# Guts and Bugs

## The Good, the Bad, the Ugly

Jenn Rowntree, DVM



## Healthy guts = healthy calves

- **Pathogens impact the small intestine's normal microbiota and barrier function**
  - Bugs talk!? Pathogen and host-microbe interactions in the gastrointestinal tract (GIT)
- **What can we do about it?**
  - Optimize management practices that contribute to GIT environment
- **How does this affect my bottom line?**
  - Diarrhea responsible for 56% of pre-weaned heifer deaths (NAHMS 2014)
  - Reduced 1st lact 305-ME, actual milk, fat, and protein (Heinrichs and Heinrichs, 2011)
  - Increased age at first calving (Heinrichs, 2005)

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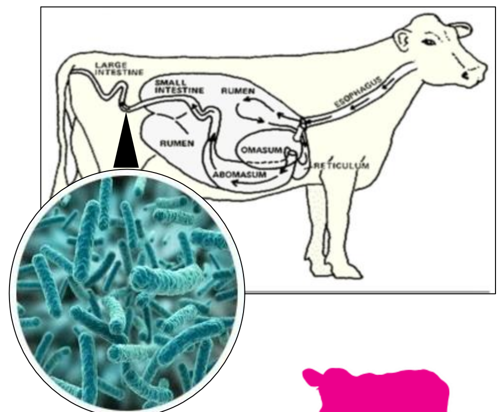
# Gut microbiome

- **Naturally-occurring organisms in the GIT**
- **Common microbiota predominating in healthy cattle:**
  - *Firmicutes, Bacteroides, Proteobacteria* (Bickhart & Weimer 2018)
  - Composition and functions vary with age and different gut locations (Malmuthuge et al., 2014)
  - Affected by physiological and health status (Bickhart & Weimer 2018)




https://medium.com/@thryve/the-development-of-the-gut-microbiome-f060a6ba41bf



# Why is a healthy gut microbiome important?

- **Critical role in metabolism, immune response, and GIT regulation (Guarner, 2006)**
  - Modulate development of intestinal epithelium and mucosal layer (Sharma et al., 1995) plus immune system (Mebius 2013)
  - Maximize nutrient absorption to promote efficient growth
- **Gut microbiota impacts overall health**
  - Sustain immune responses that detect, prevent, and eliminate bad bugs while tolerating good bugs (Bischoff, 2011)
  - Maintain homeostasis to prevent invasion of bad bugs

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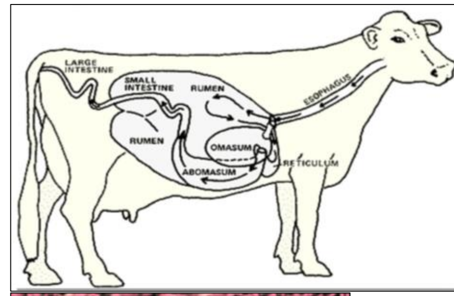
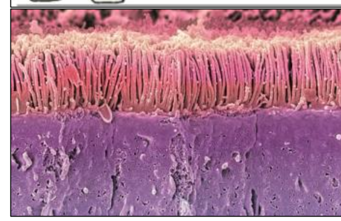
# Mucosal immune system

- **Immune responses that occur in tissues exposed to external environment**
  - Physical and chemical components
  - First week of a calf's life critical to development (Liang et al., 2014)
- **Requires a healthy gut microbiome**
  - For development, maturation, and homeostasis (Hooper et al., 2012)
- **Critical to eliminating pathogens**

http://www.lifeinharmony.me/anatomy-of-goat-digestive-system/anatomy-of-goat-digestive-system-the-systems-farm-animals-ppt-video-online-download



https://www.pharmaceutical-journal.com/news-and-analysis/features/drug-metabolism-manipulating-the-microbiome/20068240.article

