

Prebiotics and probiotics in animal production: present status and future perspectives

R. Ducatelle, F. Haesebrouck and F. Van Immerseel



Department of Pathology, Bacteriology and Avian Medicine
Faculty of Veterinary Medicine



We are born 100% human

**We are born 100% human
but we die 100% bacterial**

**We are born 100% human
but we die 100% bacterial
in between we are 90% bacterial**

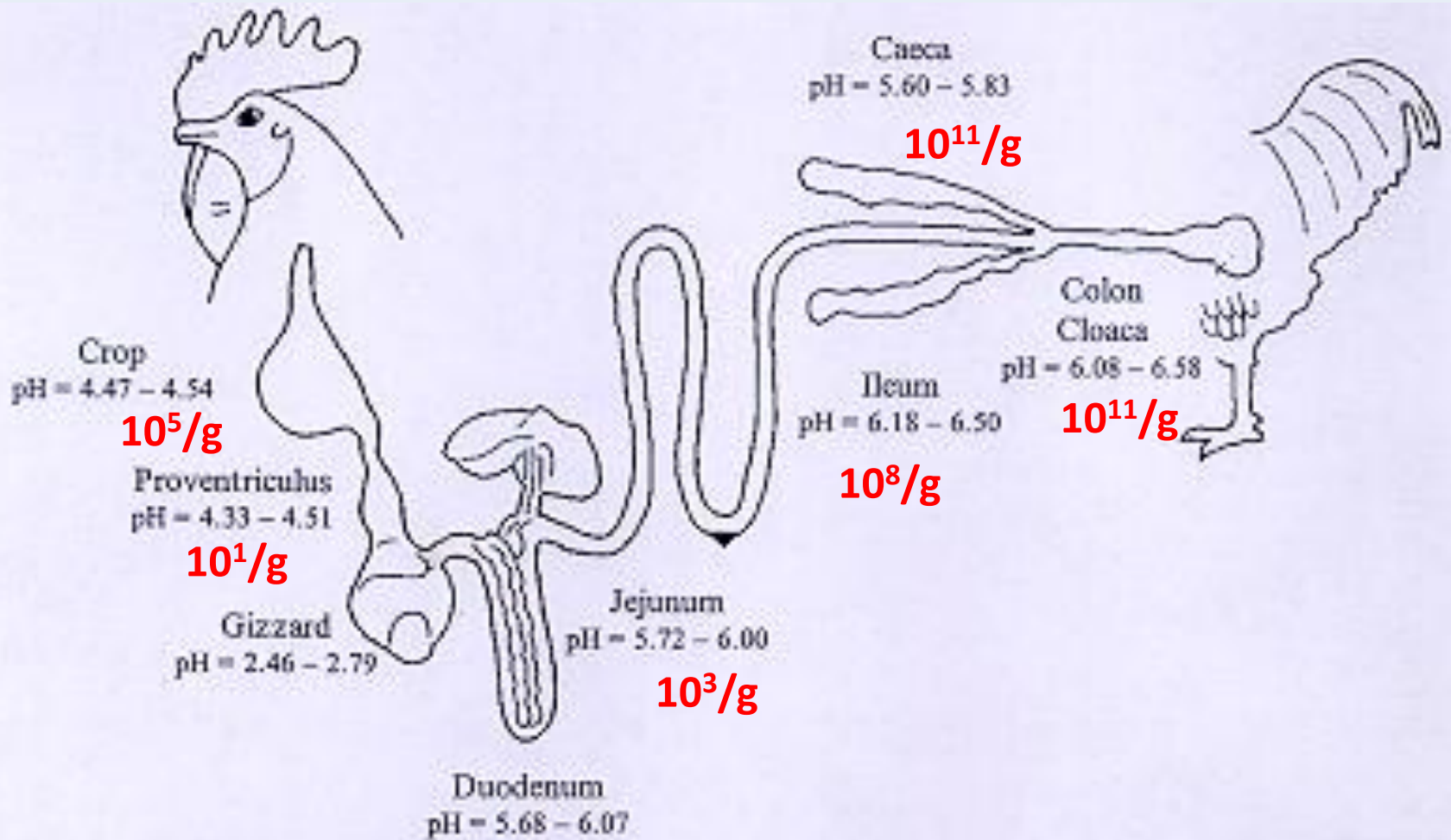


Figure 1 Diagram of the digestive tract of chickens and pH values of the digestive contents (Farner, 1942).

1. It is forbidden by law in the EU to use antibiotics for treatment of *Salmonella* in poultry
2. It is forbidden by law in the EU to use antibiotics as growth promoters in farm animals

Probiotics:

Single or mixed cultures of living microorganisms which beneficially affect the host by improving the properties of the indigenous microbiota.
(Fuller, 1992)

Prebiotics:

Non-digestible feed ingredients that selectively favor the multiplication or metabolic activity of a specific fraction of the intestinal microbiota.
(Gibson & Roberfroid, 1995)

European Union
Register
of Feed Additives

*pursuant to Regulation
(EC) No 1831/2003*

- >100 microorganisms
- >30 gut flora stabilizers
- No separate category for prebiotics



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Review

An update on alternatives to antimicrobial growth promoters for broilers

Gerard Huyghebaert^{a,*}, Richard Ducatelle^b, Filip Van Immerseel^b

^a Ministry of the Flemish Community, Institute Agriculture Fishery Research ILVO Animal Nutrition Sciences, Scheldeweg 68, B-9090 Melle, Belgium

^b Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

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(Fuller, 1992)

Enterococcus, Lactobacillus, Saccharomyces, Bacillus, Pediococcus

Prebiotics:

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Probiotics improve performance under challenge conditions:

Coccidiosis

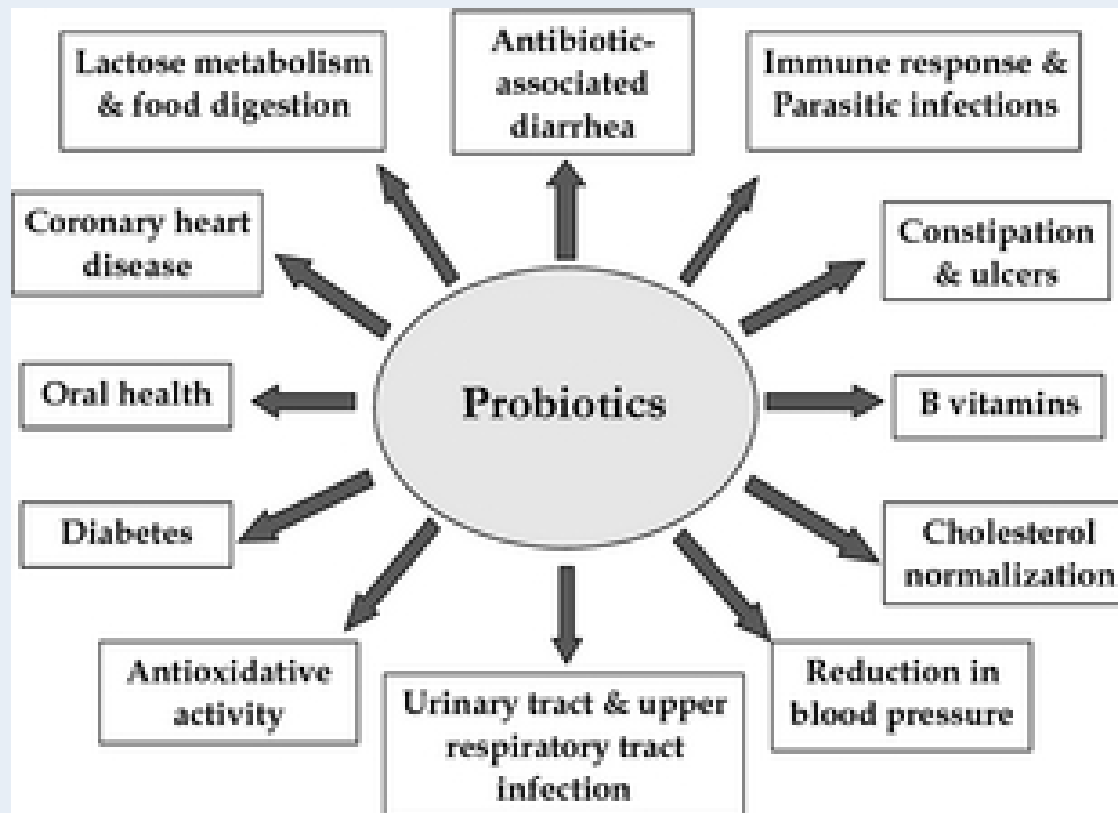
Clostridium perfringens

Salmonella

LPS (acute phase response)

Probiotics, their health benefits and applications for developing healthier foods: a review

(Nagpal et al., 2012)



