Handling and Use of Glycerin in Feed

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Objectives of Discussion

• Explain why feed manufacturers have interest in bio-diesel derived glycerin

• Describe how glycerin impacts the physical characteristics, nutritional properties and palatability of feeds

• Describe the developmental process we have used in evaluation of this material as a possible component of our products in terms of:
  – Approved supplier process/ Characterization of available products/ Evaluation of possible contaminants
  – Development of realistic specifications as an ingredient
  – Development of Standard operating procedures for this material
  – Internal evaluation of safety of various sources for use in feed
Why Glycerin?

Why Now?

Supplies of glycerin have grown dramatically with increases in bio-diesel production. This has saturated saturating historic uses. Until such time as additional applications are developed-glycerin will compete with traditional energy feeds on a price basis.

Business synergies exists between feed industry and bio-diesel industries.
Why Glycerin?

• Glycerin’s unique physical properties that make it attractive as a component of liquid feed supplements:
  – Highly water soluble.
  – Melting point of 64 degrees F if absolutely pure but when small amounts of water are present it remains fluid at temperatures near zero.
  – Mild pleasant aroma
  – Sweet Taste
  – Near Neutral ph- typical 5 to 7
  – Highly palatable
  – Decreases the viscosity of molasses and other liquid by-products
  – Hydroscopic-attracts moisture-which can help prevent feeds from “drying out” at low humidity.
Moisture Content of Crude Glycerin By Producer

Values shown in bars represent number of samples analyzed
Primary Ash Constituents in Crude Glycerin from Different Producers

- **Potassium**
- **Sodium**
- **Sulfur**
- **Chloride**
- **Ash**

<table>
<thead>
<tr>
<th>Producer</th>
<th>Potassium</th>
<th>Sodium</th>
<th>Sulfur</th>
<th>Chloride</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
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<td>C</td>
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<tr>
<td>H</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Methanol Content in Crude Glycerin by Producer

Values shown in bars represent number of samples analyzed
Glycerin Content in Crude Glycerin as a % of Organic Matter by Producer

Values shown in bars represent number of samples analyzed
Highly Toxic Heavy Metals in Crude Glycerin

![Bar chart showing heavy metal concentrations in ppm]

- Cadmium: 0.5 ppm
- Mercury: 2 ppm
- Selenium: 0.3 ppm

Legend:
- A
- B
- C
- D
- E
- G

Max Tol. In Complete Feeds
Toxic Heavy Metals in Crude Glycerin

The graph shows the concentration of Barium and Lead in various feed samples. The maximum tolerance for these metals in complete feeds is indicated. The concentrations are as follows:

- **Barium**: The concentration ranges from 0 to 30 ppm with data points for different feed samples labeled A to G. Sample B shows a concentration of 20 ppm.
- **Lead**: The concentration ranges from 0 to 35 ppm with different feed samples. Sample G shows the highest concentration at 30 ppm.
Moderately Toxic Heavy Metals in Crude Glycerin

ppm

<table>
<thead>
<tr>
<th>Metal</th>
<th>Arsenic</th>
<th>Nickel</th>
<th>Silver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max Tol. In Complete Feeds</td>
<td>0.86</td>
<td>1.67</td>
<td>0</td>
</tr>
</tbody>
</table>

A | B | C | D | E | G
Slightly Toxic Heavy Metals in Crude Glycerin

Chromium Metal

ppm

Max Tol. In Complete Feeds

A
B
C
D
E
G

0.26 0.58 1000 ppm
Impact of Glycerin on Physical Properties of Textured Feed Conditioner

Glycerin Viscosity Comparison

- Control
- 5% Glycerin
- 4% Prop Glyco
- MgCl Solution and Glycol
- 4% MgCl Solution
- 10% Glycerin
- 10% MgCl Solution

Viscosity (cps) vs. Temperature (F)
Evaluation of Liquid Supplements Added to Low Quality Forage in Continuous Culture of Rumen Microbes ’06 West Virginia University

