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Protection at the intestinal level – the role of nutrition

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Session 07, no. 1

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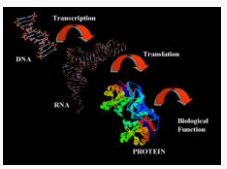

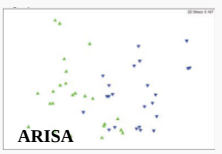

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Complex interactions

heterogenity, dosage, application time / frequency, application route

Probiotic
other dietary factors

microbiota

Taxonomical / functional diversity, inter- / intraindividual heterogenity, time dynamic, mostly unknown,…..

host

Age, genetic, race, segment of intestinal tract, intestinal defence (intestinal structure, secretion, mucus…), immune status, health status ,……..

environment

climate, housing, management, feeding, drugs, stress, treatments, ……

Adapted from Taras et al, 2009

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Microbial Composition of the Broiler Intestine (16S clone libraries)

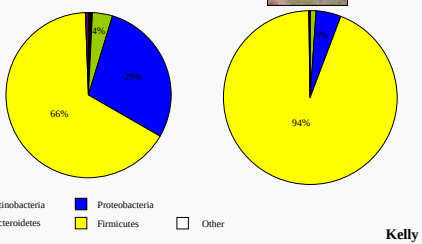
Cecum

- Lactobacillaceae(8.5%)
- Clostridia(82%)
- Bacillus(1.5%)
- Streptococcaceae(0.7%)
- Enterococcaceae(1.0%)
- Actinobacteria(0%)
- Alpha(0.8%)
- Beta(0.7%)
- Gamma(1.3%)
- Flavobacteriaceae(0.2%)
- Bacteroidaceae(5.1%)
- Unknown bacteria(1.1%)

Ileum

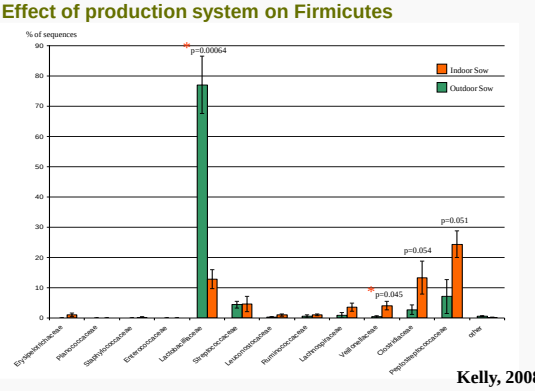
Lee, 2007

Different production systems lead to different microbiota composition

Kelly et al , 2008

Effect of production system on Firmicutes



Kelly, 2008

Complex interactions

The elucidation of the mode of action of probiotics is hinderd by

- **the complexity of the intestinal microbiota**
- **the complexity of the intestinal interactions**
- **the complexity of the probiotic microbe**
- **experimental limitations**
- **……**

Taras et al, 2009

Industry (field)constrains

- **Limited information on host, microbiota and production environment**
- **Economics**
- **Ingredient availablity**
- **Logistics**
- **Production technology**

Maintenance of a continual intestinal equilibrium,

…with the objective of nutrient recovery

…and production efficiency and host health

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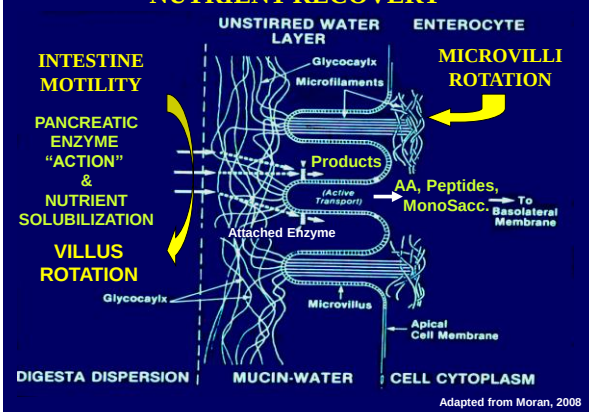
- GI system and possible adaptations
- Interactions betweem the GI system and its microbiota
- Dietary manipulation of the GI microbiota and intestinal health

NUTRIENT RECOVERY



Adapted from Moran, 2008

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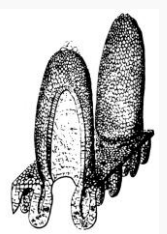
CONTINUOUS VILLUS ADAPTATION

