitic acid: 0.8:1
(2) Mean ±SE of three individually caged cockerels with ligated pancreatic ducts.
Monoglycerides: effects on lipid digestion

University of Florence, prof. Mauro Antongiovanni
<<The effect of mono-and diglycerides of Olive oil on the absorption of Distilled Free Fatty Acids in broiler chickens>> - 2002
Monoglycerides with antimicrobial effects

**GLYCEROL**
- increasing biodiesel production
- availability of glycerol in large quantities, at stable competitive prices
- price of glycerol is now 10 times lower than 5 years ago

**MEDIUM CHAIN FATTY ACIDS**

& **SHORT CHAIN FATTY ACIDS**

Combination of these 2 ingredients = monoglycerides of Medium Chain and Short Chain fatty acid having antimicrobial effects
**Monoglycerides: antimicrobial effects**

*Fatty acids and derivates as antmicrobial agents*

JON J.KABARA,
Department of microbiology - Michigan State University - 1972

MIC* comparison of free acid form with glyceride form

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pneumococci</th>
<th>Streptococcus group A</th>
<th>Streptococcus beta-hemolytic Non-A</th>
<th>Corynebacterium</th>
<th>Nocardia asteroides</th>
<th>Micrococcus</th>
<th>Candida</th>
<th>S.aureus</th>
<th>S.Epidermidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capric acid</td>
<td>1.45</td>
<td>1.45</td>
<td>2.9</td>
<td>1.45</td>
<td>1.45</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>1-Monocaprin</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
<td>0.1</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1,3 Dicaprin</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>Lauric acid</td>
<td>0.062</td>
<td>0.124</td>
<td>0.249</td>
<td>0.124</td>
<td>0.124</td>
<td>0.624</td>
<td>2.49</td>
<td>2.49</td>
<td>2.49</td>
</tr>
<tr>
<td>1-MonolaurIn</td>
<td>0.09</td>
<td>0.045</td>
<td>0.09</td>
<td>0.045</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>1,3 Dilaurin</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>Trilaurin</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
</tbody>
</table>

*Results are given in micromoles per milliliter. NI = no inhibition at the concentrations tested*
Fatty acids and derivates as antimicrobial agents

JON J. KABARA,
Department of microbiology - Michigan State University - 1972

MIC* comparison of free acid form with glyceride form

Monoglycerides can have a stronger antibacterial action than the relevant fatty acids!

In this study Monolaurin proved to be by 27 times more effective than Lauric acid against Candida and Streptococcus Aureus (see previous slide)!
MC-Monoglycerides: antimicrobial effects in vitro

H. Thormar, H. Hilmarsson <<Stable concentrate emulsion of the one Monoglyceride of Capric Acid (Mononocaprin) with microbicidian activities against the food borne bacteria Campylobacter Jejuni, Salmonella ssp., and Esherichia Coli >>— (2006)

Inactivation of 2 strains of Campylobacter d. in log 10, mixed with 10 mM of acids or monoglycerides, for 10 minutes, 37 °C
Monoglycerides: antimicrobial effects in vitro

10 mM both Capric acid and Monocaprin were active

2.5 mM (10 min) Capric acid did not show any activity

2.5 mM (1 min) Monocaprin was fully active

1.25 mM (10 min) Monocaprin was fully active

From these results it was concluded that the Monocaprin has the highest microbicidal activity against Campylobacter jejuni

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**TABLE 1.** Anticampylobacter activities of dilutions of different preconcentrated MC emulsions with or without Tween 40 stored at room temperature for various lengths of time

<table>
<thead>
<tr>
<th>Preconcentrated emulsion</th>
<th>Time in storage (mo)</th>
<th>Conc after dilution (log_{10} CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 mM MC</td>
<td>12</td>
<td>1.25 mM</td>
</tr>
<tr>
<td>200 mM MC</td>
<td>9.5</td>
<td>1.25 mM</td>
</tr>
<tr>
<td>200 mM MC</td>
<td>2</td>
<td>1.0 mM</td>
</tr>
<tr>
<td>200 mM MC-0.8% TW40</td>
<td>17</td>
<td>1.25, 0.005%</td>
</tr>
<tr>
<td>200 mM MC-0.8% TW40</td>
<td>12.5</td>
<td>1.25, 0.005%</td>
</tr>
<tr>
<td>200 mM MC-0.8% TW40</td>
<td>7</td>
<td>1.0, 0.004%</td>
</tr>
</tbody>
</table>

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*a* Final concentration(s) after the addition of bacteria (1:1). Concentrations of MC and TW40 are given in millimolar and percentages, respectively.