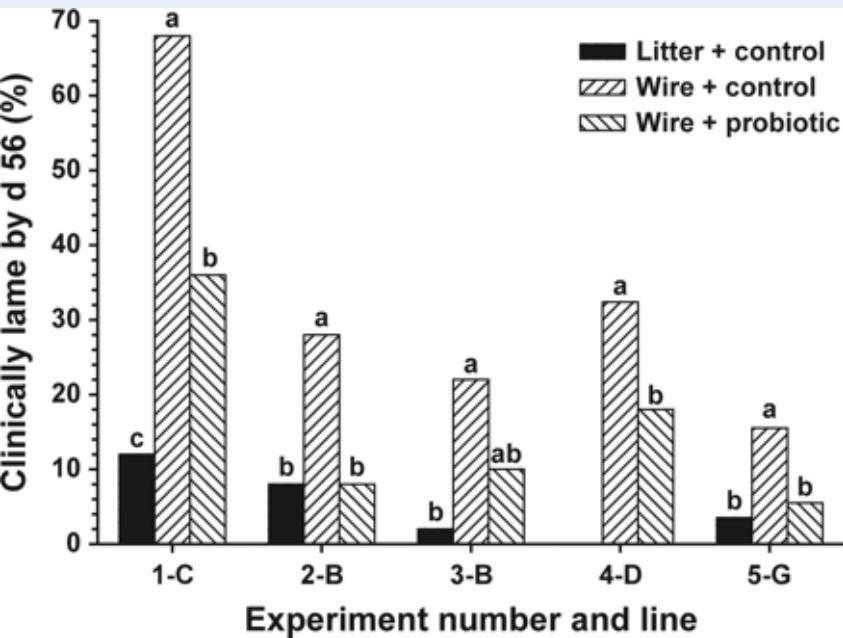
Probiotics improve performance under challenge conditions:

Coccidiosis
Clostridium perfringens
Salmonella
LPS (acute phase response)

Probiotics inhibit bacterial translocation:

Wire-floor model

(Wideman et al., Poultry Sci., 2012)



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FOS, XOS, GOS, IMO, RFO

Prebiotics :

Increase bioavailability of minerals

Stimulate the immune system

Naturally Occurring Prebiotic Oligosaccharides in Poultry Feed Mixtures

M. GRMANOVÁ^a, V. RADA^{a*}, K. SIROTEK^b, E. VLKOVÁ^a

^a*Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiolgy, Food and Natural Resources,
Czech University of Life Sciences Prague, 165 21 Prague, Czech Republic*

^b*Primagra, a.s., 262 31 Milín, Czech Republic*

Received 25 March 2010

Revised version 14 April 2010

ABSTRACT. The presence of raffinose series oligosaccharides (RSO) was determined by an enzymic method in three commercially available chicken feed mixtures. All feed mixtures contained RSO at a concentration of 2.1–2.2 %. Soya meal was identified as the exclusive source of RSO. Subsequently, the bifidogenic effect of stachyose (main soya bean RSO) was also assigned on the growth of poultry intestinal bifidobacteria. Bifidobacteria were counted in chicken intestinal tract using cultivation and FISH methods. Four out of 6 bifidobacterial strains tested grew significantly better on stachyose than on glucose. It can be thus concluded that chicken feed mixtures naturally contain prebiotic oligosaccharides in the form of RSO in higher levels (>2 %) compared with the concentration (usually up to 1 %) recommended for artificially added prebiotics. Our results therefore indicate that there is no reason for the supplementation of chicken feed mixtures with prebiotics with bifidogenic properties.

Naturally Occurring Prebiotic Oligosaccharides in Poultry Feed Mixtures

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FOS, XOS, GOS, IMO, RFO

MOS:

The effect of in ovo administration of mannan oligosaccharide on small intestine development during the pre- and posthatch periods in chickens

S. L. Cheled-Shoval, E. Amit-Romach, M. Barbakov, and Z. Uni¹

*Department of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment,
The Hebrew University of Jerusalem, Rehovot 76100, Israel*

ABSTRACT Early intestinal development is essential for chicken embryos to fulfill their maximal growth potential. Mannan oligosaccharide (MOS) is known to improve gut morphology, function, and innate immunity; therefore, we hypothesized that its administration in the prehatch period to the sterile intestine of embryos would affect intestinal development and functionality without mediation of gut microflora. The MOS was administered by in ovo feeding procedure to embryos 3 d before hatch. The effects of MOS administration on intestinal morphology, activity of the brush-border enzymes amino peptidase (AP) and sucrase isomaltase (SI) and mRNA abundance of *AP*, *SI*, sodium-dependent glucose cotransporter 1 (*SGLT1*), peptide transporter 1 (*PepT1*), secreted mucin (*MUC2*), and toll-like receptors (*TLR2* and *TLR4*) were examined and compared with saline-injected and noninjected controls. Results show that on embryonic d 20 the only parameter affected was *MUC2* mRNA abundance, which exhibited a 3-fold increase in the MOS group versus controls.

On day of hatch more parameters were affected: a 20 to 32% increase in villus area was found in the MOS group compared with controls; crypt depth and number of goblet cells per villus were higher by 20 and 50%, respectively, compared with the saline group; and AP and SI activities were higher by 44 and 36%, respectively, compared with the noninjected control. In addition, an increase in fold change mRNA abundance of *AP*, *SI*, and *TLR4* was observed in the MOS group compared with controls. However, on d 3 posthatch, a decrease in MOS effects was noted, indicating a temporally limited effect after administration of 1 dose. In ovo administration of MOS prehatch resulted in a hatching chick with more mature enterocytes and enhanced epithelial barrier and digestive and absorptive capacity at day of hatch. Results imply that the mechanism underlying the observed changes is not mediated through gut microflora but rather involves a direct effect of MOS on intestinal cells.

Key words: embryo, intestinal gene expression, mannan oligosaccharide, in ovo feeding, chicken

The effect of in ovo administration of mannan oligosaccharide on small intestine development during the pre- and posthatch periods in chickens

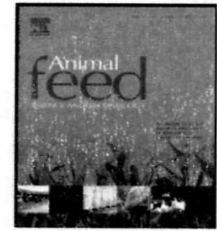
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Key words: embryo, intestinal gene expression, mannan oligosaccharide, in ovo feeding, chicken



The effect of peppermint essential oil and fructooligosaccharides, as alternatives to virginiamycin, on growth performance, digestibility, gut morphology and immune response of male broilers

N. Khodambashi Emami^{a,*}, A. Samie^a, H.R. Rahmani^a, C.A. Ruiz-Feria^b

^a Department of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

^b Texas A & M University, Department of Poultry Science, 2472 TAMU, College Station, TX 77842, USA

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Morphology
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Performance
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ABSTRACT

With the increasing concerns over food safety during these years, there has been an intense effort for substituting (eliminating) antibiotic growth promoters in poultry feeds. Therefore, in order to investigate the effect of natural alternatives for antibiotics, an experiment was conducted to examine the effect of virginiamycin, a prebiotic (Fructomix), and peppermint (*Mentha piperita*) essential oil on productive performance, digestibility, intestinal morphology and immune response of broilers. A total of 240 Ross 308 male broilers were randomly (completely randomized design) allotted to five treatments, with four replicates per treatment (12 chickens per pen). Birds were offered either a maize-soybean meal basal diet (control, CON) or the basal diet supplemented with 200 mg/kg virginiamycin (VM); 200 mg/kg peppermint oil (PO1); 400 mg/kg peppermint oil (PO2); or 500 mg/kg Fructomix (FM). After 6 weeks, daily live weight gain and feed intake were higher ($P<0.001$) for VM-fed birds compared with other groups. Feed conversion ratio was better ($P=0.039$) in chicks fed the VM (1.74), and PO1 diet (1.75) compared with birds in the CON (1.84) and PO2 (1.86) groups. Primary antibody titers against sheep red blood cell were higher ($P<0.001$) in broilers fed FM (6.37) compared with other groups. At 21d of age, crude protein digestibility was higher ($P=0.001$) in PO1 group (0.8645) compared with other groups except VM (0.8505). Finally, higher ether extract digestibility ($P=0.040$) was detected in birds fed VM (0.8831) compared with PO2 (0.7940), and FM (0.7561) fed birds. In duodenum, villus height: crypt depth was higher ($P=0.008$) in VM supplemented group (7.07) in comparison with other groups. In conclusion, this study showed that neither PO nor FM could be suggested as effective alternative for VM.

The Nonantibiotic Anti-Inflammatory Effect of Antimicrobial Growth Promoters, the Real Mode of Action? A Hypothesis

T. A. Niewold

*Nutrition and Health, Department of Biosystems, Katholieke Universiteit Leuven,
Kasteelpark Arenberg 30, 3001 Heverlee, Belgium*

ABSTRACT Societal concern and government regulations increasingly press for restricting the use of antibiotics as antimicrobial growth promoters (AGP). The search for alternatives is on, hampered by a lack of knowledge about the exact mechanism of AGP. Feed additives, such as AGP and alternatives, interact with the intestine. In the intestine, feed components, microbiota, and the mucosa interact in a very complex and dynamic way. Various mechanisms for AGP have been proposed, invariably based on the direct antibiotic influence on the microbial composition of the intestines. In the literature on antibiotics, however, the direct effects of antibiotics on host cells, in particular inflammatory cells, have been described.