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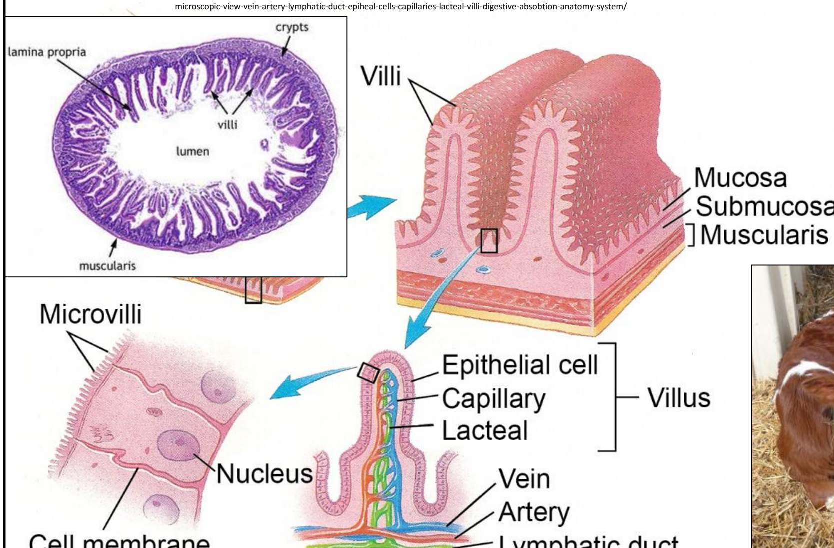
http://www.clubupton.com/best-collection-function-of-small-intestine/function-of-small-intestine-best-collection-microscopic-view-vein-artery-lymphatic-duct-epiheal-cells-capillaries-lacteal-villi-digestive-absobtion-anatomy-system/




http://histology-world.com/photoalbum//displayimage.php?pid=1133

https://www.naturalpedia.com/duodenal-cancer-causes-side-effects-and-treatments-at-naturalpedia-com.html

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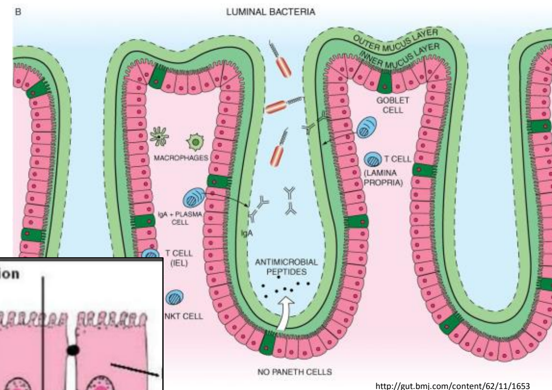
## Components of mucosal immune system

- **Physical barriers – prevent invasion of bad bugs from entering GIT tissue**
  - Mucus layer– trap microbiota
  - Epithelium – single layer of cells connected by tight junctions (Ulluwishewa et al., 2011)
    - Transport through cells and tight junctions to absorb molecules
    - Tight junctions – primary regulator of intestinal barrier function

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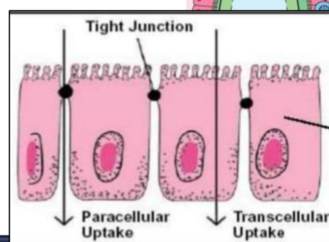


https://www.slideshare.net/NamanPant1/nasal-drug-delivery-system-75345923

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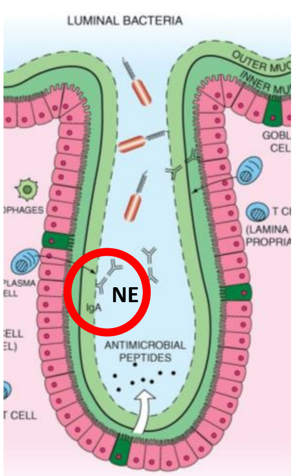
## Components of mucosal immune system




http://gut.bmj.com/content/62/11/1653

- **Chemical barriers – limit growth of bad bugs (Johansson et al., 2011)**
  - IgA – produced by mucosal immune system
    - Protect good bugs in mucosal layer (Gutzeit et al., 2014)
    - Clear bad bugs and maintain homeostasis
  - Antimicrobial peptides – can ID and kill pathogens
- **Pattern-recognition receptors**
  - Maintain integrity of intestinal barrier (Ulluwishewa et al., 2011)
  - Help immune system learn to ID bad bugs

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# Microbial endocrinology

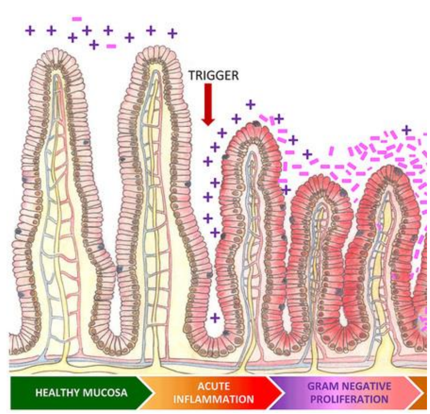
- **AKA – how good and bad bugs respond to signals from other gut bugs and their host**
- **Norepinephrine – signal molecule between host and microbiota**
  - Hormone released in response to stress
  - Intermediary for both good and bad gut bugs
  - Potential to effect changes in growth and metabolism of various microbes


https://www.jigsaw.ie/news-and-events/post/exam-season-the-fight-flight-freeze-response



