Microbial Dynamics of the Broiler Intestinal Tract — Margie Lee, Ph.D., D.V.M.

"Biography"

Ph.D., Medical Microbiology, The University of Georgia, 1990.

M.S., Medical Microbiology, The University of Georgia, 1988.

D.V.M., Va. Md. Regional College of Veterinary Medicine, 1986.

B.S., Biology (major), Chemistry (minor), Virginia Tech, 1982.

Areas of Research Emphasis:

1) Development of molecular tools for studying the pathogenesis of *Pasteurella multocida* infection.

2) Molecular epidemiology of food safety pathogens.

3) Contribution of neuraminidase to microbial virulence and value as a protective or diagnostic antigen.

4) Molecular mechanisms of antimicrobial resistance.

5) Molecular characterization of intestinal microflora.

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Microbial Dynamics of the Broiler Intestinal Tract

"Abstract":

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The microbial ecology of the chicken small intestinal flora is relatively ill defined primarily because studies have focused on the cecum. In order to better understand the ecology of this environment, we used 16S ribosomal DNA gene sequencing to identify the dominant members of the bacterial flora. More than 68.85% of sequences, at all the tested ages, were related to those of *Lactobacillus*. However, the sequences of bacterial populations varied significantly by age of the birds. At all ages, sequences were identified in the library showing homology to the genus *Clostridium*. There was a unique community structure at 3 days age with the sequences homologous to culturable bacteria such as *L. delbrueckii*, *C. perfringens* and *Campylobacter coli*. From 7 days of age to 21days, a similar community structure was maintained with dominant sequences related to *L. acidophilus*, *Enterococcus* and *Streptococcus*. To some extent the bacterial community at 49 days of age was similar to those at age 28 with the abundant sequences homologous to *L. crispatus*, but it was significantly different from those sequence from the other ages. The role of those bacteria nutrient acquisition, intestinal heath and growth promotion remain to be defined.

Introduction

It has long been known that densely colonized intestinal bacteria play an important role in the health and performance through its effect on gut morphology, nutrition, and pathogenesis of intestinal disease and immune response of animal. Intestinal bacteria are primarily responsible for degrading the copious amounts of mucus produced by goblet cells in the intestinal mucosa. The microbial flora is may also protect against colonization of the intestines by pathogens and to stimulate the immune response (Mead, 1989).

Studies based on the culturable bacteria flora of chickens have been extensively conducted. The predominant bacteria present in the chicken ceca are obligate anaerobes (10^{11}/g) (Barnes, 1972). There have been at least 38 different types of anaerobic bacteria isolated from the chicken ceca (Barnes *et al.*, 1972) with more than 200 total bacterial strains isolated (Mead, 1989).

However, it is believed that only between 10 and 60% of the bacteria in the cecum grew in culture (Barnes *et al.* 1972; Barnes, 1972; Salanitro 1974).

Netherwood *et al.* (1999) used hybridization methods to monitor the response of bacterial flora in chicken cecum to probiotics and diet related differences were analyzed by Apajalahti *et al.* (1998) based on a percent G+C profiling. These studies demonstrated showed that many of the 16S rDNA sequences found in the chicken cecum were not closely related to any previous known bacterial genus. Zhu *et al.* (2002) isolated 243 unique partial 16S rRNA gene sequences from DNA isolated from the cecal content and the cecal mucosa.