• Procedures
  – A poor quality hay was selected for the study so as to compare well to dormant season grazing pasture conditions. Hay contained 6.3% crude protein and 72.6% Neutral Detergent Fiber
  – Comparisons Included Hay alone vs. Conventional Liquid supplement – exclusively Molasses based (CLS), and Conventional Liquid Supplement containing Molasses + Glycerol (GLY)
  – Liquid supplements were added at 10 % Dry Basis to the Hay in treated cases in an in-vitro continuous culture system.
Evaluation of Liquid Supplements Added to Low Quality Forage in Continuous Culture of Rumen Microbes

Digestibility of Dietary Nutrients %

Results

– Dramatic impact on digestibility of dietary fiber (Neutral Detergent Fiber) was observed – Increased by a factor of nearly 8 times
– Digestibility of Non-Structural Carbohydrates (NSC) was high in all cases as expected
– Note TDN est = sum of digestible NDF, NSC and CP

![Bar Chart showing digestibility of dietary nutrients for different treatments: Hay Alone, Hay + CLS, and Hay + GLY. The chart indicates the percentage of digestibility for Neutral Detergent Fiber (NDF), Non-Structural Carbohydrates (NSC), Crude Protein, and Total Digestible Nutrients estimated (TDN est).]
Impact on Measured Parameters
Evaluation of Liquid Supplements (Conventional and 40% Glycerol)
Added to Medium Quality Forage

TK Miller, RFPL- WVA10-06

- Study was designed to compare impact replacing molasses with glycerin on dry basis in a conventional liquid supplement. Approximately 40% glycerin was included in the LFS with 12.5% molasses vs typical all molasses based LFS.

- Results
  - Dry matter digestibility not different
  - Ammonia content and yield of microbial Nitrogen not different
  - Total production of Volatile Fatty acids tended to be higher in glycerin supplement with amount of acetic acid lower and butyric acid higher
  - Ph data implies drop post feeding was less with the glycerin based feed. (next slide)

- Conclusion - replacement of molasses with glycerin should not impact digestive performance of liquid supplements.
Impact of Glycerin (40%) on rumen culture pH

![Graph showing the impact of Glycerin (40%) on rumen culture pH over time. The graph compares pH levels with and without Glycerin addition, highlighting the fluctuation and recovery process.](image-url)
TABLE 5. Effect of Liquid Supplement on Volatile Fatty Acid Production and pH

<table>
<thead>
<tr>
<th>Component</th>
<th>CLS</th>
<th>GLY</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total VFA</td>
<td>181</td>
<td>178</td>
<td>0.73</td>
</tr>
<tr>
<td>Acetic</td>
<td>121</td>
<td>107</td>
<td>0.06</td>
</tr>
<tr>
<td>Propionic</td>
<td>41</td>
<td>42</td>
<td>0.22</td>
</tr>
<tr>
<td>Iso-butyric</td>
<td>0.46</td>
<td>0.55</td>
<td>0.06</td>
</tr>
<tr>
<td>Butyric</td>
<td>16</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Isovaleric</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Valeric</td>
<td>2.4</td>
<td>2.4</td>
<td>0.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Molar %</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>67.1</td>
<td>59.9</td>
<td>0.0011</td>
</tr>
<tr>
<td>Propionic</td>
<td>22.5</td>
<td>23.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Iso-butyric</td>
<td>0.26</td>
<td>0.31</td>
<td>0.02</td>
</tr>
<tr>
<td>Butyric</td>
<td>8.8</td>
<td>15.0</td>
<td>0.0009</td>
</tr>
<tr>
<td>Isovaleric</td>
<td>0.0</td>
<td>0.0</td>
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</tr>
<tr>
<td>Valeric</td>
<td>1.3</td>
<td>1.4</td>
<td>0.79</td>
</tr>
</tbody>
</table>
CONSUMPTION STUDIES

Effect of adding USP glycerin to equine feed to determine if feed refusal is an issue:

Added 1% glycerin to normal ration. Fed eight horses - 1 colt, 4 Thoroughbred mares, 1 Arab mare, 1 Arab gelding, 1 Thoroughbred gelding. Ages range from 6 months to 29 yrs. Everything was cleaned up in normal time. Each horse took the first bite, chewed it up, and went back for the next with no hesitation. Bottom line is 1% glycerin caused no noticeable difference to the horses.