# Damage to microbiome and mucosal immune system due to:

- **Stress**
- **Antibiotics**
- **Invasive and opportunistic pathogens**

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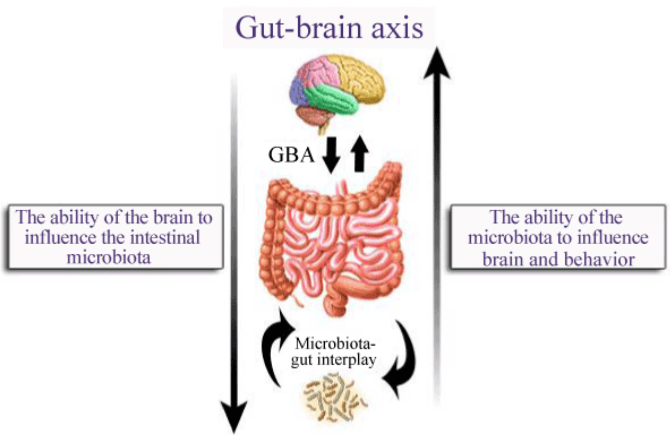
## Stress impacts the good gut bugs




http://drselakovic.rs/novosti/mikrobiom-u-crevima-moze-uticati-na-rad-mozga/

- **Good bugs**
  - Key regulators of gut-brain axis (Foster et al., 2017)
- **Alters microbiota (Lyte 2016)**
- **Study in mice by Bailey et al., 2011:**
  - Decreased abundance of *Bacteroides*
  - Increased relative abundance of *Clostridium* species

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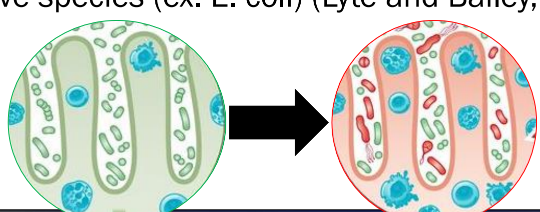
## Stress impacts the bad gut bugs

- **Norepinephrine released at high concentrations =>**
  - Acts on bacteria (ex. O157:H7 E. coli) to promote movement towards intestinal surface, enhance growth and virulence (Green et al., 2004, Lyte and Ernst 1992)
- **Release of signal factors from injured GIT nerves**
  - Microbial population changes from mostly gram-positive good bugs to a single gram negative species (ex. E. coli) (Lyte and Bailey, 1997)



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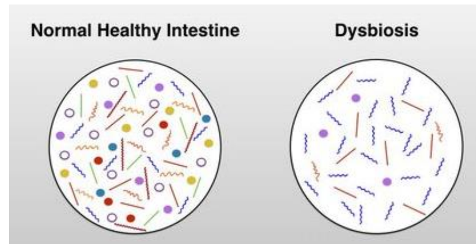
## How do antibiotics impact the good bugs?

- **Reduce total bacterial populations (Ubeda et al., 2010)**
  - Decrease beneficial bacteria (Xie et al., 2013)
  - Precipitate *Salmonella* infections
- **Increase antibiotic resistance in opportunistic bugs (Ubeda et al., 2010)**
  - Ex. *E. coli, Enterococcus,* (Xie et al., 2013)
- **Cause microbial imbalance in GIT**




https://reportshealthcare.com/what-is-dysbiosis-and-what-is-it-treatment/



## Antibiotics and the microbiome

- **Avoid use of oral antibiotics for treatment of scours (Constable 2004)**
  - Current label Rx options not consistently effective
  - Goal for scours treatment:
    - Control growth of *E. coli* in small intestine
    - Minimize damage to beneficial gut microflora

- Diarrhea, normal appetite, no fever – monitor, administer electrolytes
- Diarrhea, no appetite, fever – administer broad-spectrum antibiotic, electrolytes
  - Ex – ceftiofur, amoxicillin or ampicillin
  - NOT enrofloxacin

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# What about waste milk?