It is curious that this has never been considered in the literature on AGP. Presently, a case is being made that AGP most likely work as growth promoters by inhibiting the production and excretion of catabolic mediators by intestinal inflammatory cells. Concomitant or subsequent changes in microflora are most likely the consequence of an altered condition of the intestinal wall. This common, basic mechanism potentially offers an excellent explanation for the highly reproducible effects of AGP, as opposed to those obtained by alternatives aimed at microflora management. Therefore, the search for alternatives could be aimed at nonantibiotic compounds with an effect on the inflammatory system similar to that of AGP.

Key words: antimicrobial growth promoter, inflammation, inhibition, catabolism, growth permitting

2007 Poultry Science 86:605–609

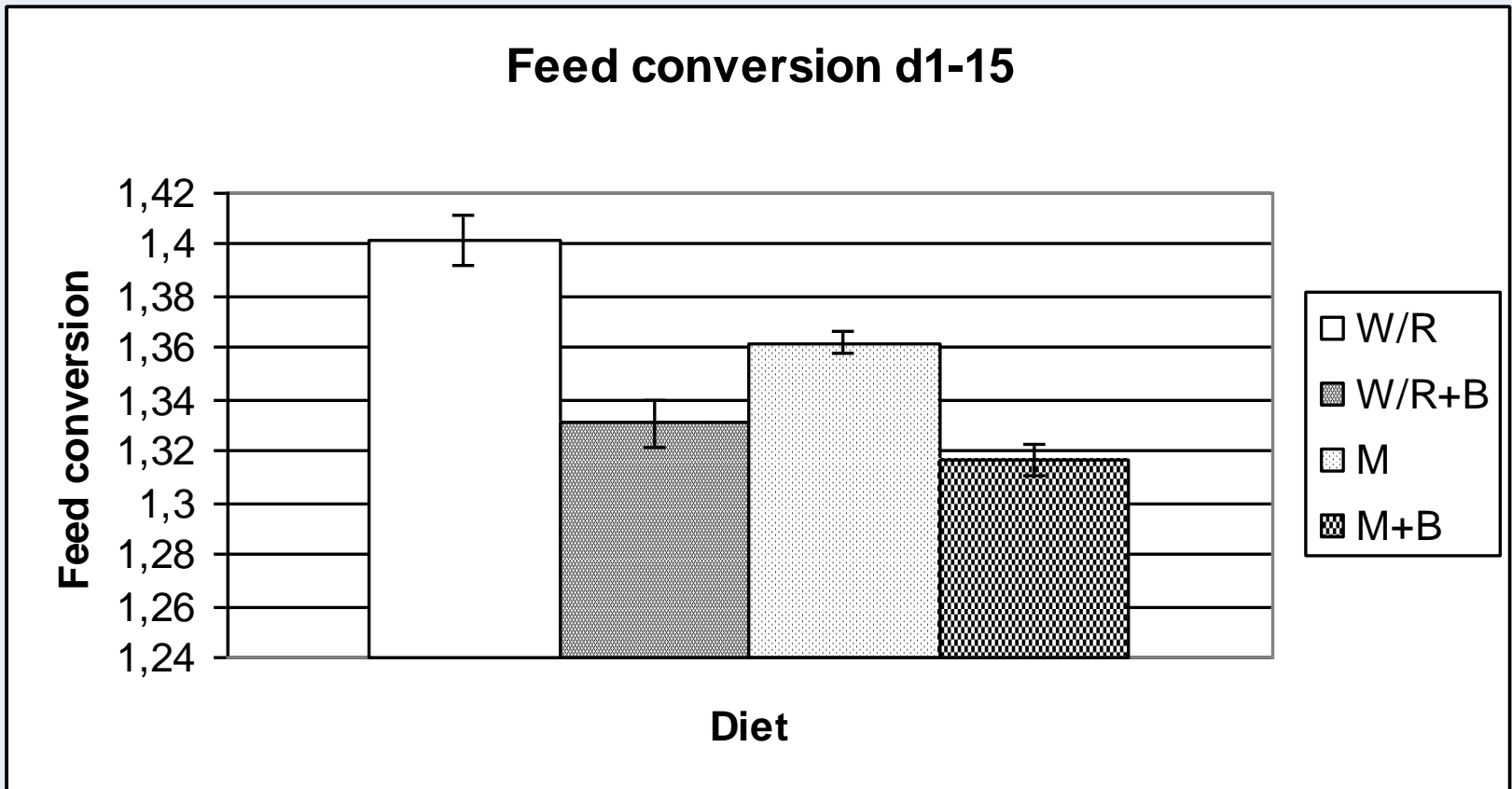
Experimental model 1

Experimental set up

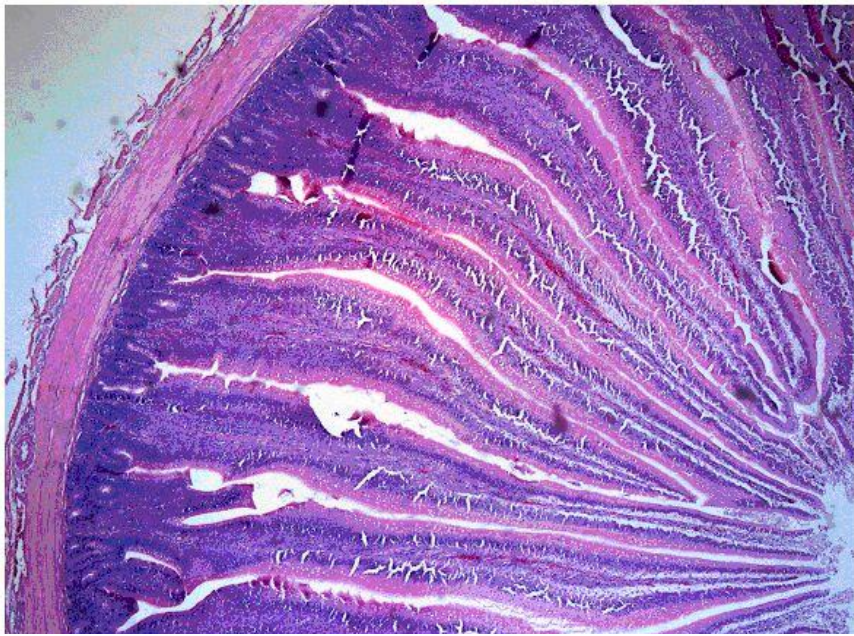
- Antimicrobial growth promoter
(Zn-bacitracin 100 mg/kg)
- 2 ≠ carbohydrate sources



