

#### Poultry Gut Development and Function: Brief Review

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#### **Presentation Summary**

- 1. Introduction/ Importance of GIT
- 2. Anatomy
- 3. Physiology
- 4. Microbiome
- 5. Development of the GIT
- 6. Ways to assess the GIT



## Today, a newly hatched chick increases its body weight by 25% overnight and 5000% by 5 weeks, to 2kg.

0 d

28 d

56 d

This astonishing performance of the modern chicken comes from:

- (a) Intensive selection for growth rate;
- (a) Meticulous attention to health and husbandry;
- (a) And advances in feed formulation, matching the nutrient contents of the feed with the nutrient requirements of the bird.





## Negative consequences of Genetic Selection for Growth

- Metabolic Disorders:
  - Ascites, fatty liver, leg problem, sudden death syndrome
  - lower immune response
    - decreased adaptive immune response but increase cell mediate and inflammatory response (Cheema et al., 2003)
  - decreased resistance to pathogens
    - (Tottori et al., 1997; Cheema et al., 2003)



Table 3. Cumulative feed conversion ratio (g of feed:g of BW gain) of University of Alberta Meat Control (AMC) strains unselected since 1957 and 1978, and Ross 308 broilers (2005)

Age (d)	AMC-1957	SEM	AMC-1978	SEM	2005	SEM	<i>P</i> -value
0 to 7	$2.553^{a}$	0.258	1.382 <sup>b</sup>	0.030	$1.108^{c}$	0.026	< 0.0001
0 to 14	3.300 <sup>a</sup>	0.362	$1.506^{b}$	0.019	$1.275^{c}$	0.017	< 0.0001
0 to 21	$3.188^{a}$	0.170	$1.608^{b}$	0.013	$1.379^{c}$	0.006	< 0.0001
0 to 28	$3.084^{a}$	0.093	$1.706^{b}$	0.019	1.483 <sup>c</sup>	0.008	< 0.0001
0 to 35	3.003 <sup>a</sup>	0.118	$1.832^{b}$	0.030	1.573 <sup>c</sup>	0.012	< 0.0001
0 to 42	$2.882^{a}$	0.101	$1.899^{b}$	0.026	$1.674^{c}$	0.012	< 0.0001
0 to 49	$2.871^{a}$	0.103	$2.018^{b}$	0.017	$1.808^{c}$	0.018	< 0.0001
0 to 56	$2.854^{\rm a}$	0.096	$2.135^{b}$	0.037	$1.918^{c}$	0.015	< 0.0001

<sup>a-c</sup>Means within rows with no common superscript differ significantly (P < 0.05).



#### The Complexity of the GIT is Amazing

- Harbors more than 600 different species of bacteria
- Approximately 1010-12 cfu/gm total bacteria
- Contains over 20 different hormones
- Digests and absorbs the vast majority of nutrients
- And accounts for 20% of body energy expenditure.
- It is also the largest immune organ in the body
- Many adaptations for flight/weight
- Relatively short GIT







#### **Total Surface Area**









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#### **Intestinal Anatomy and Function**



#### The Chicken GIT –









- Beak/Mouth
  - Light jaw
  - absence of teeth→ ridged palate
  - Choanal slit connect the oral and nasal cavity
  - Some amylase digestion
- Esophagus





- Crop
  - Pocket of the esophagus
  - Food storage
  - Digestion : starch hydrolysis
  - Absorption: glucose
  - Bacterial fermentation (10<sup>8</sup>-10<sup>9</sup>)
    - Lactobacillus, Bifidobacterium and Enterobacter
  - Crop fill
    - indicator of appetite
    - chicks have found feed





- Proventriculus
  - Secretes mucus, HCl and pepsinogen
- Ventriculus (Gizzard)-
  - thick and thin muscles, koilin lining
  - mechanical digestion
  - gastric proteolysis
  - Pyloric region: limits particle size of digesta entering the duodenum



- Small intestine
  - Villi (absorption)
    - Duodenum: 1.5mm
    - Ileum 0.4-0.6mm
    - Number of villi decrease from 1-10 days of age → remains constant
    - Zigzag arrangement
  - Crypt
- 4 Layers
  - Mucosal
  - Submucosal
  - Muscle tunic
  - Serosal







- Cell types
  - Crypts of Lieberkuhn
  - Paneth cells
  - Goblet cells  $\rightarrow$  produces mucin
  - Neuroendocrine cells
  - Absence of lacteals
  - Submucosa (lamina propria
  - Inner circular muscle
  - Outer longitudinal muscle
  - Serosa
  - Vagus and sympathetic innervation
  - Afferent nerves and endocrine signals which markedly influence hunger and homeostasis