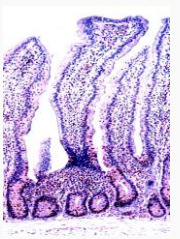


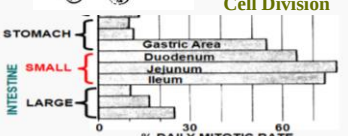
Extrusion

Optimum Function

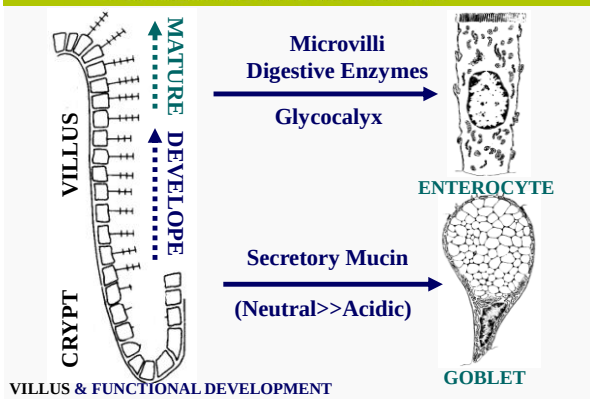
Instituting Alterations

Cell Division

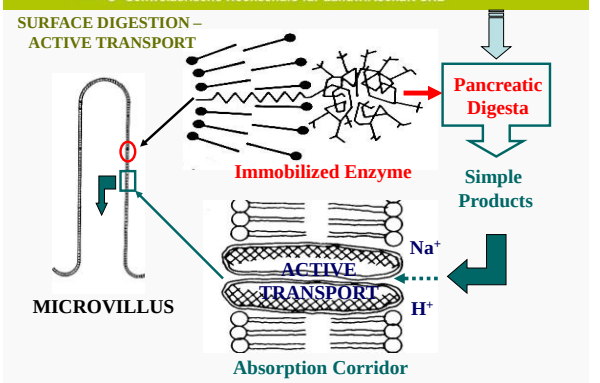




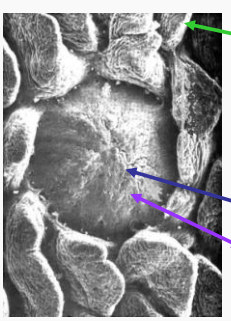
Moran (2008), Uni et al. (1998)



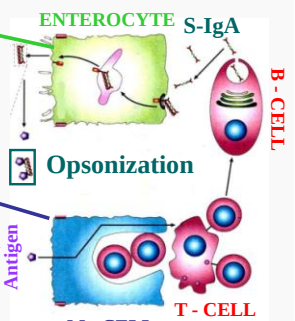
VILLUS & FUNCTIONAL DEVELOPMENT



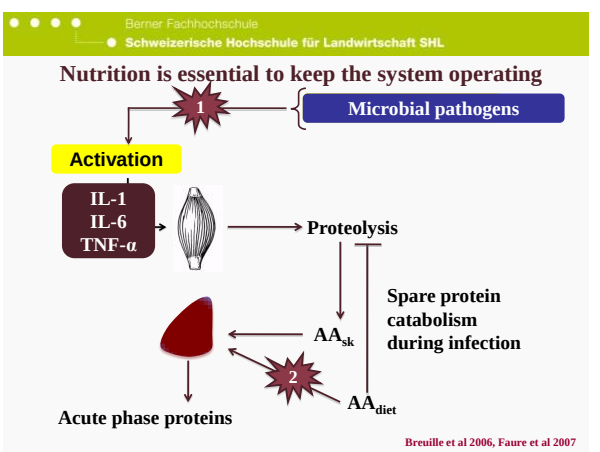
GALT



PEYER'S PATCH
Komuro & Hashimoto (1990)



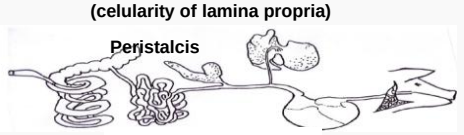
Rojas & Apodaca (2002)



Breuille et al 2006, Faure et al 2007

Effect of microbiota

Intestinal mass
(celularity of lamina propria)



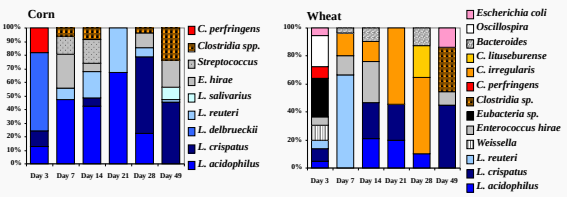
Peristalcis

Cell turnover
Villus : crypt
Mucus dynamics
Brush border enzymes
Absorption

Gastric acid

immunological defence

Composition of broiler ileum (T-RFLP analysis of 16S rRNA)



Lee, 2007

Commensalism or Symbiosis?