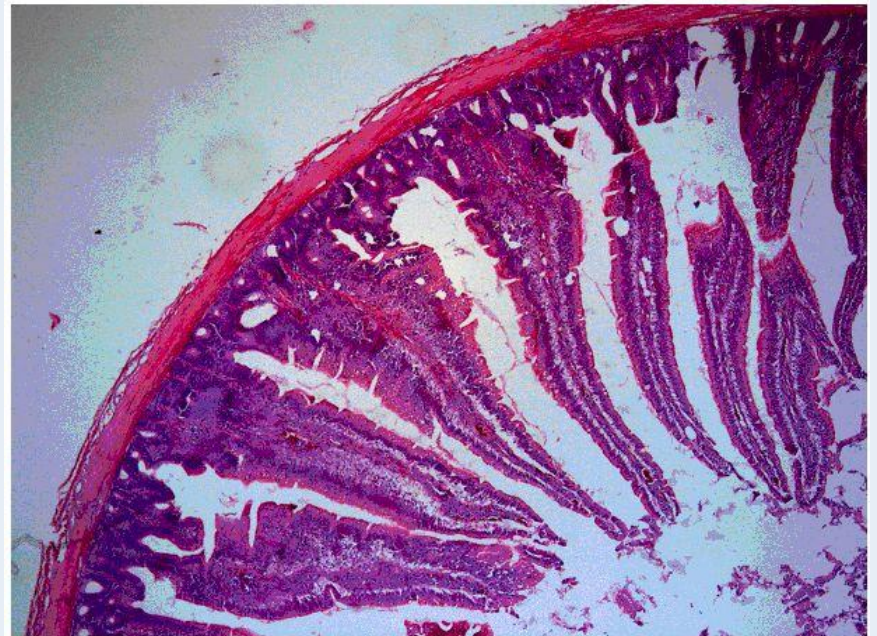
Feed conversion



Villus length



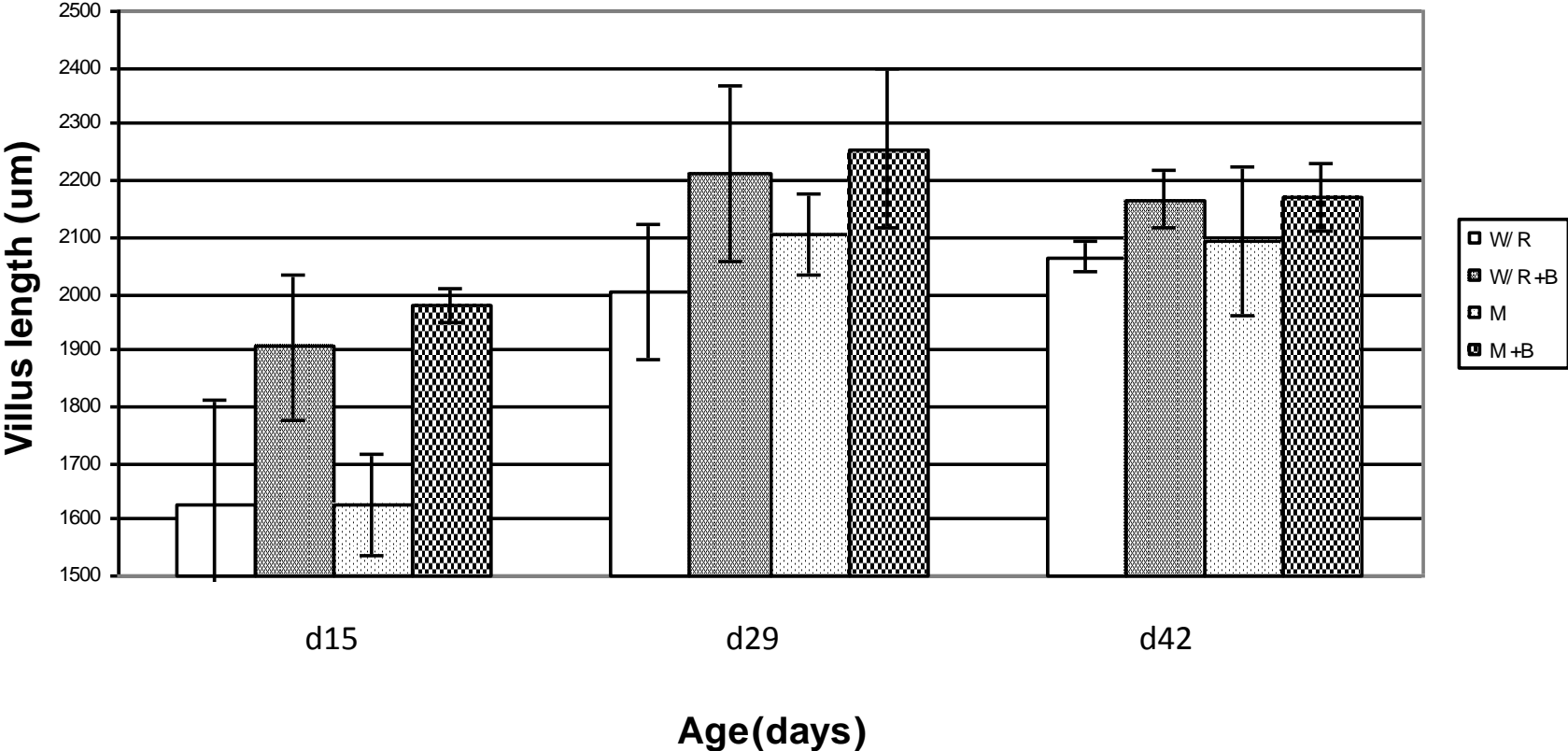
Villi Duodenum M+B diet (dag 15)



Villi Duodenum W/R diet (dag 15)

Villus length

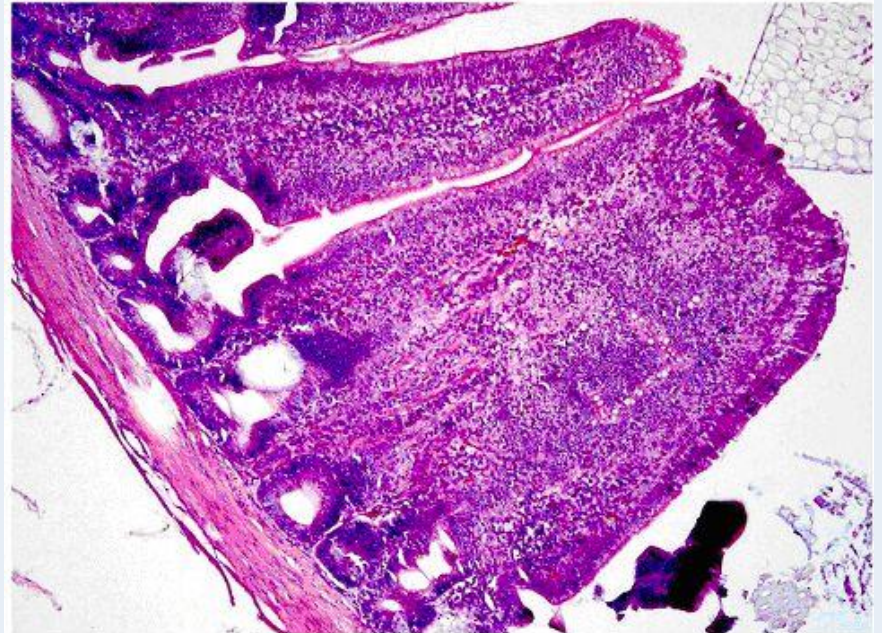
Villus length in duodenum



Villus fusion

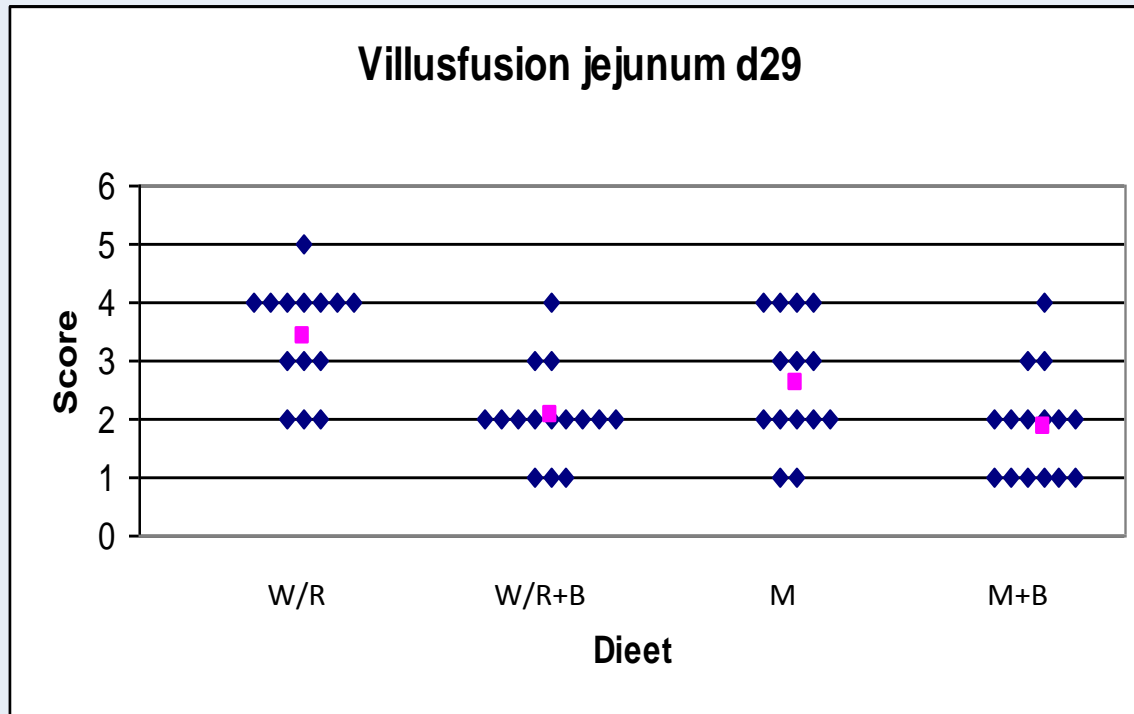


Villi Jejunum M+B dieet (dag 15)

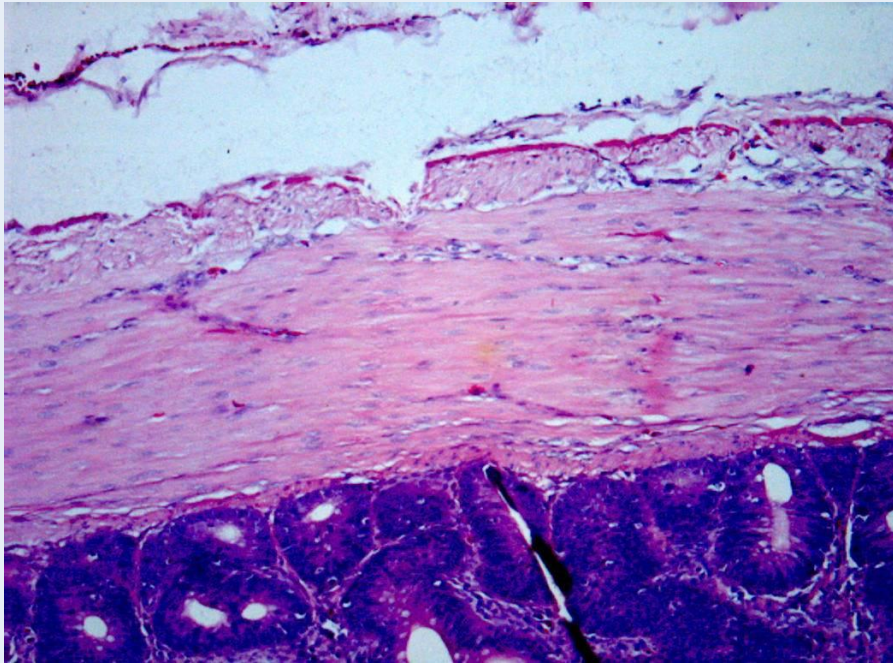


Villi Jejunum W/R dieet (dag 15)