- Duodenal loop
  - Nutrient digestion
    - Pancreatic juices
      - Neutralize pH
      - Enzymes
  - Bile secretion
- Jejunum/upper lleum
  - no histological differences
  - Nutrient digestion and absorption
  - Weight 20-50% more than the ileum
  - Retention time 40-60 mins
- Ileum/Lower Ileum arbitrarily delineated by Meckel's diverticulum
  - Retention time 80-120 mins
  - Mineral and water reabsorption
  - Starch digestion → diet dependent





# Development of Digestive, Absorptive, and Protective Functions





### Nutrient Absorption:

- Intestinal uptake capacity matches the birds needs → Limiting factor?
- Carbohydrates:
  - Glucose: Active transport =80% via SGLT-1
  - Occurs primarily in duodenum but also has a high affinity in the lleum
- Protein
  - amino Acids: Secondary active transport depends on classification of functional group
    - Ceca is able to transport amino acids → microbial fermentation of Uric acids
  - Tri and di-Peptides: PEPT1 and paracellar transport
    - Absorbed more rapidly than amino acids
  - Fats
    - Digestion occurs in the duodenum

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 Fatty acids absorbed via diffusion in the distal jejunum and ileum



#### Anatomy and Function: Ceca

- The cecum are blinded ended pouches
- Ileocecal junction interdigitating villi →allow fluid and finely ground particles with a low molecular weight
- Anti-peristaltic movement → filling and mixing
- peristaltic movement  $\rightarrow$  mixing and emptying of the ceca
- Digesta retention time:
- Cecal Function:
  - Microbial fermentation and nutrient digestion
  - Microbial action 

     beneficial and pathogenic
  - Water and electrolyte absorption
  - Site of immunoglobulin and antibody production
- Cecal Avian Microbiome
  - The average number of bacteria in ceca ranges from  $10^{10} 10^{11}$  bacteria per gram of digesta
  - Predominant Cecal Bacteria:
    - Firmicutes (44-54% )
    - Bacteroidetes (23-46%)
    - Proteobacteria (1-16%)





- Rectum ("colon")
  - extends from ileocecal junction to cloaca short villi
- Proctodeum external (vent)





- Liver bi-lobed, left hepatic duct- directly to duodenum, right to gall bladder, bile ducts join duodenum (distal loop)
- Pancreas, secretions via three ducts





### **Chicken GIT- Notes on Function**

- Motility regulated by vagus and sympathetic NS
- Fasted chicken may bypass feed directly to the proventriculus
- Crop fullness influences proventricular secretions and gizzard motility
- Feed (digesta) is easily and quickly retrograded from gizzard to crop, or from lower ileum to gizzard by retroperistalsis (refluxes unique to birds)
- Peyers patches, cecal tonsils, Meckel's diverticulum, post-crop esophageal tonsils, and dendritic cells (crop)





#### **Intestinal Dysfunction**



# Integrity of GIT Barrier Through Tight Junctions



http://www.dbriers.com/tutorials/2012/12/junctions-between-cells-simplified/





# Inflammation Leads to Bacterial Translocation

- Normal closure of tight junctions
- Normalized by commensal bacteria
- Stress, low digestibility feed, feed restriction, therapeutic antibiotics cause inflammation



http://allnaturaladvantage.com.au/how\_gastrointestinal\_he alth\_affe.htm





http://www.dbriers.com/tutorials/2012/12/junctions-between-cells-simplified/



Subclinical forms enteritis are financially more devastating than acute, short-term, mortality-associated infections.

Focus for some has shifted from "sanitation" to providing beneficial pioneer Git colonizing bacteria

Dietary factors that modulate the immune system and gut microbiota should be considered when formulating diets and managing feeding practices.







#### Nutrient metabolism and the immune system



#### Microbiome





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# There is a coevolved relationship between hosts and their gut microbiota





Fraune and Bosch. Bioessays. 2010 Jul;32(7):571-80.

## **Gnotobiotic (Germ Free) Animals**

- Develop limited villi and almost no acquired immune function within the GIT
- GIT is the "largest Immune Organ" almost no function
- Very low digestive enzyme production
- Markedly attenuated growth rate
- Very high nutritional requirements for survival
- Highly sensitive to many ingested toxins





#### Microbiome

- Many recent reviews have described the known interactions between the innate and adaptive immune system and the trillions of microbes within the GIT
- The Microbiome is literally an "organ", consisting of many times the genes of the chicken





### **Chick Microbiome**

In Humans→ appropriate newbon micrbiome colonization is required to stimulate the innate and adaptive immune system preventing inflammatory, allergies and autoimmune diseases