Pathogenic ⟷ **Commensal** ⟷ **Symbiotic**

E. coli ⟷

Clostridia ? ⟶

⟵ ***Enterococcus/Streptococcus*** ⟶

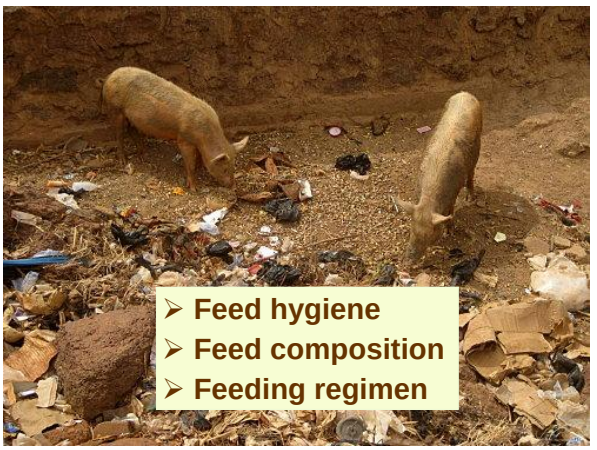
⟵ ***Bacteroides*** ⟶

Lactobacillus ⟶

CE- cultur for piglets

Farm	Treatments	n	Mortality and cull
A	Control	3242	9.06
	CE culture	10402	2.80
B	Control	6318	3.33
	CE culture	4900	2.54
C	Control	3068	3.30
	CE culture	3127	2.45
D	Control	1331	9.00
	CE culture	1288	4.20

Harvey et al., 2003



- Feed hygiene
- Feed composition
- Feeding regimen

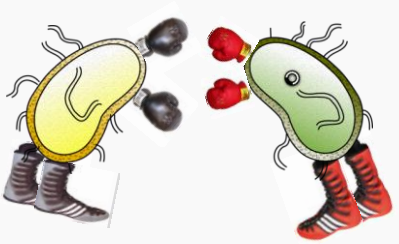
Nutrients for host (in particular digestive system

Undernutrition

- Weaning
- Disease

Specific nutrients

- Energy for gut (SCVA, AA,…
- Threonin for mucus syntheses
- FA for immune modulation
- Antioxidants
- Nucleotids
- ……

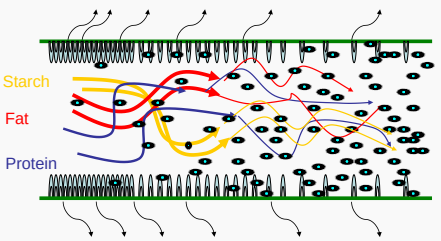


What are match decisive factors?

Key factors

- Nutrient availabily
- Intestinal environment / passage rate
- Inhibiting substances
- Surface environment and adherence
- Immunstatus of host
- …..

Nutrient concentration and availability



80% ileal digestibility
Increase of 2%: 10% less in the large intestine

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Reducing undigested protein material at ileum

Dietary CP, g/kg	224	204	184
Faeces consistency, % (days as % of total days on trial)			
Hard faeces	81.9	82.0	95.4
Soft faeces	14.7	14.5	4.1
Liquid faeces	3.4	3.5	0.5
N excretion, g/d	10.7	9.4	6.8

Le Bellego and Noblet, 2002

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Effect of dietary CP concentration on post weaning diarrhea

Days	1 - 7	8 - 14	15 - 21	22 - 28
High CP 14				
Low CP 7				
Low CP 14				

256 g CP
175 g CP
213 g CP

Challenge: 72, 96 and 120 h after weaning with ETEC

J. Anim. Sci: 87:2833-2843 (2009)

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Effect of dietary CP concentration on diarrhea score post weaning

Period	HP14	LP7	LP14[4]
d 1 to 7	16.7	10.1	
d 8 to 14	22.6[a]	7.1[b]	8.3[b]
d 1 to 14	19.6[a]	9.5[b]	8.3[b]

J. Anim. Sci: 87:2833-2843 (2009)

Effect of dietary CP concentration on diarrhea score post weaning(with challenge)

Period	HP14	LP7	LP14[4]
d 1 to 7	48.8	29.7	
d 8 to 14	40.5[c]	20.2[a]	26.2[a]
d 1 to 14	44.6[c]	21.4[a]	31.5[d]

J. Anim. Sci: 87:2833-2843 (2009)

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Effect of dietary CP concentration on piglet performance (with challenge)

Period	HP 14	LP 7	LP 14
ADG, g			
d 1 to 14	55	63	63
d 15 to 28	352	334	296
d 1 to 28	204	179	199
ADFI, g			
d 1 to 14	196	206	189
d 15 to 28	501	486	418
d 1 to 28	349	304	346
G:F, g/g			
d 1 to 14	0.28	0.31	0.34
d 15 to 28	0.70	0.71	0.70
d 1 to 28	0.58	0.58	0.59

J. Anim. Sci: 87:2833-2843 (2009)

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Role of protein fermentation in intestinal health

Intestinal bacterial groups
- ***Bacteriods***
- ***Clostridium***
- ***Enterobacterium***
- ***Lactobacillus***
- ***Streptococcus***

→ **Decarboxylation of amino acids; Breakdown of polyamines**

↓

Produce toxic amines
- **Putrescine**
- **Cadaverine**
- **Histamine**
- **Tyramine**

Ammonia, BCFA, indole, phenols etc.