Consumption of high crude glycerin biodiesel origin content liquid supplement by pregnant beef cows confined in a dry lot: Two groups of beef cows were separated into pens in a dry lot. Both groups were offered a liquid supplement that contained 42.43% glycerin (formula shown below) and a diet consisting of cotton seed burrs and a 30% burr ration. One group was fed with a typical lick wheel feeder while the other group was fed using an open trough feeder. The trial period for the consumption study was 18 days. Diet and performance data are summarized below. As expected, the cattle consuming the liquid supplement from the open trough consumed more than the cattle exposed to the lick wheel feeder (10.6 vs. 7.0 lbs/hd/day). From this information it is ascertainable that feed refusal due to glycerin is not an issue. Further more the cows performed well on the product averaging 1.7 lbs/hd/day gain over the 18 day period.
Observation on -C- Cattle at Nolan County Feeders

Group 1  |  Group 2
-------:|:-------
103 Cows | 113 Cows
18 days  | 18 days

Batts
30% Batts Rations | 4.2 | 3.6

Liquid (Liquid Whole)* | 7.0 | 6.6
Total IBD/day | 28.2 | 28.2

Dry Matter IBD/day | 24.3 | 23.3

# Grainy | 1.7 | 1.7

Cows Description
Medium frame size, solid mouth, Brangus type cows
Central Texas sale barn origin, Average wt. 1075 lbs

Westway Experiment Blend
Methanol Considerations

- Human metabolism includes conversion to formaldehyde and then formic acid-formic acid responsible for toxic effects- in some species excretion via respiration and urine is documented

- CFR 573.460 permits use of formaldehyde in feeds wherein approximately 25 % of animals diet is comprised of a protein meal treated with up to 1% formaldehyde. This would equate to approximately 0.25% dietary formaldehyde. Molecular weight of formaldehyde is 30.03 and molecular weight of methanol is 32. .25*(32.04/30.03) = .2667 % Substituting methanol for formaldehyde and assuming 20% glycerin in diet would equate to .2667/20 or 1.333 % methanol in glycerin source

- CFR 573.480 describes use of formic acid in hay crop silages as a preservative not to exceed 2.25% on dry weight basis- assuming 50% silage on a dry basis in diet would provide 1.125% formic acid. Formic acid m.wt. = 46.02. Methanol m.wt. = 32.04. Adjusting for molecular wt. 1.125 *(32/46) = .783% . .783/20 = would equate to 3.915% methanol in a glycerin source fed at 20% of diet to ruminant animals.

- CFR 573.640 describes the use of “methyl-esters” of higher fatty acids for use in animal feeds. Methyl-esters are considered non-toxic with LD 50 > 17.4 g/kg in rats. Digestion includes the release of methanol from the fatty acid. As a portion of molecular weight assuming C-16/0 as an average- methanol yield equals approximately 11.83% of inclusion of the methyl-esters. If diets contained 5 % methyl esters 5 X 0.1183= methanol contribution (.5915%). Again using a 20% inclusion of glycerin source .5915/20 = 2.957% methanol concentration in glycerin would be equivalent.

- Numerous literature references are available relative to feeding formic acid or its’ calcium/potassium salts at levels near 1% on a formic acid basis. Methanol concentrations in order to reach this level and again adjusted for relative molecular weights would equal 1*(32/46) = 0.695 %. With glycerin content of 20% in diets .695/20 = 3.478% methanol would need to be present to provide these levels.
EPA, 1994 cites a no observed adverse effect level (NOAEL) of 500mg/kg/day for rats fed 90 days.
- Assuming a dietary dry matter intake of 2.3% of body weight (23 grams/kg) a value of 0.5g/23g= 2.17% of total diet. If glycerin was source at 20% of diet 2.17/20 = 10.85% methanol concentration tolerable in glycerin.
- NOAEL level can be extrapolated in number of ways. A factor for interspecies differences could be applied and a factor for sensitive sub-populations could be applied. Assuming a safety factor of 3 for possible species differences and a factor of 10 for possible sensitive sub-populations an acceptable daily intake (ADI) would be calculated as 500/(3x10) = 17 mg/kg/d