Discussion and Conclusions

We used a molecular ecological approach to identify the bacterial composition and to determine community succession in the ileum of chickens fed a corn-soy diet lacking coccidiostats and growth-promoting antibiotics. We isolated random clones of 16S ribosomal DNA gene sequences after multiple PCR amplification of bacterial genomic DNA isolated from the ileum of chickens at 3, 7, 14, 21, 28 and 49 days of age. From analysis of 614 clones isolated from the 16S rDNA libraries, we identified four major phyla. These phyla included low and high G+C gram-positives, proteobacteria and the CFB group (Table A-1 and Fig. A-1). Eleven families or groups and sixteen genera were identified among the 16S rDNA sequences analyzed. The bacterial microbiota consisted predominantly of low G+C gram-positive bacteria, whose representative distinct sequences were shown in Fig. A-1, with *Lactobacillus* accounting for 68.85% of the total 16S rDNA sequences in the libraries. The low G+C gram-positives consisted of five families or groups represented by nine genera. Identification of members of dominant genera *Lactobacillus*, *Enterococcus* and *Streptococcus* were culturable and have been often isolated from normal ileum (Salanitro, 1978). However, we did not anticipate finding that *Clostridium* was a dominant group at age 3 and age 49 in the ileum according to previous studies (Barnes *et al.* 1972; Salanitro, 1978). We detected *Clostridium* spp. in the ileal flora at all ages. Stutz and Lawton (1984) reported detection of clostridia, including *C. perfringens*, by culture of the ileum of 2-day-old chicks. About 15% of our total sequences at 3 days of age had homology to *C. perfringens,* which is important cause of necrotic enteritis in broilers (George, 1982; Long, 1973). We also detected sequences of segmented, filamentous *Clostridium* spp., commonly found in healthy animals, at 14d of age (Snel, 1995).

There are various formulations of antibiotics used as growth promotants. In the US, many companies use Virginiamycin in the grower and finisher feed for broiler chickens. In order to determine its effect on the ileum microflora, we sequenced 16S rDNA genes isolated from libraries prepared from these birds at 28 and 49 days of age. Birds fed Virginiamycin contained significantly fewer *Lactobacillus* species in the ileum than controls at both ages. In addition, the ratios among the dominant *Lactobacillus* species and the dominant *Clostridium* species were different. Changes in the other bacterial populations appeared to be minor.

These findings may allow us to identify ways to achieve present day growth rate and feed efficiency without use antibiotics by manipulation of the intestinal flora. It may also identify methods to predict intestinal disease prior to the clinical manifestation of symptoms and methods to prevent colonization of pathogens, such as, *C. perfringens*, *Salmonella* spp. or *Campylobacter* spp.

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Fig. A-1. Percentage of bacterial sequences belonging to particular phylogenetic groups or subdivisions present in the ileum of chickens at different ages.