← **Implicated on intestinal health in particular in the aetiology of PWD**

Modified from Gaskins, 2001

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Animal protein feed restrictions to be eased 22 Jul 2010

The European Commission (EC) has proposed allowing the use of animal meal to feed fish, chickens and pigs.

This practice is currently prohibited to prevent the spread of bovine spongiform encephalopathy (BSE), known "mad cow disease", reports FIS.

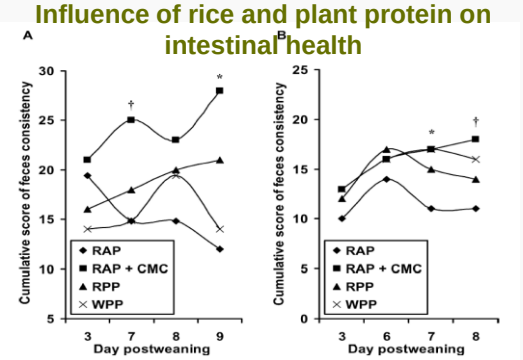
The commission approved a proposal to ease the restrictions in force because it believes that, thanks to the efforts to combat diseases of the group of transmissible spongiform encephalopathies (TSEs), there was a significant decrease in the number of animals affected.

"We're finally on the verge of eradicating the disease in the European Union (EU)," said EU Health Commissioner, John Dalli, adding that any change in the measures will not affect the aim of protecting citizens.

From 2011, a certain "tolerance" level of processed animal proteins will be accepted in the feed used for animals other than ruminants. However, EU authorities want to maintain the ban on the use of animal protein for ruminating mammals.

http://www.allaboutfeed.net/news/animal-protein-feed-restrictions-to-be-eased-id4621.html

Influence of rice and plant protein on intestinal health



1 = normal; 4 = diarrhea

(J Anim Sci 2004. 82:2364-2374)

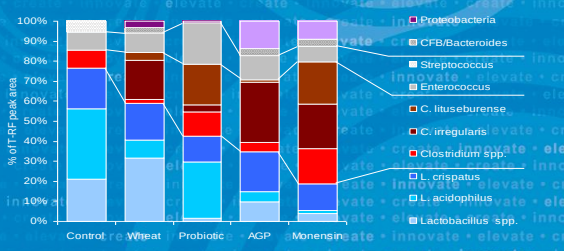
***C. perfringens* proliferation in various digested diets incubated at 40°C**

	Corn-based diet (n = 6)	Barley-based diet (n = 7)	Wheat-based diet (n = 7)
Median ($\times 10^8$ CFU/ml)	3.78^A	5.90^B	5.80^B
First quartile ($\times 10^8$ CFU/ml)	3.41	4.90	5.25
Third quartile ($\times 10^8$ CFU/ml)	4.06	7.95	6.90

Avian Pathology, 31: 6, 598 — 601 (2002)

Broilers fed different diets

(all ages combined community of ileum)



Lee et al. 2004

Influence on rice and plant protein on hemolytic E. coli

(Swab scores, % hemolytische E. coli)

	RAP	RAP+CMC	RPP	WPP	SEM	P-value
Ileum	16.7^a	53.3^b	13.3^b	36.7^{ab}	4.3	0.012
Colon	16.7^a	70.0^b	23.3^b	63.3^b	4.3	0.001

Lower digestive tract weight with RAP

(J Anim Sci 2004. 82:2364-2374)

Comparison wheat vs. extruded rice with and without oat hulls

Cereal source…	Extruded rice		Wheat		
OH (g/kg)…	0	20	0	20	SEM
Pigs with diarrhoea (*n*)	5/12	2/12	0/12	1/12	
Incidence of PWD†	12·5[a]	3·6[b]	0·0[b]	1·8[b]	2·03

(Br J Nutr 2008: 99:1217-25)

Role of NSP after infection with B. hyodysenteriae

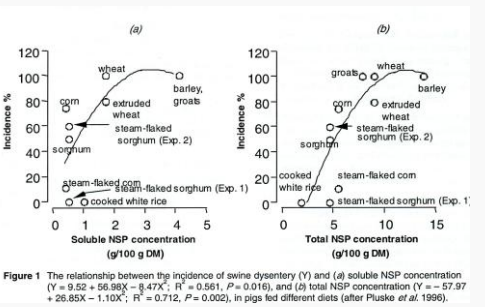
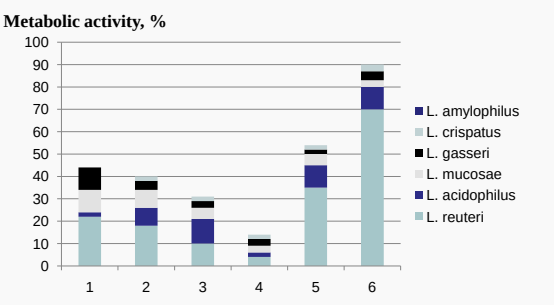