*b* Compared with count of bacteria mixed 1:1 with the control solution.

*c* After 160-fold dilution (1.25 mM [0.03%] MC).

*d* After treatment for 10 min at room temperature.

*e* After treatment for 1 min at room temperature.

*f* After 200-fold dilution (1.0 mM [0.024%] MC).
36-d old broilers from a Campylobacter positive flock were received at the time of slaughter and divided in 2 groups of 5 birds each; the following day cloacal swabs were collected, and 1 group was then started on a treatment with 10 mM of Monocapryn (0,24%) + 0,04% of polysorbate for 3 days (administered in water and in feed) 2 trials, A & B

Table 2. Campylobacter counts in cloacal swabs of chickens naturally infected with Campylobacter and treated for 3 d with 10 mM (0.24%) monocaprin and 0.04% polysorbate 40 emulsion added to their drinking water and feed

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Day</th>
<th>Treated group (log_{10} cfu/mL)</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>7.2 ± 0.4</td>
<td>6.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.4 ± 1.3</td>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>4.7 ± 0.8</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.2 ± 0.3</td>
<td>5.4 ± 0.3</td>
</tr>
</tbody>
</table>

1The control group was not treated.
2Mean for 5 cloacal swabs ± SD.
3Significantly (P < 0.05) less than the control group on d 3 and significantly (P < 0.05) less than the treated group before beginning of treatment on d 0.
4Significantly (P < 0.01) less than the control group on d 3 and significantly (P < 0.05) less than the treated group before beginning of treatment on d 0.
The study included 4 experiments. Here below some conclusions of the authors are reported:

• The treatment with monocaprin in water and feed did not prevent spread of Campylobacter from artificially infected to noninfected 24-d-old chickens;

• The average weight increase of 2 treated groups during the 3 d of treatment was 13.6% compared with 19.6% for the untreated control groups. This may suggest a slight reduction in growth rate due to the concentration of 10 mM (0.24 %) monocaprin in their drinking water and feed (palatability)

• but Campylobacter counts in cloacal swabs were significantly reduced, particularly during the first 2 d of treatment. There was a significant reduction in the Campylobacter counts in cloacal swabs of naturally infected 36-d-old broilers that were treated for 3 d prior to slaughter.

• Further studies are needed to determine whether this would reduce carcass contamination.
Expression of the virulence genes hilA of Salmonella Typhimurium grown in acidified LB-broth, relative to Salmonella Typhimurium grown in LB-broth


SMCFA: antimicrobial effects in vitro
Invasion rate in the porcine intestinal epithelial cell line IPI 21 of Salmonella Typhimurium


**SMCFA: antimicrobial effects in vitro**

Invasion rate in the porcine intestinal epithelial cell line IPI 21 of Salmonella Typhimurium
M. Mahu, F. Boyen, R. Ducatelle, F. van Immerseel
Coated fatty acids alter virulence properties of Salmonella Typhimurium and decrease
intestinal colonization of pigs - (2008)

**Salmonella counts in feaces of 6 weeks old piglets fed supplemented diets after oral inoculation with Typhimurium – 7*10^7**

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unsupplemented feed</strong></td>
<td>![Graph](Unsupplemented feed)</td>
<td>![Graph](Day 2)</td>
<td>![Graph](Day 3)</td>
</tr>
<tr>
<td><strong>Coated butyric acid</strong></td>
<td>![Graph](Day 2)</td>
<td>![Graph](Day 3)</td>
<td>![Graph](Day 3)</td>
</tr>
<tr>
<td><strong>Coated caprylic acid (C8)</strong></td>
<td>![Graph](Day 2)</td>
<td>![Graph](Day 3)</td>
<td>![Graph](Day 3)</td>
</tr>
</tbody>
</table>

The group receiving coated butyric acid showed a strong trend (p = 0.082), of decreased Salmonella shedding at the first 3 days after inoculation. Faecal shedding was approximately 100 times lower in the group fed coated butyric acid compared to the control group. Coated caprylic acid did not significantly (p = 0.89) reduce fecal shedding of Salmonella Typhimurium.
SMCFA: antimicrobial effects in vivo

Comments to previous slides

Despite the relatively low MIC values at low pH, a direct antimicrobial effect of these acids in the intestines is not expected. In order to achieve a direct antimicrobial effect in the porcine gut to combat Salmonella Typhimurium, quite high concentrations of SCFA (160 mM at pH 6) and MCFA (40 mM at pH 6) are needed.