- **Maynou et al., 2017 – calves fed waste milk vs. milk replacer:**
  - Whole waste milk contained B-lactam residue =>
    - Higher number of antibiotic-resistant *E. coli* bacteria in feces
      - To enrofloxacin, florfenicol, and antibiotics with B-lactamase genes
    - Higher prevalence of colistin-resistant *Pasteurella multocida* in nasal swabs
- **Antibiotic resistance changes with age**
- **How will this impact effectiveness of antibiotics in sick calves?**
  - More research needed

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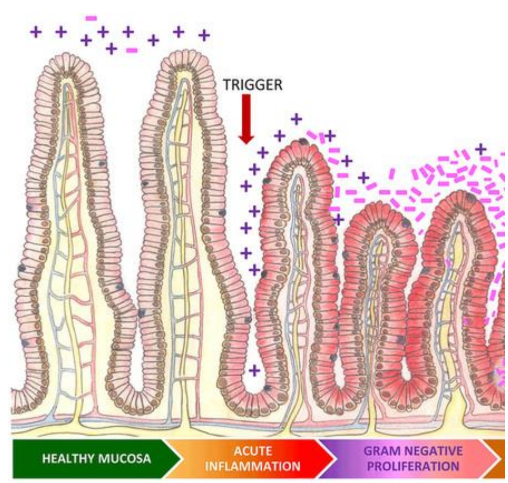
# Bad gut bugs

**Invaders and commensals that overstay their welcome**

- **Common perpetrators:**
  - *Clostridium perfringens*
  - Rotavirus and coronavirus
  - *Cryptosporidium parvum*
  - Coccidia

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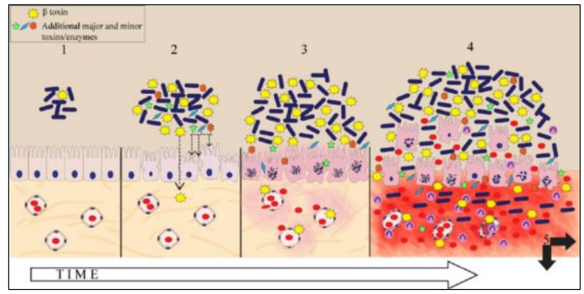
## *Clostridium perfringens*

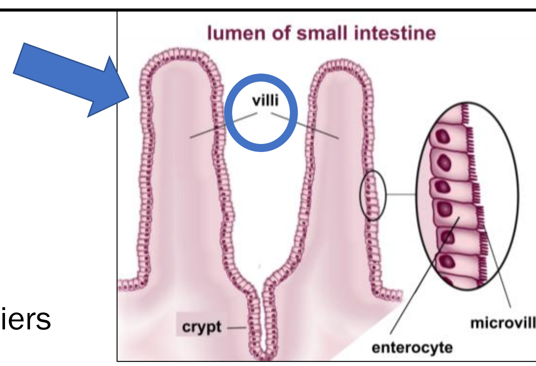
- **Gram positive, anaerobic bacteria**
  - Found in environment, GIT microflora
  - Multiple types
- **Toxins cause damage**
  - Presence of bacteria ≠ disease
- **Risk factors for toxin production:**
  - Large quantities of soluble carbs and/or protein
  - Presence of signaling molecules – ex. norepinephrine, epinephrine
    - Epinephrine increases growth rate and decreases infective dose (Cooper 1946)




Roos et al., 2015



## Rotavirus



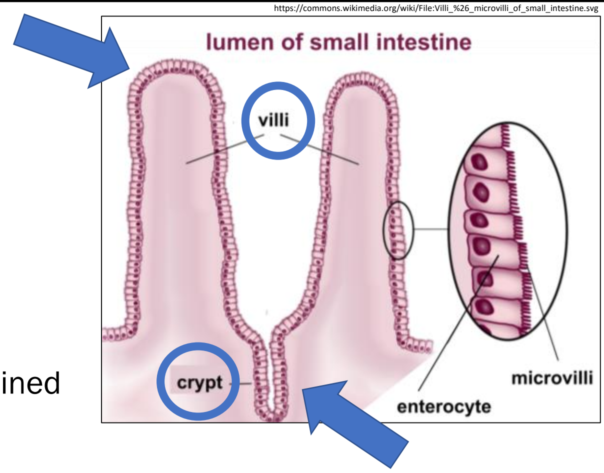

https://commons.wikimedia.org/wiki/File:Villi_%26_microvilli_of_small_intestine.svg