#### The Chicken Microbiome – Little is Known

#### Diversity and Succession of the Intestinal Bacterial Community of the Maturing Broiler Chicken

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#### ABSTRACT

The diversity of bacterial floras in the ilea and ceca of chickens that were fed a vegetarian corn-soy broiler diet devoid of feed additives was examined by analysis of 1,230 partial 16S rRNA gene sequences. Nearly 70% of sequences from the ileum were related to those of *Lactobacillus*, with the majority of the rest being related to *Clostudiaceae* (11%), *Streptococcus* (6.5%), and *Enterococcus* (6.5%). In contrast, *Clostridiaceae*-related sequences (65%) were the most abundant group detected in the cecum, with the other most abundant sequences being related to *Fusobacterium* (14%), *Lactobacillus* (8%), and *Bacteroides* (5%). Statistical analysis comparing the compositions of the different 16S rRNA libraries revealed that population succession occurred during some sampling periods. The significant differences among cecal libraries at 3 and 7 days of age, at 14 to 28 days of age, and at 49 days of age indicated that successions occurred from a transient community to one of increasing complexity as the birds aged. Similarly, the deum had a stable bacterial community structure for birds at 7 to 21 days of age and



#### Microbiome Establishment and Maintenance

Microflora exposure is key

Quality feed is essential

Early (and continuous) feeding is critical

Avoidance of stress is crucial





http://laurenbeatssugar.blogspot.com
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#### The Brooding Package –as it once was





#### Early Colonization- at Pip (or before)

- FIND WHERE THEY GET BACTERIA
- Very recent studies some LAB survive well on shells and may penetrate and persist within shell membranes
- Many opportunistic bacterial pathogens in hatchers may be of human origin
- Early mortality can be markedly reduced by hatchery treatment with beneficial microflora
- Well known effect of the "recycled litter paradox"
- Substantial bacterial colonization by placement, for better or worse



#### Early colonization



# The early establishment of the intestinal microbiota promotes:

- Assembly of the gut-associated lymphoid tissue
- Regulates intestinal motility
- Educate the immune system
- Affects the integrity of the intestinal mucosal barrier
- Proliferation and differentiation of its epithelial lineages
- Regulates angiogenesis, and mucin production
- Modifies the activity of the enteric nervous system
- Extracting and processing nutrients in the diet
- Decreasing pH







#### **Early Intestinal Development**



#### **Intestinal Development**

Innate and adaptive immune system are immature in until the first two weeks of life (Schokker et al., 2009)

#### Maternal Ig's

Role of micrbiome

early exposure to probiotics to intestinal epithelial cells is essential because the bacteria can program the gene expression of the host to favour a envrionemt specific to that bacteria (Gaggia et 2010)



#### Factors affecting development

- Probiotics
- In ovo feeding



## Early Life Programming

- Theory functional and structural changes in genes, cells, tissue, organs
- nutrition is a major driver of early exposure affecting phenotype (Korotkova et al., 2005)
- Early age bacterial supplementation leads to development of a distinct microbiome (advantage of early colonization) and alters gene expression in the gut (Yine et al., 2010)
- Antibiotics treatment on day of hatch caused a modulation in the microbiome that decrease the number of macrophage like cells (Schokker et al., 2017)



#### Post Hatch

- Proportional growth in small intestine is greater than total BW
- First week post hatch is critical for intestinal growth Duodenum weight peaks at 3days of age
- Number of enterocytes per villi increased with age and was greater in broiler chick compared to layers



#### Yolk Sac

- Located at the Meckel's diverticulum
- Incubation nutrients pass through yolk sac membrane and its surround vascular systems
- Yolk Sac: 15-20% of BW at hatch
- Yolk sac is a major source of glucose and glycogen storage to source the embryonic before hatch
- Yolk Sac is THE source of maternal immunity... this is expensive energy!



## Assessing the Intestine: PHL Research



- Morphology doesn't always relate to function
  - Increased villus height correlates with improved function and dysfunction (Feed restriction) (Svihus , 2014)



## **UA-PHL : Intestinal inflammation**

### Models and Ways to detect it

- Stressors increase inflammation
  - High viscosity feeds (NSP)
  - Non-digestible proteins (or undercooked soy)
  - Feed restriction
  - DDS
  - Dexamethasone
  - Coccidiosis
  - Necrotic Enteritis

#### All the above cause bacterial translocation!





#### Procedure



# Detection of FITC-D in serum of broilers after enteric inflammation induction



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DSS – dextran sodium sulfate High fat – 6.5% poultry fat in Rye diet – rye substituted for FR – 24 h feed restriction

# Detection of FITC-D in serum of broilers after enteric inflammation induction

Serum FITC-D



#### **Immune related Biomarkers**



#### Summary

- Absorptive capacity of the chick markedly increases within the first week of age, surface area and enzyme secretions
- Development of both digestive and immune function is optimally dependent upon beneficial microbiota development
- Early and active feeding, nutrition quality, and absence of stressors, are critical for microbiota development
- Early mortality and perpetuated growth/FE phenotype may be markedly affected by the brooding period



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