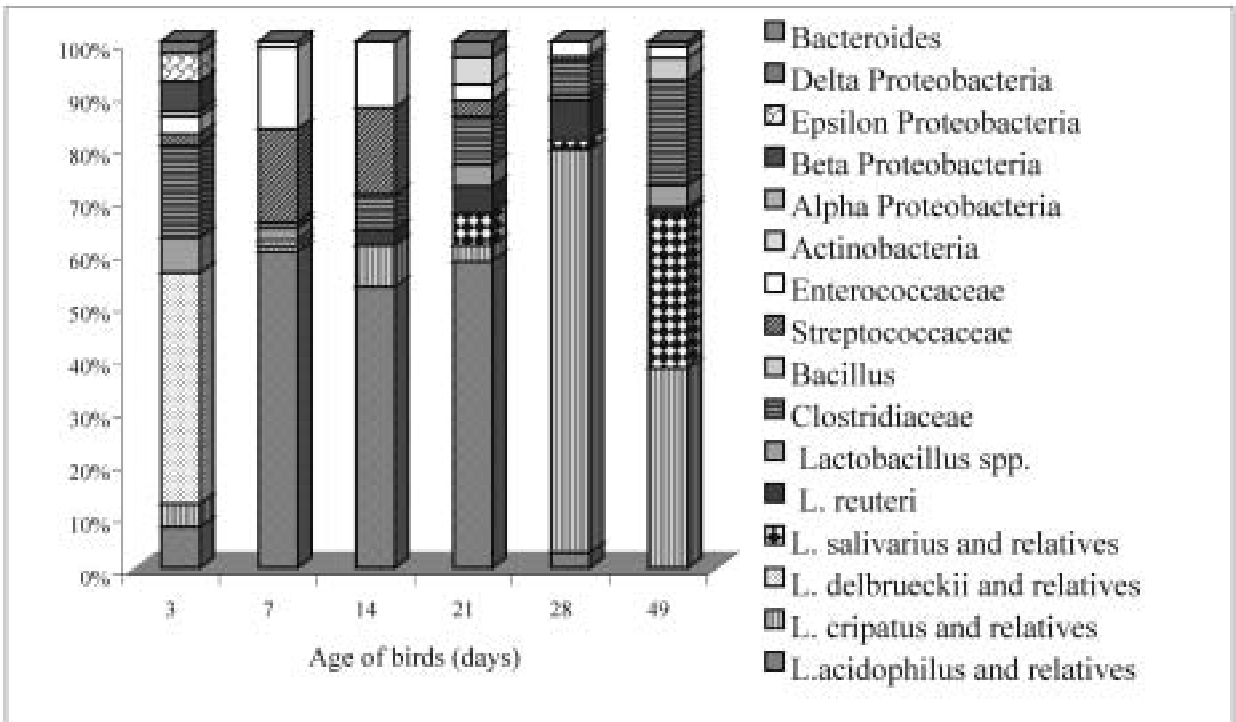

Table A-1. rDNA frequencies in ileum of chickens fed corn soy diet without growth-promoting antibiotics or coccidiostats.

Group		Genus or species	3 day		7 day		14 day		21 day		28 day		49 day	
			# of seq	**# of seq**	**# of seq**	**# of seq**	**# of seq**	**# of seq**	**# of seq**	**# of seq**	**# of seq**	**# of seq**	**# of seq**	**# of seq**
Low G+C Gram-positive (LGC)	*Lactobacillaceae*	*Lactobacillus spp.*	57	60.00	58	64.44	65	63.73	75	65.79	96	87.27	69	69.70
		L. acidophilus,	7		54		54		57		3			
		L. crispatus,	4		1		8		3		82		36	
		L. reuteri					3		5		8		1	
		L. delbrueckii	40		1									
		Weisella spp.	6											
		L. salivarius							6		2		28	
		L. gasseri							3					
	Clostridiaceae	*Clostridium spp.*	16	16.84	1	1.11	7	6.86	9	7.89	7	6.36	19	19.19
		C. perfringens	15											
		Ruminococcus							3					
		Eubacterium spp.							5					
	Bacillaceae	*Bacillus*											4	4.04
	Staphylococcaceae	*Staphylococcus*	2	2.11					3	2.63				
	Streptococcaceae	*Streptococcus*	2	2.11	16	17.78	17	16.67	3	2.63	1	0.91		
	Enterococcaceae	*Enterococcus*	3	3.16	14	15.56	13	12.75	3	2.63	3	2.73	2	2.02
High G+C Gram-positive (HGC)	*Actinobacteria*	*Fusobacter*							5	4.39				
		prausnitzii												
		Bifdobacter			1	1.11								
	Bacteroides													
Proteobacteria (gram-negative)	Alpha	*Ochrobactrum*	1	1.05										
	Beta	*Alcaligenes*	4	5.26										
		A. faecalis,	1											
	Epsilon	*Campylobacter*	5	5.26										
	Delta	*E. coli*	1	2.11										
		Salmonella enterica	1											
CFB phylum	Bacteroides	*Bacteroides spp.*							3	2.6			1	1.01

Table 2. rDNA frequencies in ileum of chickens fed corn soy diet with and without growth-promoting antibiotics.

Group		Genus or species	28 day virginiamycin		28 day no promotants		49 day virginiamycin		49 day no promotants	
			# of seq	# of seq	# of seq	# of seq	# of seq	# of seq	# of seq	# of seq
Low G+C Gram-positive (LGC)	*Lactobacillaceae*	*Lactobacillus spp.*	30	32.25	96	87.27	27	45.76	69	69.70
		L acidophilus,			3					
		L. crispatus,	16		82		12		36	
		L. aviarius					12		28	
		L. reuteri	11		8		3		1	
	Clostridiaceae	*Clostridium spp.*	58	62.37	7	6.36	26	44.06	19	19.19
		C. irregularis	58							
		C. Lituseburense					25			
	Bacillaceae	*Bacillus*	1	1.08			3	5.08	4	4.04
	Streptococcaceae	*Streptococcus*	17	1.08	3	2.63	1	0.91		
	Enterococcaceae	*Enterococcus faecium*	3	3.23	3	2.63	3	5.08	2	2.02
Proteobacteria (gram-negative)	Alpha	*Ochrobactrum anthroopi*	1	1.08						
	Beta	*Achromobacter spp.*	2	2.16						
CFB phylum	*Bacteroides*	*Bacteroides spp.*							1	1.01

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“Slide Presentation”

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Figure A-2.