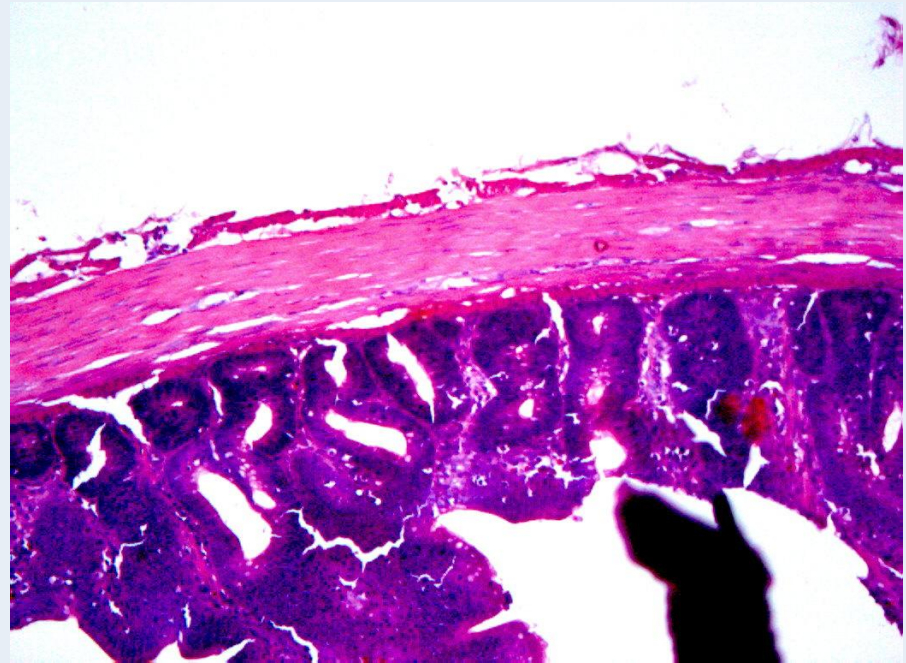
Villus fusion



Muscularis thickness

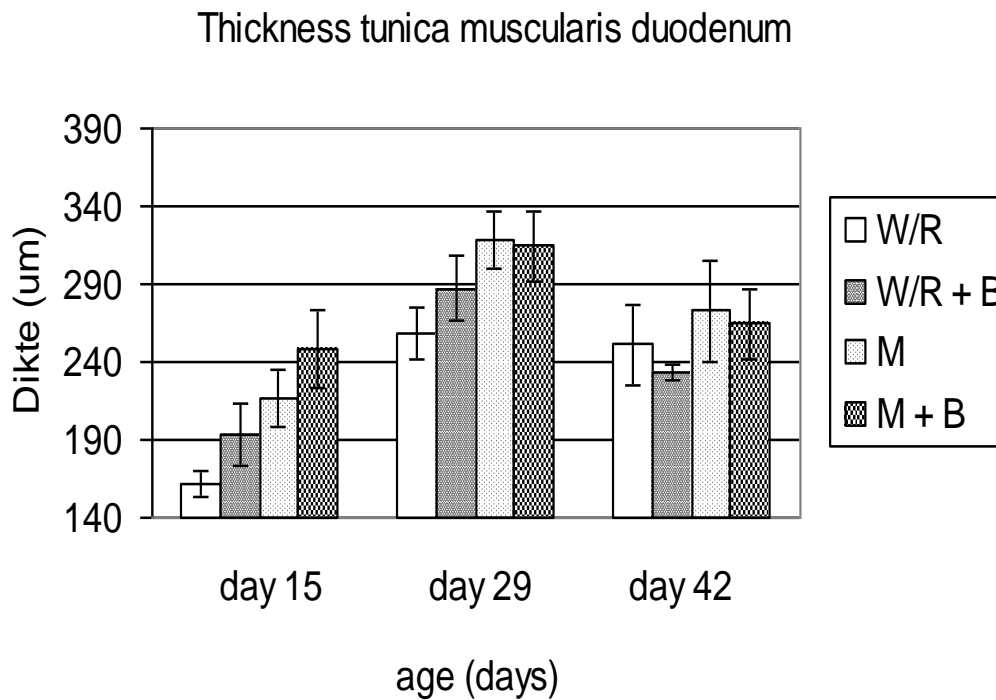


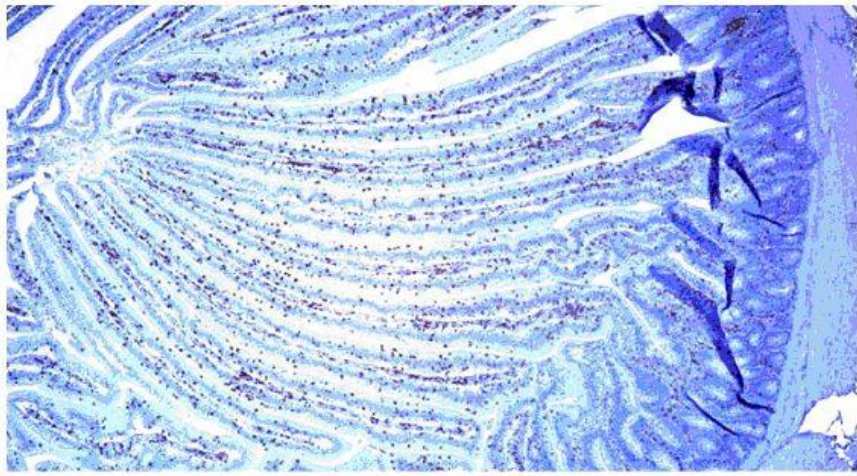
**M+B: Dikte tunica muscularis
duodenum (dag 15) (20x)**



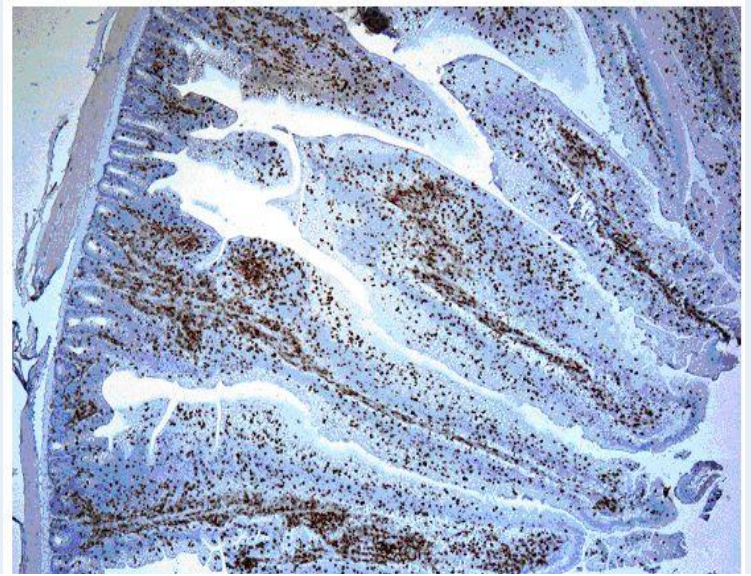
**W/R: Dikte tunica muscularis
duodenum (dag 15) (20x)**