Figure 1 The relationship between the incidence of swine dysentery (*Y*) and (*a*) soluble NSP concentration ($Y = 9.52 + 56.98X - 8.47X^2$; $R^2 = 0.561$, $P = 0.016$), and (*b*) total NSP concentration ($Y = -57.97 + 26.85X - 1.10X^2$; $R^2 = 0.712$, $P = 0.002$), in pigs fed different diets (after Pluske *et al.* 1996).

Pluske et al., 1996

Enzyme addition and metabolic activity of lactobacilli



Simon, 2001

Feed structure as a tool to control nutrient availability: Effect on salmonella

Parameters*	Fine grinding	Coarse grinding
Meat juice, % positive	24.4	5.95
Tonsils, % positive	38.8	13.2
Bile, % positive	4.3	1.66
Ileal-cecal lining	19.5	13.7
Cecal content	45.1	15.3
Carcass surface	8.15	3.85

***Average of 3 problem farms**

Kampheus , 2010

NSP: Concentration and from

Soluble NSP
↑ Water holding capacity
↑ Viscosity

↓ Contact between digesta and enzymes
↓ Digesta flow
↑ Thickness of mucosal layer
↑ Proliferation of microbes
↓ Pancreatic enzyme activity
↓ Absorption rates in the SI
↑ Endogenous secretions

↑ Undigested material entering the LI

negative effect on microbiota

Insoluble NSP
↑ Water holding capacity but lesser extent and not viscous
Dilute nutrient concentration
↑ Digesta flow

↓ Nutrient digestibility

Slowly fermented in the LI, reduced AA break down

Positive effect on microbiota

Adapted from Mullen, 2010

Acids and fecal score in weaning piglets

	Group							
Day	1 NC	2 LS	PA	LA	FOA	MA	CA	FA
1–7	4·57*	0·89‡	3·11†	1·07†	1·79†‡	2·86†	2·50†	2·00†‡
8–14	11·39*	3·93¶	8·04†	4·46¶	5·54§	6·82‡	6·25‡	6·68†
15–21	4·57*	1·36**	3·89†	1·64**	1·89¶	3·25†‡	2·79†§	2·39§¶
22–28	1·96*	0·61§	1·54*†	0·57§	0·79§	1·04†§	1·29†‡	0·93†§
1–14	7·98*	2·41¶	5·57†	2·77§¶	3·66†§	4·84†‡	4·38‡	4·34†
15–28	3·27*	0·98¶	2·71†	1·11¶	1·34§¶	2·14‡	2·04‡	1·66†§
1–28	5·63*	1·70**	4·41†	1·94¶**	2·50§¶	3·49†‡	3·21†§	3·00†§

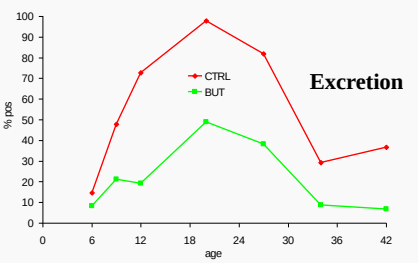
Mean values in one row with at least one same superscript symbol are not statistically different ($P > 0.05$);
*, †, ‡, §, ¶, **Mean values in one row with different superscript symbols differ ($P < 0.05$)
NC, negative control; LS, lincospectin 22 premix; PA, propionic acid; LA, lactic acid; FOA, formic acid; MA, malic acid; CA, citric acid; FA, fumaric acid.

Research in Veterinary Science 2001, 70, 287–293

Acid and cecal salmonella colonization in broilers

Van Immerseel, 2008

Butyrate and cecal salmonella colonization in broilers



Van Immerseel, 2008

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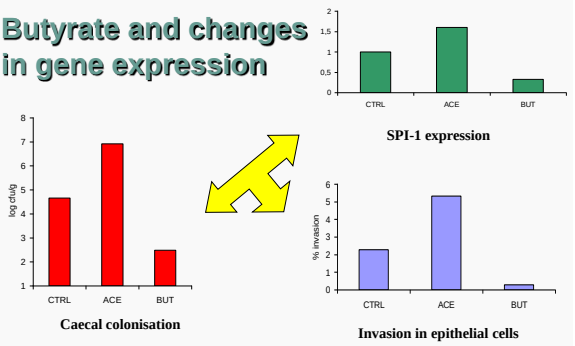
Butyrate and changes in gene expression