Figure A-3.

Figure A-4.

Figure A-5.

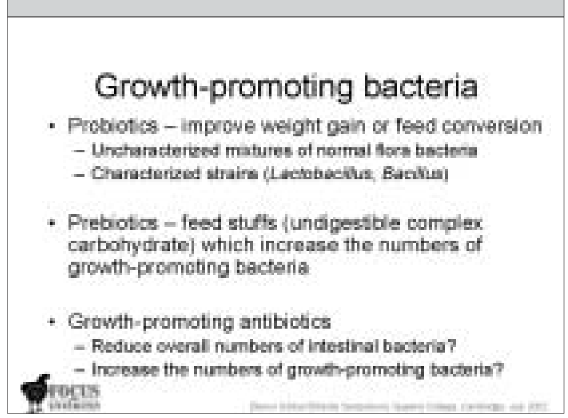

Figure A-6.

Normal flora

- Skin
- Mucosal surfaces
  - Mouth
  - Gastrointestinal system
  - Genito-urinary system
    - vagina
    - distal urethra
  - Respiratory system
    - nares
    - trachea

Figure A-7.

Normal flora of GI

- Approximately 10^{11} CFU/gram
- Bacteria primarily associated with mucus and macromolecular food matrix (fiber)
- Composition varies in different portions of GI
- Composition varies in different animals

Figure A-8.

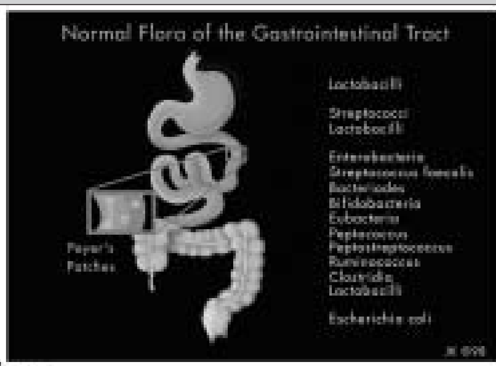

Figure A-9.

Drasar & Barrow, Intestinal Microbiology, ASM 1985.

Animal	Stomach	Ileum	Cecum	Feces
Man	none	E. coli 10[illegible] Lactobacilli 10[illegible] Bacteroides 10[illegible]	E. coli 10[illegible] Lactobacilli 10[illegible] Enterococci 10[illegible] Bacteroides 10[illegible]	E. coli 10[illegible] Lactobacilli 10[illegible] Enterococci 10[illegible] Bacteroides 10^{11}
Cow	E. coli 10[illegible] Lactobacilli 10[illegible] Streptococci 10[illegible] Bacteroides 10[illegible]	E. coli 10[illegible] Lactobacilli 10[illegible] Enterococci 10[illegible]	E. coli 10[illegible] Lactobacilli 10[illegible] Enterococci 10[illegible]	E. coli 10[illegible] Lactobacilli 10[illegible] Enterococci 10[illegible]
Pig	E. coli 10[illegible] Lactobacilli 10[illegible] Streptococci 10[illegible]	E. coli 10[illegible] Lactobacilli 10[illegible] Streptococci 10[illegible]	E. coli 10[illegible] Lactobacilli 10[illegible] Enterococci 10[illegible] Bacteroides 10[illegible]	E. coli 10[illegible] Lactobacilli 10[illegible] Enterococci 10[illegible] Bacteroides 10[illegible]
Cat	E. coli 10[illegible] Clostridia 10[illegible] Streptococci 10[illegible]	E. coli 10[illegible] Clostridia 10[illegible] Enterococci 10[illegible] Lactobacilli 10[illegible]	Clostridia 10[illegible] Lactobacilli 10[illegible] Enterococci 10[illegible] Bacteroides 10[illegible]	Clostridia 10[illegible] Lactobacilli 10[illegible] Enterococci 10[illegible] Bacteroides 10[illegible]
Chicken	Lactobacilli 10[illegible] Streptococci 10[illegible]	Lactobacilli 10[illegible] Enterococci 10[illegible]	E. coli 10[illegible] Lactobacilli 10[illegible] Enterococci 10[illegible] Bacteroides 10[illegible]	E. coli 10[illegible] Lactobacilli 10[illegible] Enterococci 10[illegible] Bacteroides 10[illegible]

Figure A-10.