Muscularis thickness

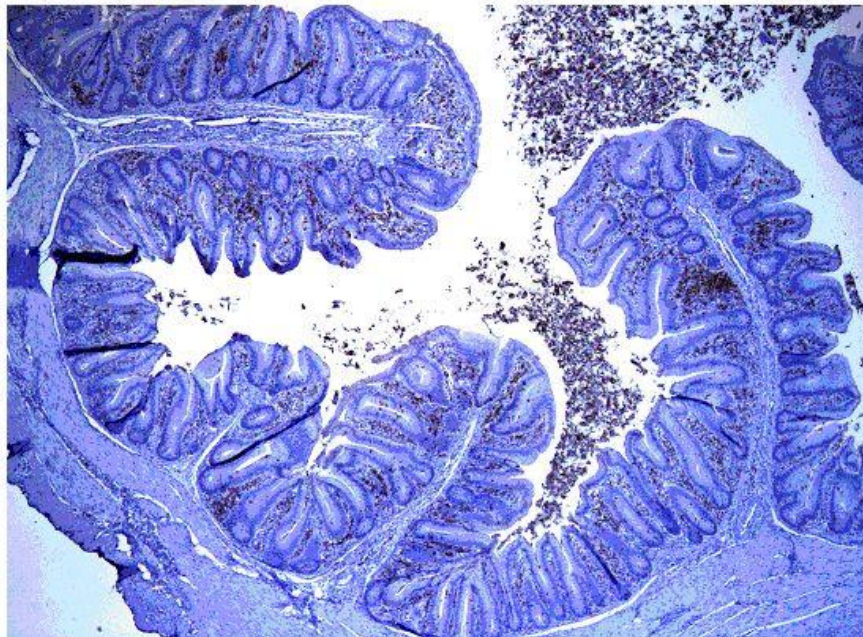




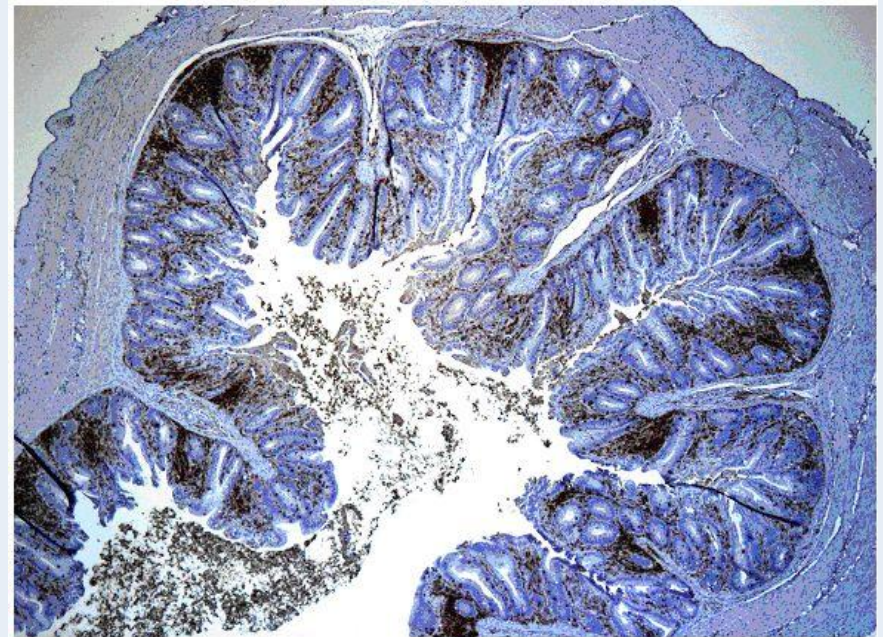
M+B: T-cel infiltration in duodenum



W/R: T-cel infiltration in duodenum



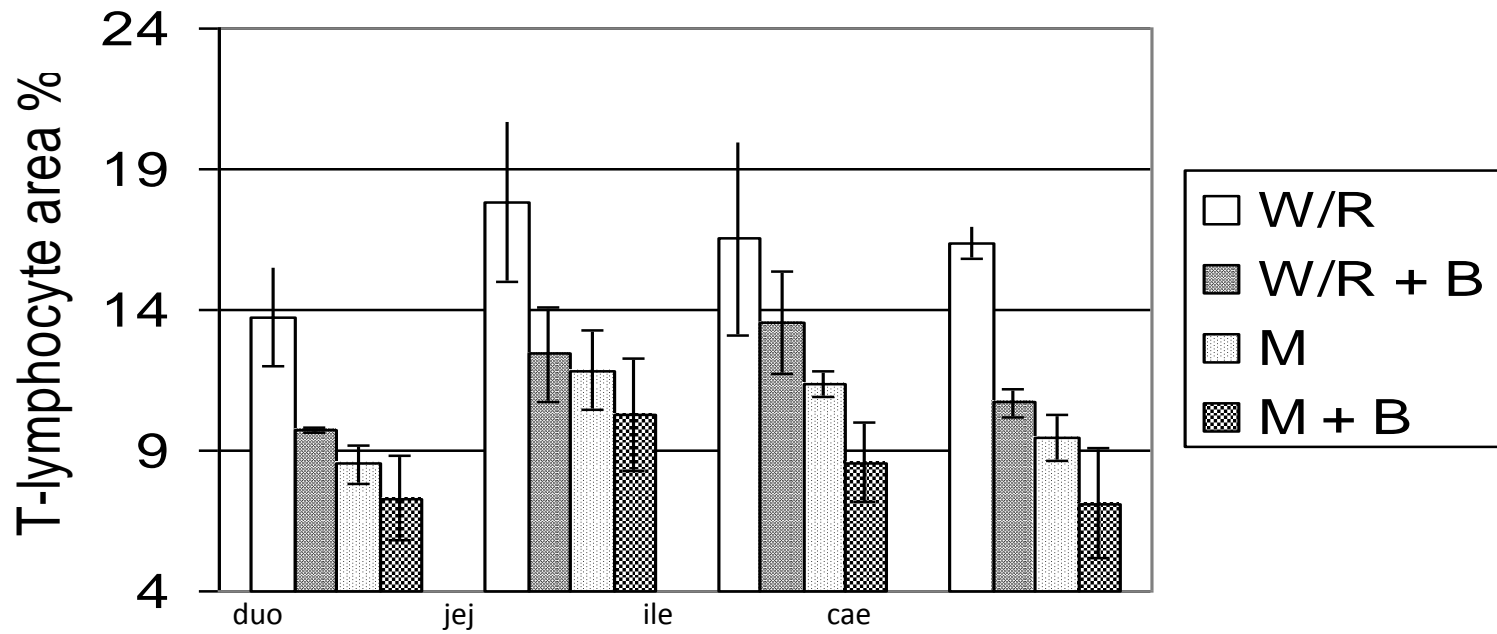
M+B: T-cel infiltration in caecum



W/R: T-cel infiltration in caecum

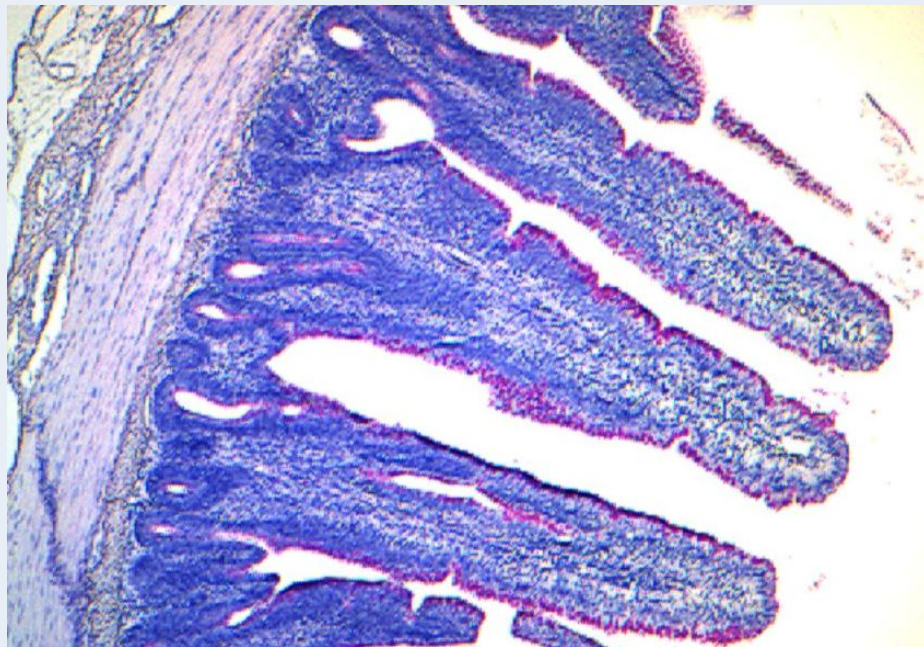
Immune cell infiltration

T-lymphocyte infiltration d15

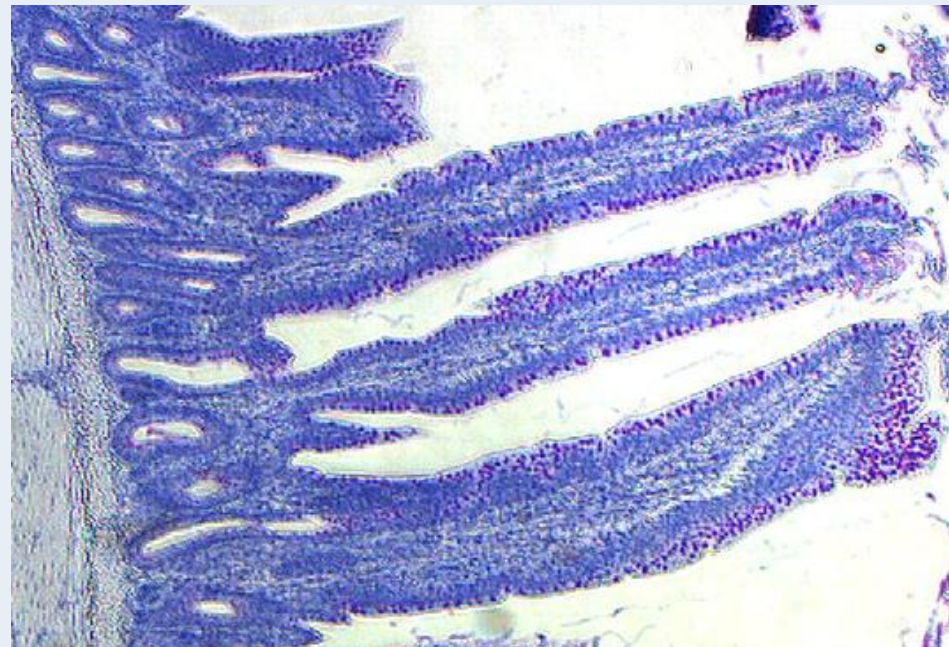


Results

Goblet cells



W/R: ileum (100 x)