Van Immerseel, 2008

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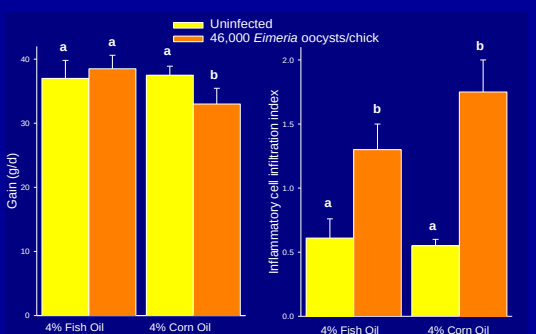
Effect of plasma and different feed additives on health in weaning piglets

	Dietary treatments[b]					
	SDPP – EYA	PPI – EYA	PPI + EYA	PPI + ZnO	PPI + FA	PPI + AB
Scours scores[c]						
8 h after *E. coli* challenge	1.9[xy]	2.4[z]	1.5[y]	2.3[z]	2.1[xy]	1.4[y]
24 h after *E. coli* challenge	2.0[z]	2.7[y]	1.6[x]	1.9[z]	1.9[z]	1.6[x]
48 h after *E. coli* challenge	1.6[z]	2.7[y]	1.3[x]	1.4[z]	1.3[x]	1.1[x]
7 d after *E. coli* challenge	0.5[x]	2.2[y]	0.3[x]	0.6[x]	0.5[x]	0.2[x]
Percentage of pigs shedding *E. coli* (K88)						
8 h after *E. coli* challenge	73	80	67	87	80	53
24 h after *E. coli* challenge	80	100	53	67	73	67
48 h after *E. coli* challenge	53[xy]	85[y]	27[x]	62[xy]	64[xy]	29[x]
7 d after *E. coli* challenge	29[z]	81[y]	23[x]	42[xy]	31[z]	21[x]
Scouring days[d]	4	7	3	4	5	4
Mortality, No.	1	6	1	2	1	2
Mortality, %	6.6[x]	40.0[y]	6.6[x]	13.3[x]	6.6[x]	13.3[x]

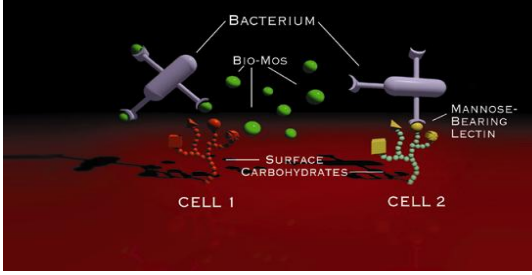
(J Anim Sci 2003. 81:1790-1798)

n-3 PUFAs can ↓ body weight loss during immune responses

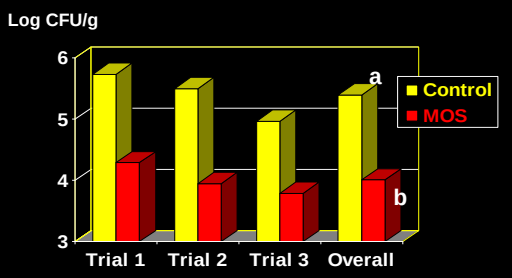


Effect of dietary oil source on broiler performance and immune response.
Data adapted from Klasing and Korver, 1995.

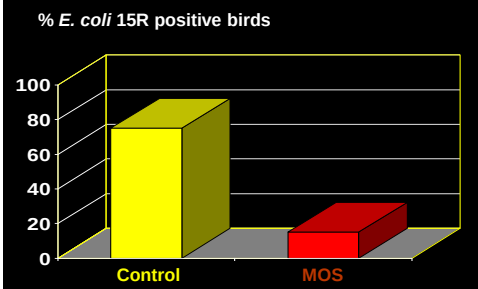
MOS blocks microbial attachment on the enterocyte



Effect of MOS on Cecal *Salmonella typhimurium* 29E Concentrations



Effect of MOS on Cecal *Escherichia coli* 15R Concentrations



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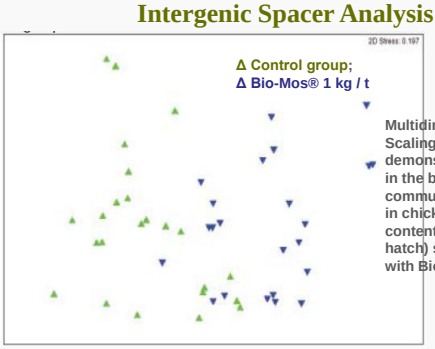
ARISA-Analyses : Automated Ribosomal Intergenic Spacer Analysis



Multidimensional Scaling (MDS) plot demonstrating changes in the bacterial community composition in chicken caecal contents (14 days post hatch) supplemented with Bio-Mos®.