- **Non-enveloped virus**
  - No outer shell = resilient
- **Transmission:**
  - Older calves and adult cows serve as carriers
- **Diarrhea at 7-14 days of age**
  - Lasts 3-7 days – calves shed millions of viral particles/gram of feces
  - 50-100% of calves affected, varying death rates
  - Often coexists with other pathogens
- **Damage limited to small intestines**
  - Decreased absorption and digestion of nutrients

https://commons.wikimedia.org/wiki/File:Villi_%26_microvilli_of_small_intestine.svg

# Coronavirus

- **Enveloped virus**
  - Outer shell easily damaged
- **Fecal-oral transmission**
- **Diarrhea at 7-10 days of age**
  - Lasts ~1 week
  - Severe – death rate >50% when combined with other bad bugs
- **Mass destruction to SI and LI**
  - Reduced ability to absorb electrolytes
  - +/- IV fluids, systemic antibiotics



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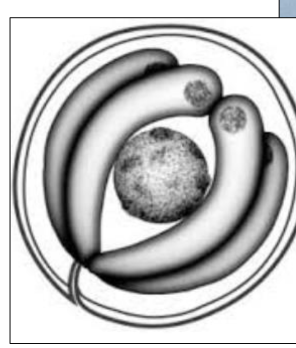
# *Cryptosporidium parvum*

- **Protozoan**
  - Extremely hardy, persists for months
- **Transmission:**
  - Fecal-oral – infective dose <100 oocysts
  - ZOONOTIC
- **Diarrhea in calves 5-28 days old**
  - Calves shed millions of oocysts per gram of feces
- **Destroys host cells along entire GIT**
  - Cell death and damage predispose calf to other infections – ex. *E. coli*, viruses, *Salmonella*

https://www.researchgate.net/figure/Cryptosporidium-scophthalmi-Line-drawing-of-oocyst-Scale-bar-2-m_fig1_8082232

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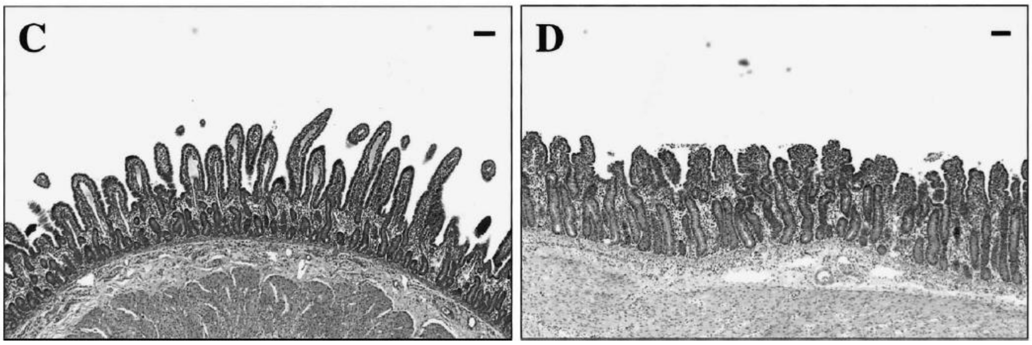
# Response to cryptosporidium infection




Gookin et al., 2002



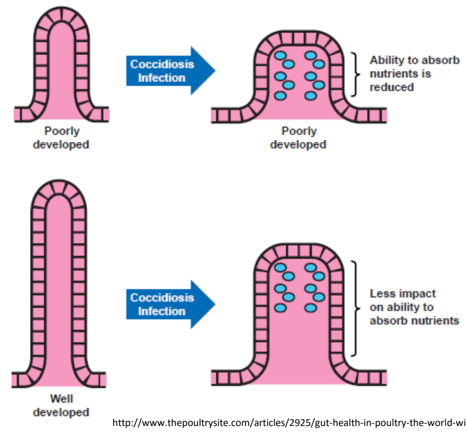
# The Ugly Gut Bugs

## Quorum-sensing culprits

- ***E. coli, Salmonella, Klebsiella*** **(Curtis et al., 2014, Moreira et al 2016)**
- **Quorum-sensing proteins**
  - Involved in virulence factors, bacterial growth, and colony density (Lyte et al., 2018)
  - Release of NE enhances ability to infect host:
    - Improves *E. coli* O157:H7 attachment to SI and fluid secretion (Vlisidou et al., 2014)
    - Increases replication of *Salmonella typhimurium* in GIT (Pullinger et al., 2010)
    - Bacteria adhere to intestinal lining => use NE to call other bacteria (Pasupuleti et al., 2014)