Chicken Microflora Study

- Purpose – characterize the true composition of the intestinal microflora in order to identify populations affected by the growth-promoting antibiotics.
- Goal – develop reliable microflora modification approaches to replace growth-promoting antibiotics in broiler chicken production.

Figure A-11.

Methods – PCR library of 16S sequences

- PCR amplification using universal bacterial 16S primers
- Clone PCR products
- DNA sequence individual clones
- Compare sequence to known taxonomic groups for identity

Figure A-12.

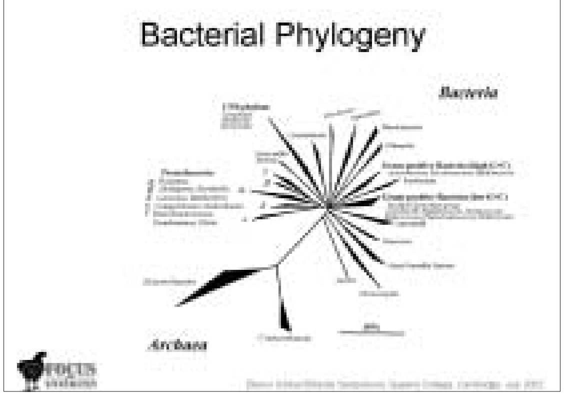

Figure A-13.

Microbial Composition of the Cecum (%)

Group	3d	7d	14d	21d	28d	49d
LGC	82	90	84	56	62	80
HGC		2	10	31	35	9
Proteob	16		1			
CFB	1	7	5	12	5	6

Figure A-14.

Microbial Composition of the Ileum (%)

Group	3d	7d	14d	21d	28d	49d
LGC	84	99	99	93	99	99
HGC		1	1	4		
Proteob	13					
CFB	1			3		1

Figure A-15.

Microbial Composition of the Cecum (%)

Group	Species	3d	7d	14d	21d	28d	49d
LGC	*Lactobacillus*	23			1		
	Clostridia	42	90	83	54	60	80
	C. perfringens	13					
	Steptlenterocc	3					
HGC	*Actinobacterium*		1	9	31	35	9
Proteob		16		1			
CFB	*Bacteroides*		7	5	11	5	6

Figure A-16.

Microbial Composition of the Ileum (%)

Group	3d	7d	14d	21d	28d	49d
Lactobacillus spp	60	64	64	66	88	70
Clostridiaceae	17	1	7	9	7	19
Bacillus						4
Staphylococcus	2			3		
Streptococcus	2	18	17	3	1	
Enterococcus	3	16	13	3	3	2
Bifidobacter		1				
α-Proteobacteria	1					
β-Proteobacteria	5					
ε-Proteobacteria	6					
δ-Proteobacteria	2					
Bacteroides				3		1

Figure A-17.

Ileum *Lactobacillus* species
(% of total sequences)

species	3d	7d	14d	21d	28d	49d
L. acidophilus	7	60	53	50	3	
L. crispatus	4	1	8	3	75	36
L. reuteri			3	5	8	1
L. delbruickii	42	1				
L. salivarius				6	2	28
L. gasseri				3		

Figure A-18.

Ileum *Clostridiaceae* (% of total sequences)

species	3d	7d	14d	21d	28d	49d
Clostridium spp.	1	1	7	8	7	19
C. perfringens	16					
Ruminococcus spp.				3		
Eubacterium spp.				5		

Figure A-19.

Conclusions

- Bacterial microflora of chickens is primarily gram positive.
- Major components are low G+C gram positive bacteria
  - Lactobacilli
  - Clostridia
- Virginiamycin greatly affects clostridia/lactobacillus ratio

Notes:

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