Depending on the used feed, concentrations of butyric acid in the porcine caecum contents vary around 10 mmol/kg (10 mM) (Mikkelsen et al., 2004). Therefore, fatty acid concentrations currently used in supplemented feed (10–30 mmol/kg feed) will not be able to increase the intraluminal concentrations to antimicrobial concentrations (160 mM at pH 6).

IN VITRO, SCFA and MCFA were shown to have an indirect effect on Salmonella pathogenicity. Even non-bacteriostatic concentrations as low as 2 mM for caproic or caprylic acid and 10 mM for butyric and propionic acid considerably decreased virulence gene expression and epithelial cell invasion by Salmonella Typhimurium.
SMCFA: antimicrobial effects in vivo

M. Mahu, F. Boyen, R. Ducatelle, F. van Immerseel

Coated fatty acids alter virulence properties of Salmonella Typhimurium and decrease intestinal colonization of pigs - (2008)

IN VIVO, MCFA, coated caprylic acid, was not able to control/decrease salmonella counts in feces: at day 3 after infection the salmonella counts was almost the same of control group

Protected butyric acid, even if at a low concentration, proved to be able to control salmonella proliferation. Faecal shedding was approximately 100 times lower in the group fed coated butyric acid compared to the control group

This means that an increase of only a few mM butyric acid in the gut contents could result in reaching the threshold concentration for activation of the indirect effect of the SCFA.
Microencapsulated Short-Chain Fatty Acids and Salmonella challenge

Ghent University Belgium Faculty of Veterinary Medicine

**Broiler trial:**
- 5*4 groups of 20 chickens
- feed supplementation:
  - control: no supplement
  - 2. acetic acid 0.24% (microencapsulated)
  - 3. formic acid 0.22% (microencapsulated)
  - 4. propionic acid 0.27% (microencapsulated)
  - 5. butyric acid 0.15% (microencapsulated)
- challenge with 5*10³ cfu S. Enteritidis at day 5 & 6 posthatch

Van Immerseel et al., 2004 Poultry Science 83:69–74
Colonization of the caeca at day 8 after inoculation (10^3 cfu S.enteritidis) at day 5 & 6 posthatch, 5*4 groups of 20 chickens

Van Immerseel, Ducatelle- 2004 Poultry Science 83:69–74

SCFA: antimicrobial effects in vivo
Severity of infection
mean log colony-forming units per gram in caeca

Van Immerseel et al., 2004 Poultry Science 83:69–74

Van Immerseel, Ducatelle- 2004 Poultry Science 83:69–74

SCFA: antimicrobial effects in vivo
In this study, it was demonstrated that:

“butyrate down-regulated the expression of 19 genes common to both serovars S.enteritidis and S.typhimurium by a factor of twofold or more, and 17 of these genes localized to the Salmonella pathogenicity island 1 (SPI1)”

Ghent University Belgium
Faculty of Veterinary Medicine
In a preliminary study recently conducted by the Public Animal Health Institute Forlì (Italy) with Salmonella Typhimurium in culture broth, it was proved that monobutyrin (butyric acid monoglyceride) at a concentration of 1.24 mM, was ten times more effective in reducing Salmonella Typhimurium CFU than butyric acid.

<table>
<thead>
<tr>
<th>Product</th>
<th>Tested concentration</th>
<th>pH</th>
<th>Salmonella Typhimurium cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td></td>
<td>7</td>
<td>120 $\times 10^5$</td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
<td>4.5</td>
<td>96 $\times 10^5$</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>1.24 mM</td>
<td>7</td>
<td>65 $\times 10^4$</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>1.24 mM</td>
<td>4.5</td>
<td>25 $\times 10^3$</td>
</tr>
<tr>
<td>Monobutyrin</td>
<td>1.24 mM</td>
<td>7</td>
<td>74 $\times 10^3$</td>
</tr>
<tr>
<td>Monobutyrin</td>
<td>1.24 mM</td>
<td>4.5</td>
<td>32 $\times 10^2$</td>
</tr>
</tbody>
</table>
Monoglycerides of short-chain fatty acids, such as monoprionin and monobutyrin, show a particular chemical behaviour: unlike monoglycerides of MCFA (Monocaprin, Monocaprylin, which have a longer chain) they are hydrodispersible without emulsifying agents.

An hypothesis is that, due to this characteristic, the expected antibacteric action in vivo is not reduced due to partial inactivation through solubilization in lipid fractions (triglycerides) as commonly contained in feeds.

This allows these new molecules to be active in several different environments: water, feed, stomach and intestinal tract.

Monoglycerides of SCFA seem to open new prospects in the fight against pathogenic bacteria.