# What can we do about it?

1. **Promote diverse gut microbiome**
   - Robust mucosal immune system
   - Focus on nutrition
2. **Limit stress**
   - And exposure of GIT to stress-related hormones
3. **Reduce exposure to pathogens**
   - Implement biosecurity protocols
   - Even if exposure is inevitable




http://www.thepoultrysite.com/articles/2925/gut-health-in-poultry-the-world-within



# 1. Focus on nutrition: start with colostrum

- **Provide clean, high-quality colostrum ASAP**
  - 4 quarts in 4 hours
  - Calves need a source of glucose
- **Helps *beneficial* bacteria colonize SI (Malmuthuge et al., 2015)**
  - Single feeding of heat-treated colostrum soon after birth (<12 hours) promoted colonization with *Bifidobacterium* and reduced colonization with *E. coli*
  - Natural prebiotic = help the good bugs beat the bad bugs to the SI

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# Colostrum during disease challenge

**Study by Chamorro et al., 2017: 2 treatment groups of 100 calves**

- Control group – no colostrum supplement
- Treatment group – 150g colostrum supplement powder twice daily for first 14 days of life

- **Results**
  - Mean body weight, ADG at weaning *not* significantly different among treatment groups
  - Reduced antibiotic therapy in treatment group (18.8%) vs. control group (76.5%)
  - Reduced disease in treatment group
- **On-farm:**
  - Freeze good quality colostrum (BRIX >20) in ice-cube trays
  - Feed 1 cube per calf per feeding for 1st 14 days of life



# 1. Focus on nutrition: milk

- **Quality**
  - Clean and free of bad bugs
  - Osmolality similar to cow's milk
  - Fed at 12-14% total solids
- **Quantity**
  - Must meet energy requirements for growth
  - 100lb calf requires 5.7 lbs (2/3 gallon) of whole milk for maintenance per day (Drackley, 2008)
  - Enough to support immune function, temperature extremes

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# 1. Focus on nutrition: quality calf starter

**Early introduction to high quality calf starter:**

- **Promotes rumen development and facilitates early weaning**
  - Diverse microbial community (Malmuthuge et al., 2013)
  - Improved growth rates
- **Increased ability to fight disease**
  - Earlier expression of antimicrobial defense molecules that help ID and kill pathogens (Malmuthuge 2015)
  - Influences GIT barrier function and immune responses

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# 1. Focus on nutrition: probiotics

**Probiotic – a source of *live*, viable good bugs or yeast**

- Interact with microflora, GIT epithelium, immune cells
- *Bacillus subtilis* – increased ADG, feed efficiency, decreased weaning age by 7 days (Sun et al., 2010)
- *Saccharomyces cerevisiae* – decreases susceptibility of calves to GI infections
  - Decrease # of days with diarrhea in calves with failure of passive transfer (Galvao et al., 2005)
  - Feeding with grain decreases incidence of diarrhea and death rate in calves <70 days of age (Magalhães et al., 2008)
- **Potential problems**

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# 2. Limit stress to calves

- **Minimize pain associated with procedures whenever possible**
- **Avoid simultaneous stressors – dehorning, vaccines, moves**
- **Gradual weaning**
- **Temperature control – avoid heat or cold stress (40-70°F ideal)**
- **Dry, well-bedded environment**
- **Adequate ventilation, volume and area per calf**
- **Fly control**

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# 3. Minimize exposure to pathogens

- **Biosecurity and biocontainment practices can reduce risk of pathogen transmission**
  - Effective cleaning and disinfecting protocols
  - Manage animal movement
- **Infection and disease result of:**
  - I. Innate resistance of host animal
  - II. Infectious dose received
  - III. Virulence of particular strain

http://agavaceae.info/clipart/balance-scale-clip-art.html

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# Conclusion

**Maximize resistance of animal and reduce pathogen exposure**

- **Promote healthy gut microbiome to increase GIT immune system**
  - Colostrum, starter intake
  - Dry cow vaccines
  - Optimal facilities and ventilation
- **Reduce exposure to triggers that break down GIT barrier function**
  - Stress
  - Poor hygiene
  - Pathogen load

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