ROLE OF BACTERIA IN RUMINANT NUTRITION

The newborn calf

- Calf is sterile at birth
- Early microbial colonization - rather haphazard?
- Risk of colibacillosis? Early colonization by E. coli.
- Can dosing calf with “good”bacteria colonize intestine and prevent establishment of E. coli.

Probiotics and the health of newborn calves?

Bob James
Dept. of Dairy Science

Probiotic product from Salem, VA?

- Lactobacilli grown on wheat bran
- Dose calf with probiotic in shipped in calves housed at Vet Science
- Challenge with enteropathogenic strain of E. coli.
Problems with probiotics – according to W. E. C. Moore – VPI Anerobe Lab

- Most intestinal organisms are anaerobes not aerobes
- Microflora is a mixed culture of organisms.
- Likelihood of single species or few species to successfully populate is nil
- Intestine is rapidly populated by organisms at birth.

Source of meaningful probiotic?

- Source of organisms for probiotic?
  - Older milk-fed calf?
  - Duodenal cannulated
  - Collect and mix with whey solution
    - Intestinal fluid is of low palatability.

Influence of "probiotic" on resistance to enteropathogenic E. coli challenge
James et al, 1976

- Calves entered our facility @~ 3 hour of age
- Colostrum fed @ ~ 5 hours of age
  - No E.coli challenge
  - E. coli challenge @ 12 hours
  - E. coli challenge @ 24 hours
  - All with or without "probiotic" inoculum of 200 ml of duodenal fluid from milk-fed calf.
    - Whey solution as carrier

Results

- Calves receiving probiotic inoculum had less diarrhea and higher ADG for 1st 14 days.
Serum gamma globulin – g/100 ml @ 24 h

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>0</th>
<th>12 h challenge</th>
<th>24 h challenge</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inoculum</td>
<td>1.1</td>
<td>0.94</td>
<td>1.68</td>
<td>1.24</td>
</tr>
<tr>
<td>Inoculum</td>
<td>.5</td>
<td>0.49</td>
<td>.59</td>
<td>.53</td>
</tr>
<tr>
<td>Means</td>
<td>.80</td>
<td>.72</td>
<td>1.14</td>
<td>.89</td>
</tr>
</tbody>
</table>

Follow up study
James et al., (1978)

- Three Treatments
  - Colostrum @ 2.5 hours of age
  - Colostrum and inoculum (200 ml duodenal fluid) concurrently @ 2.3 hours of age
  - Inoculum followed three h later by colostrum @ 5.6 hours of age
- No E. coli challenge

Mean total protein and gamma globulin (g/dl) @ 24 hours of age.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total serum protein</th>
<th>Gamma globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inoculum</td>
<td>5.97+/-.94</td>
<td>1.05+/-.36</td>
</tr>
<tr>
<td>Colostrum and inoculum</td>
<td>6.07+/-.64</td>
<td>1.08+/-.41</td>
</tr>
<tr>
<td>concurrent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed colostrum</td>
<td>5.22+/-.59</td>
<td>.76+/-.31</td>
</tr>
</tbody>
</table>

Delayed colostrum calves had lower protein and gamma globulin

Cause of apparent depression in Ig absorption?

- Live bacteria?
- Cell walls of bacteria (endotoxins)?
- Carrier for the inoculum – dried whey?
Intensive study

- Utilized “gut loops” in newborn calves ~ 8.6 hours at beginning of treatments
- Constructed beginning 1.8 M anterior to ileocecal junction
- Each calf received all treatments
- Incubated for 4 hours
- Inject loops with $^{125}$I – gamma globulin in electrolyte solution incubated for 75 min.

Treatments

- Live bacteria – .3ml duodenal fluid – 9 ml anaerobic rumen fluid glucose cellobiose broth (RGC). **Anaerobic culture**
- Autoclaved culture
- Sterile RGC broth

### Treatment means for uptake, bacterial no. in tissue and inoculum

<table>
<thead>
<tr>
<th>Uptake of gamma globulin $^a$</th>
<th>Bacterial growth in tissue $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live bacteria</td>
<td>Sterile broth</td>
</tr>
<tr>
<td>2.09 +/- 1.15</td>
<td>3.18 +/- 1.50</td>
</tr>
</tbody>
</table>

$^a$ milligrams gamma globulin internalized/g tissue

$^b$ Bacteria (X10^6)

Uptake reduced only with live bacteria culture – (P<.05)

# organisms significantly related to uptake

Three Q’s of colostrum management

- Quickness – feed as soon as possible with a goal of less than 6 hours
- Quantity – 4 liters in 1st 12 hours
- Quality - >50g/liter

- Not colostrum again!

**WARNING**

I’M NOT LISTENING
Add another letter to the list?

• “C” for cleanliness

Microbial risks associated with feeding colostrum

Review Ig absorption in the calf

Ig Absorption
• Large MW macromolecules absorbed from Jejunum and proximal ileum
• 1st 2 – 24 hours of life
• Variable efficiency
• Little selectivity in uptake
• Some selectivity in transfer through serosal side

Cessation of Ig absorption

• Cessation termed closure
• Differs by species
• In the calf
  • Not as diet dependent
  • Very variable onset – 6 – 24 hours
  • Precipitous decline in absorptive efficiency
Closure - One step process where uptake and transfer ceases

- Theories
  - Development of gastric and enzymatic function
  - Reduction in permeability of villus epithelial cells
  - Replacement by generation of cells incapable of pinocytosis

Replacement by generation of cells incapable of absorption.