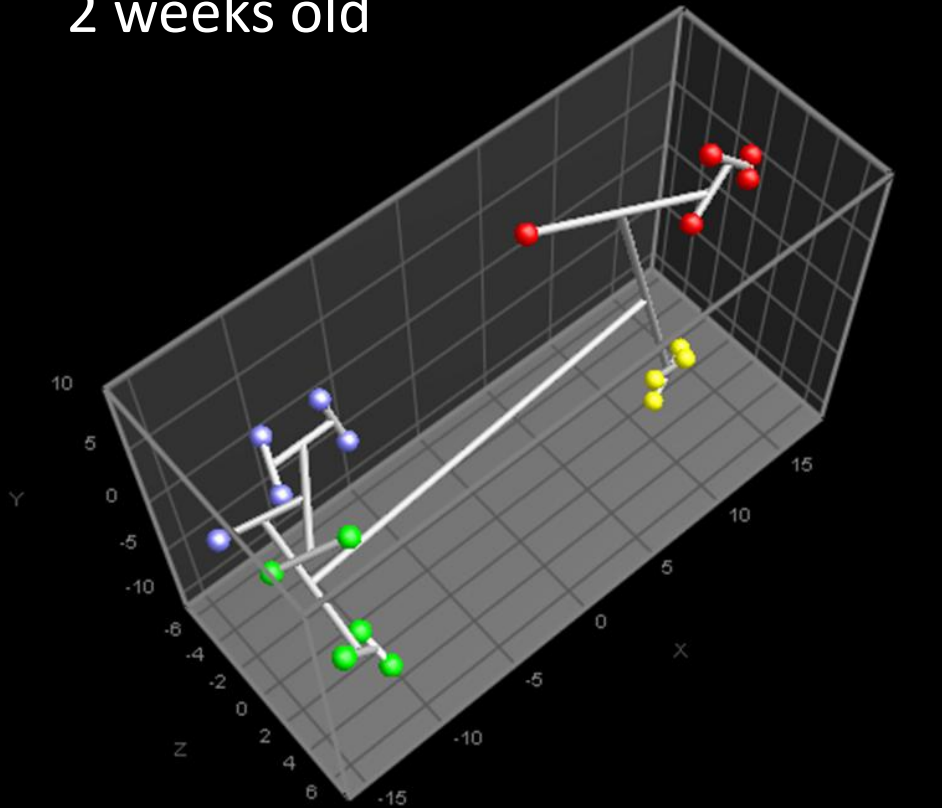
M: ileum (100x)

→ Protective mucin layer

Results t-RFLP

Effect of diet

2 weeks old



Green: wheat/rye

Blue: wheat/rye + Zn-
Bacitracine

Yellow: maize

Red: Maize + Zn-Bacitracine

Statement 1:

Growth promoters cause a shift in the microbiota of caeca and colon

Statement 1:

Growth promoters cause a shift in the microbiota of caeca and colon

Statement 2:

Growth promoters promote shift from inflammation to oral tolerance

Experimental model 2

High molecular weight pectins

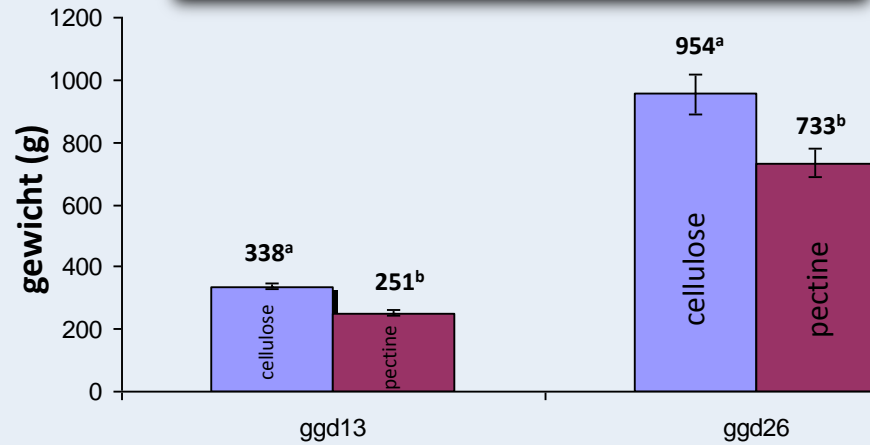
Langhout et al., Poultry Sci. 2000

Feed composition

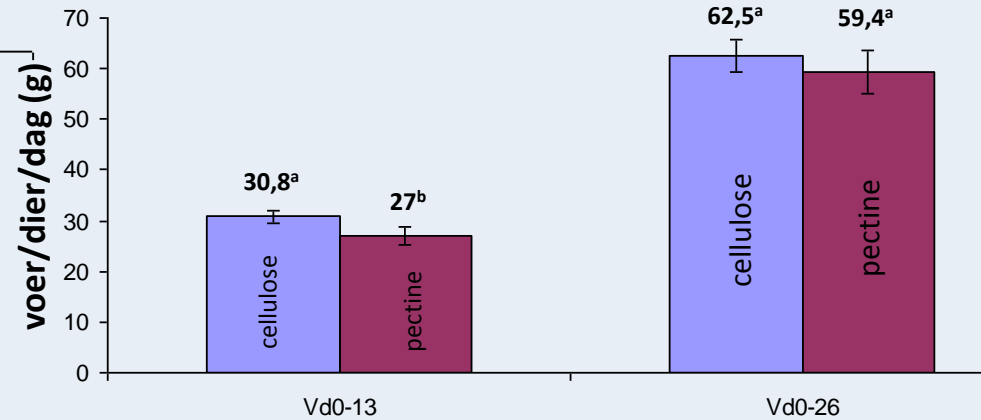
	Starter		Grower		Finisher	
	Cellulose d0-d13 %	Pectine d0-d13 %	Cellulose d13-d26 %	Pectine d13-d26 %	Cellulose d26-d39 %	Pectine d26-d39 %
Corn	47,35	47,35	48,64	48,64	50,90	50,90
Soybean meal 48 (46)	31,95	31,95	29,60	29,60	27,44	27,44
Soybeans	4,68	4,68	4,68	4,68	4,68	4,68
Animal fat	3,60	3,60	5,45	5,45	5,00	5,00
Soybean oil	1,85	1,85	1,45	1,45	2,04	2,04
CaCO ₃	0,33	0,33	0,27	0,27	0,27	0,27
D-Ca-phosphate	1,81	1,81	1,60	1,60	1,30	1,30
NaCl	0,22	0,22	0,24	0,24	0,26	0,26
NaHCO ₃	0,14	0,14	0,11	0,11	0,10	0,10
L-Lys HCl	0,205	0,205	0,134	0,134	0,174	0,174
DL-methionine	0,295	0,295	0,236	0,236	0,237	0,237
L-threonine	0,050	0,050	0,025	0,025	0,025	0,025
Vit. & Tr. el. Premix	1,00	1,00	1,05	1,05	1,05	1,05
Phytase	0,02	0,02	0,02	0,02	0,02	0,02
Cellulose	6,50	3,50	6,50	3,50	6,50	3,50
Pectines	0,00	3,00	0,00	3,00	0,00	3,00
NSP (Bio FW)	no	no	no	no	no	no
Sum	100,00	100,00	100,01	100,01	100,00	100,00

Performance parameters

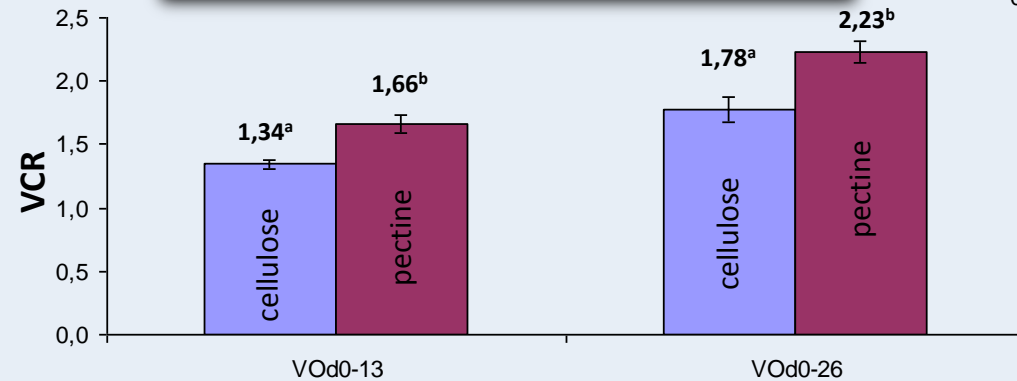
Weight



Feed intake



Feed conversion



Gemiddeldes met verschillend superscript zijn significant verschillend (P = 0,001)