<table>
<thead>
<tr>
<th>Species</th>
<th>Age (wks)</th>
<th>bwt (kg)</th>
<th>FI (g/kg bwt)</th>
<th>ppm MtOH (max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickens, broilers</td>
<td>2</td>
<td>0.3</td>
<td>160</td>
<td>1063</td>
</tr>
<tr>
<td>Chickens, broilers</td>
<td>7</td>
<td>2.1</td>
<td>62</td>
<td>2742</td>
</tr>
<tr>
<td>Chickens layers</td>
<td>20</td>
<td>1.3</td>
<td>46</td>
<td>3696</td>
</tr>
<tr>
<td>Chickens layers</td>
<td>40</td>
<td>1.9</td>
<td>47</td>
<td>3617</td>
</tr>
<tr>
<td>Swine young</td>
<td>4</td>
<td>62</td>
<td>2742</td>
<td></td>
</tr>
<tr>
<td>Swine mature</td>
<td>100</td>
<td>31</td>
<td>5484</td>
<td></td>
</tr>
<tr>
<td>Cattle growing</td>
<td>135</td>
<td>27</td>
<td>6296</td>
<td></td>
</tr>
<tr>
<td>Cattle beef mature</td>
<td>500</td>
<td>20</td>
<td>8500</td>
<td></td>
</tr>
<tr>
<td>Cattle, dairy lactating</td>
<td>600</td>
<td>32</td>
<td>5313</td>
<td></td>
</tr>
<tr>
<td>Horses (concentrate)</td>
<td>500</td>
<td>12</td>
<td>14167</td>
<td></td>
</tr>
</tbody>
</table>
## Proposed Quality / Usage Parameters (Glycerin-Feed Grade)

### Chemical Properties:

<p>| | |</p>
<table>
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<tbody>
<tr>
<td>Moisture</td>
<td>5-25%</td>
</tr>
<tr>
<td>Glycerol</td>
<td>&gt;= 95% of the organic matter</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.3% max in dry matter</td>
</tr>
<tr>
<td>Potassium</td>
<td>3% max in dry matter</td>
</tr>
<tr>
<td>Sodium</td>
<td>3.9% max in dry matter</td>
</tr>
<tr>
<td>Chloride</td>
<td>Guaranteed by supplier</td>
</tr>
<tr>
<td>Sulfur</td>
<td>Guaranteed by supplier</td>
</tr>
<tr>
<td>Methyl Esters</td>
<td>1% max in dry matter</td>
</tr>
<tr>
<td>Fat</td>
<td>1% max in dry matter</td>
</tr>
<tr>
<td>Methanol (Method AOAC 973.23 GCFID 16th ed. 1995)</td>
<td>0.75% max in dry matter</td>
</tr>
<tr>
<td>Total Ash</td>
<td>12% max in dry matter</td>
</tr>
<tr>
<td>Lead</td>
<td>30 ppm max in dry matter</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.5 ppm max in dry matter</td>
</tr>
<tr>
<td>Nickel</td>
<td>50 ppm max in dry matter</td>
</tr>
<tr>
<td>Mercury</td>
<td>2.0 ppm max in dry matter</td>
</tr>
<tr>
<td>Selenium</td>
<td>2.0 ppm in the dry matter</td>
</tr>
<tr>
<td>Arsenic</td>
<td>50 ppm max in dry matter</td>
</tr>
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</table>
Proposed Quality/Usage Parameters (cont.)
Glycerin-Feed Grade

Physical Properties:
• Mild pleasant aroma
• Near neutral pH: 5 to 7.0

Usage Guidelines: via Labeling
• Not to be used in combination in diets containing formic acid, formaldehyde and methyl-esters.
• Guarantees for moisture, ash, sodium, potassium, sulfur and chloride maximums
• Limited inclusion rate in feeds i.e. poultry diets (5-10%), equines and swine (10%) and ruminants (20%)
Summary

- Glycerin from bio-diesel production is:
- An energy dense, palatable material for use in feed
- Of high purity—particularly when evaluated on an organic matter basis
- Low in concentrations of heavy metals
- Low in methanol concentration compared when compared levels of either metabolites approved for feed use or published toxicity values
- Can represent a value to animal feeding programs