- Micropinocytotic IgG transfer by newborn calf enterocytes
  - Existence of a receptor mediated transport system?
  - Relationship between apoptosis in cessation of Ig transfer

Apoptosis and IgG absorption in goats  Castro-Alonso et al. (2008)

- 10 new born kids
- Fed colostrum – 2,000 mg IgG/kg body weight – 2X @ 2 and 14 h
- Sacrificed - birth – 60 d of age.
- Assessed for apoptotic cells and stained for IgG.

IgG staining in duodenum of goat kids harvested at ......

Birth - no IgG immunoreactivity
Day 1 – IgG internalized
Day 2 + IgG was bound to enterocytes but minimal internalization
Day 60 – No staining for IgG
Terminal deoxynucleotidyl transferase nick end labeling (TUNEL) – identifies apoptotic cells

Birth – TUNEL staining at center of villus
Day 1 – staining is significantly reduced after day 1
Day 60 – only cells at villus terminus are staining

Conclusions

- Relationship of IgG absorption with apoptosis in the intestine?
- Apoptosis is influenced by composition of colonizing intestinal bacteria in pigs (Willing and Van Kessel, 2007)
- How to delay apoptosis?

Feeding heat-treated colostrum or unheated colostrum with two different bacterial concentrations to neonatal calves - Elizondo-Salazar and Heinrichs, 2009

- Treatments
  - Colostrum frozen - low bacteria
  - Colostrum pasteurized – 60°C / 30 min. then frozen
  - Colostrum stored at 20°C for 24 h then frozen
  - First feeding 3.8 liters / 68g IgG/liter - 1.5 – 2 h after birth with esophageal feeder.

IgG (g/L) and SPC of colostrum 
Serum protein and IgG @24 h

<table>
<thead>
<tr>
<th>Item</th>
<th>Low bacteria</th>
<th>High bacteria</th>
<th>Heat – treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (g/L)</td>
<td>69.55</td>
<td>69.55</td>
<td>66.17</td>
</tr>
<tr>
<td>SPC x10^3/ml</td>
<td>9.332</td>
<td>40.738</td>
<td>.645</td>
</tr>
<tr>
<td>Serum protein @24h (g/L)</td>
<td>57</td>
<td>56.2</td>
<td>62.5</td>
</tr>
<tr>
<td>IgG @ 24 h (g/L)</td>
<td>20.2</td>
<td>20.1</td>
<td>26.7</td>
</tr>
<tr>
<td>AEA of IgG @ 24 h (%)</td>
<td>35.4</td>
<td>32.4</td>
<td>43.9</td>
</tr>
</tbody>
</table>

Heat treating improved AEA and IgG at 24 h. High bacteria load was relatively low / less than 100,000 goal (McGuirk)
Role for microbial colonization and Ig absorption?
- Rate of intestinal cell production in the crypts
- Migration of cells up the villus and desquamation from the tips influenced by microbial colonization?
- Microbes may occupy binding sites on the apical plasma membrane.

Intestinal microflora and the absorptive surface
- Colostrum deprived calf receiving E. coli O55
- Exfoliation of microvilli
- Intracellular penetration of epithelial cells

Corley et al., 1977

How is colostrum managed on many dairies?
- Colostrum deprived calf
- Colostrum fed calf
How is colostrum managed on dairies?

Field studies of colostrum quality

- Swan et al., 2007 –
  - 12 Minnesota and Wisconsin dairies
  - Median TPC – $6.15 \times 10^8$
  - Varied from $7 \times 10^7$ to $10^9$
  - 93% of samples over goal of 100,000 TPC
- Poulsen et al., 2002
  - 82% of samples over 100,000 TPC

Critical control points for colostrum contamination

Samples from cow, bucket and tube feeder
64% of samples collected within 20 min of harvest were <100,000 TPC
Growth during storage

![Graph showing growth during storage](image)

Goal – 100,000

Batch pasteurization of colostrum

- 60° – 60 minutes
- Batch pasteurization
- Higher temperatures
  = cottage cheese

Total Bacteria Counts in Minnesota Colostrum

(Swan et al. 2007. JDSci. 90)

Median TPC = 615 million cfu/ml (73 to 104 billion)

93% of samples > 100,000 cfu/ml TPC

“We are feeding ‘fat-laden’ manure” Rob Trembley, 2006

From - S. Godden

Raw vs. pasteurized colostrum

Johnson et al., 2007

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Raw</th>
<th>Pasteurized</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG – mg/ml</td>
<td>72.6</td>
<td>67.3</td>
</tr>
<tr>
<td>Total plate count</td>
<td>46,000</td>
<td>872</td>
</tr>
</tbody>
</table>

Correct table in proceedings
Recent UMN Field Study
M. Donahue, S. Godden

- 1,000 calves / 6 herds
  - ½ fed raw and ½ fed heat-treated colostrum
- Colostrum total plate count and serum IgG – negative
- Colostrum IgG concentration – positive effect
- Heat treatment – positive – independent of Total plate count

30 years later - lots of common sense

Did some “West Virginia” rub off?
I hope so!!!!

Common Sense and Colostrum

- Clean cows – infection and sanitation
- Don’t pool colostrum
- Milk fresh cows first and process colostrum immediately.
- If refrigerating – cool rapidly in ice bath
- Clean buckets, bottles and esophageal feeders
- If herd size and herd infection status warrants – pasteurize or use replacer
- Avoid use of probiotics for 24 hours

Thanks, Carl!