Effects of MOS and Virginiamycin on Jejunum morphology of 14 d Turkey Hens

Measurement	Control	Bio-Mos	VM
Villus Height (mcm)	905	823	855
Crypt Depth (mcm)	104^{a}	86^{b}	98^{ab}
Goblet Cells/mm villus	116^{b}	169^{a}	137^{ab}

Parks et al. (2000)

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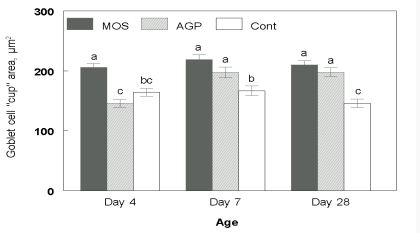
Effect of Bio-Mos on Mucin dynamics: Goblet cell "cup" area of Broilers



MOS

Control

Uni, 2006

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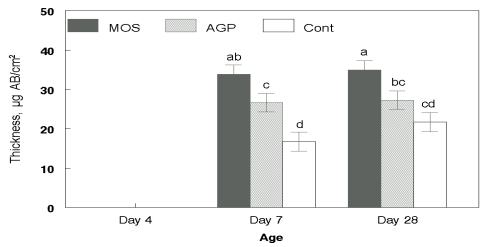
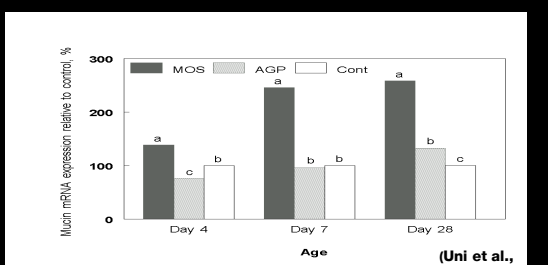
Effect of Bio-Mos on Mucin dynamics: Thickness of the jejunal mucus adherent layer of Broilers

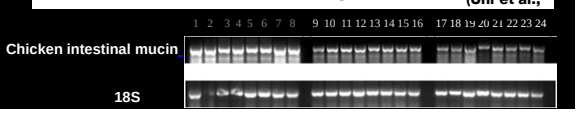


Uni, 2006

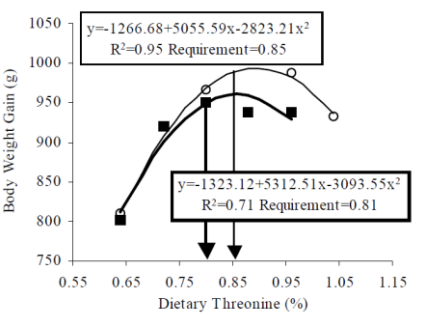
Effect of Bio-Mos on Mucin dynamics: Mucin mRNA expression of broilers



(Uni et al.,



MOS and threonin interaction

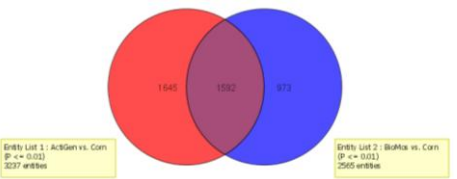


Seng Huan Chee, 2009

Description of wheat challenge model and transcript analyses

- 9 replicate pens (6- 1d old chicks/pen)
- Standard balanced corn-soy diet
- Wheat challenge (corn replaced with wheat)
  - Wheat challenge with Bio-Mos (2 kg/t)
  - Wheat challenge with: Actigen (2nd gegeration MOS product)
- Measured growth and feed efficiency
- Gene expression in jejunum measured at 21 days (7 birds per treatment)
  - Microarray "GeneChip Chicken Genome Array" with 37,703 probe sets representing 28,000 genes

Some key changes in gene expression patterns measure relative to the corn based diet



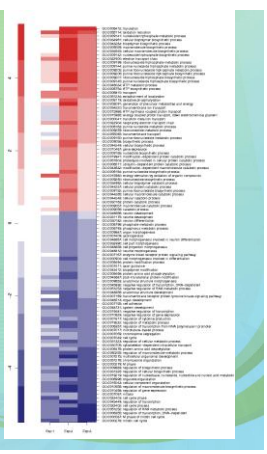
- Of the 4210 genes modified by Bio-Mos and Actigen supplementation of challenge diets 1592 (38%) were effect the same way by both supplements

Key metabolic processes in the jejunum affected by BioMos

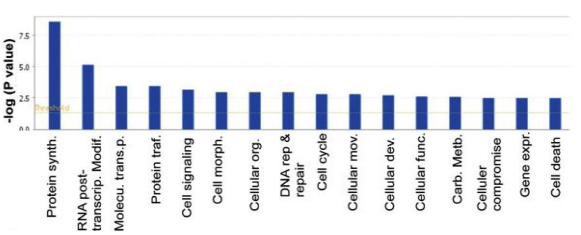
PAGE Biology processes evaluation

(Corn as Control vs. Wheat, BioMos, P<0.01, FDR<0.05)

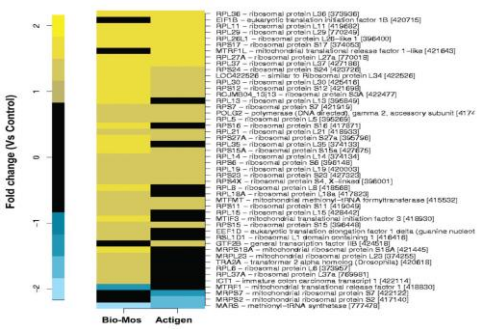
- Up-regulated
  - Protein translation
  - Energy production (e-transport and ATP production)
  - Transport systems
  - Digestive enzymes
  - Components of immune response
- Down-regulated
  - Stress responsive pathways
  - Protein degradation pathways



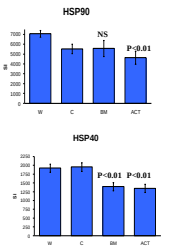
Molecular and cellular functions regulated by Bio-Mos



Protein synthesis related genes up-regulated by Bio-Mos® or Actigen

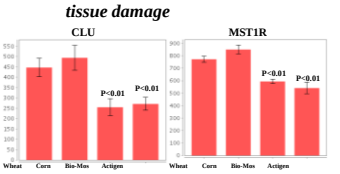


Effects of Bio-Mos and Actigen on gene expression: Decreased expression of stress protein and indicators of tissue damage in the small intestine



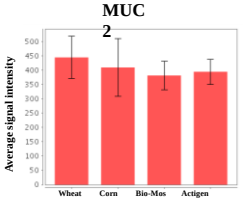
Both supplements decreased expression of stress related genes

Both supplements decreased expression genes associated with tissue damage

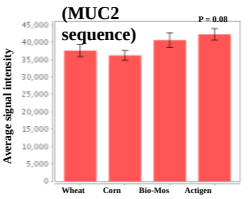


Biomarkers: Less tissue stress and damage

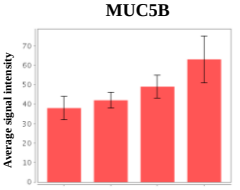
MUC 2



LOC771289 (MUC2 sequence)

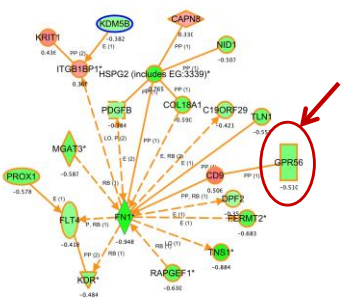


MUC5B



Regulation of mucin associated genes in intestinal tissue of broilers by Bio-Mos or Actigen

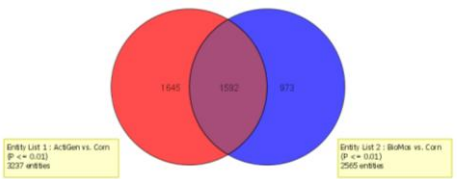
Complex effects of mannan oligosacchrides on Fibronectin associated gene interactions



Repressor of mucin and cell surface glycosylation controlling mannan levels: influencing mucin structure and function

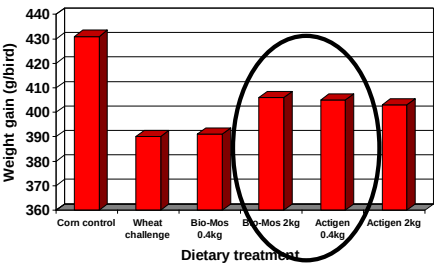
(Weimer, 2010)

Some key changes in gene expression patterns measure relative to the corn based diet



Of the 4210 genes modified by Bio-Mos and Actigen supplementation of challenge diets 1592 (38%) were effect the same way by both supplements

Dose effects of BioMos and Actigen on the 14-d gain of broiler chick fed a wheat-based challenge diet



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Key factors

- Nutrient availabily
- Intestinal environment / passage rate
- Inhibiting substances
- Surface environment and adherence
- Immunstatus of host
- …..

Nutrigenomics is providing new tools for evaluating nutritional response

- **Rapid product development (screening)**
- **Fewer resources are used**
- **Process based on physiological responses**
- **Defines new areas for development (hidden nutrition)**

Designing specific animal studies (under field like conditions) to allow for quantitative evaluation of responses