day 22

3 animals/pen

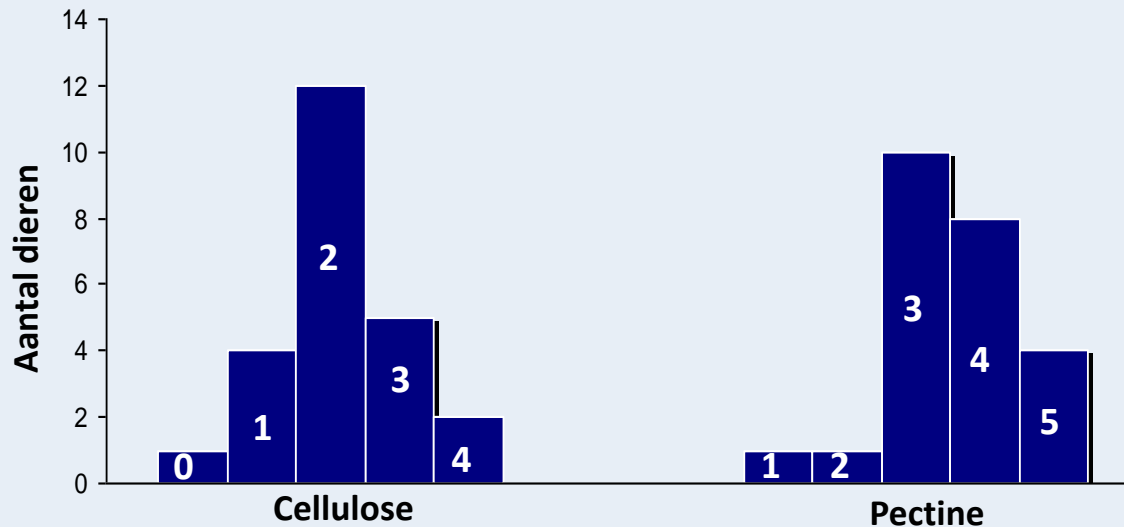


24 animals/group

Dysbacteriosis scoring

(according to Teilynck et al., Avian Pathol. 2011)

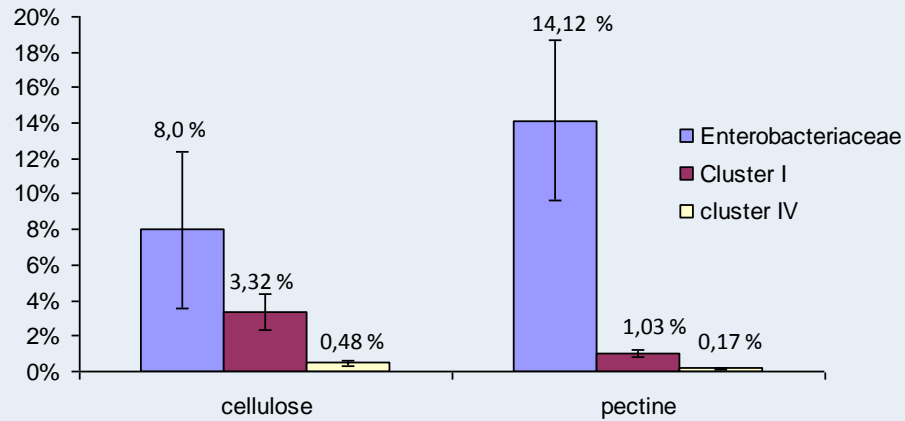
Cage	Treatment	Chicken	Gut Ballooning	Cranial				Caudal				Un-digested feed
				Inflamma-tion Vessels	Flaccid	Content	Thickness Translucen cy Fragility	Inflamma-tion Vessels	Flaccid	Content	Thickness Translucen cy Fragility	
33	cellulose	1	1	0	0	1	0	0	0	0	0	0
35	pectine	1	0	0	0	1	0	0	0	1	1	0



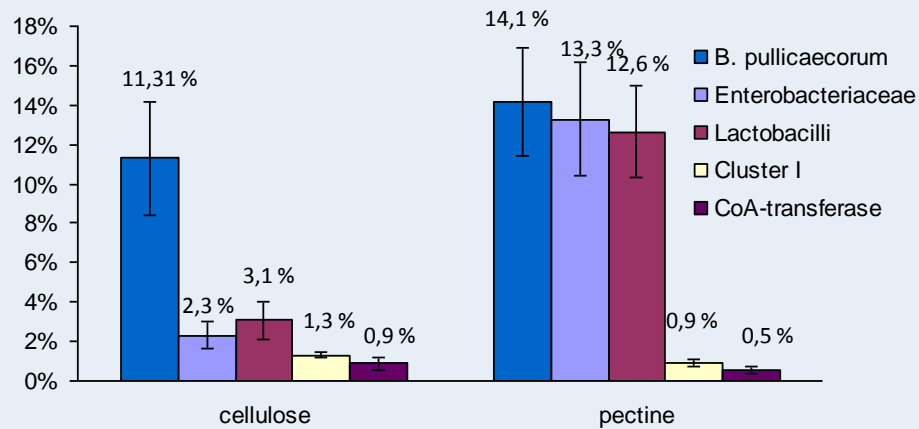
Group	Score	Stdv
Cellulose	2,12	0,946
Pectine	3,54	0,997

day 22

Ileum (relative percentage)

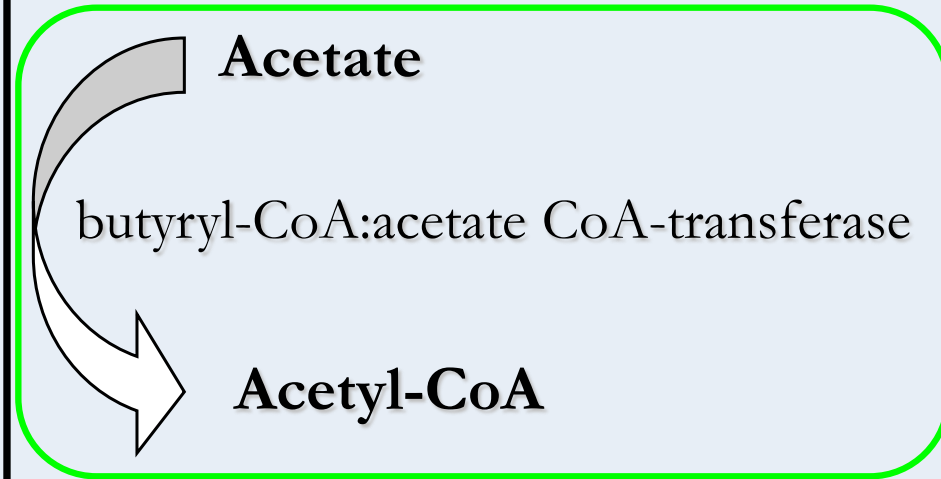
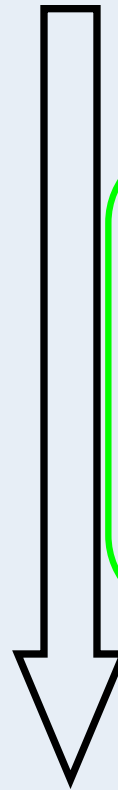


Caecum (relative percentage)



Butyrate production

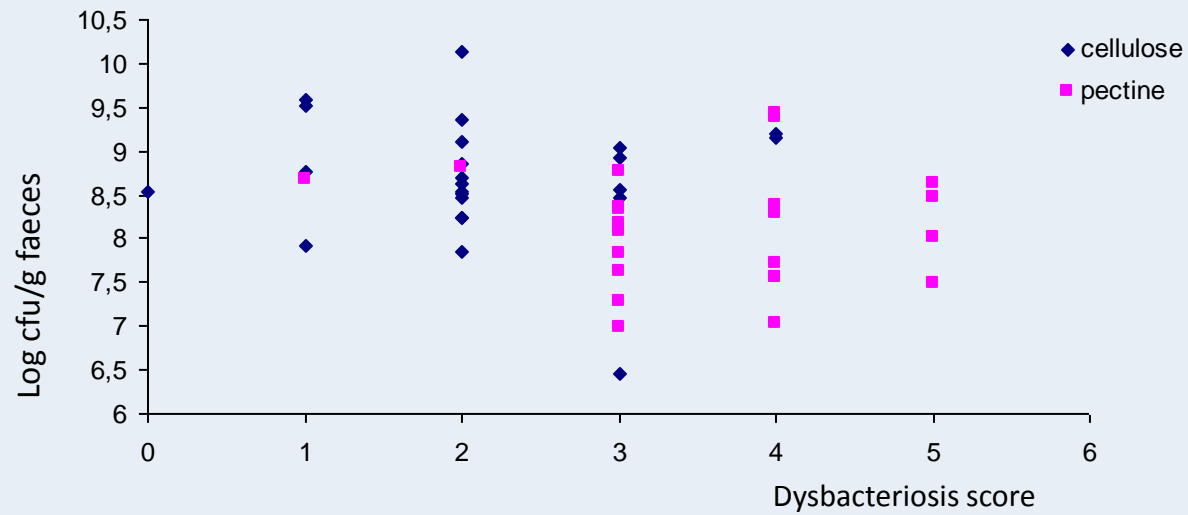
Butyryl CoA



Butyrate

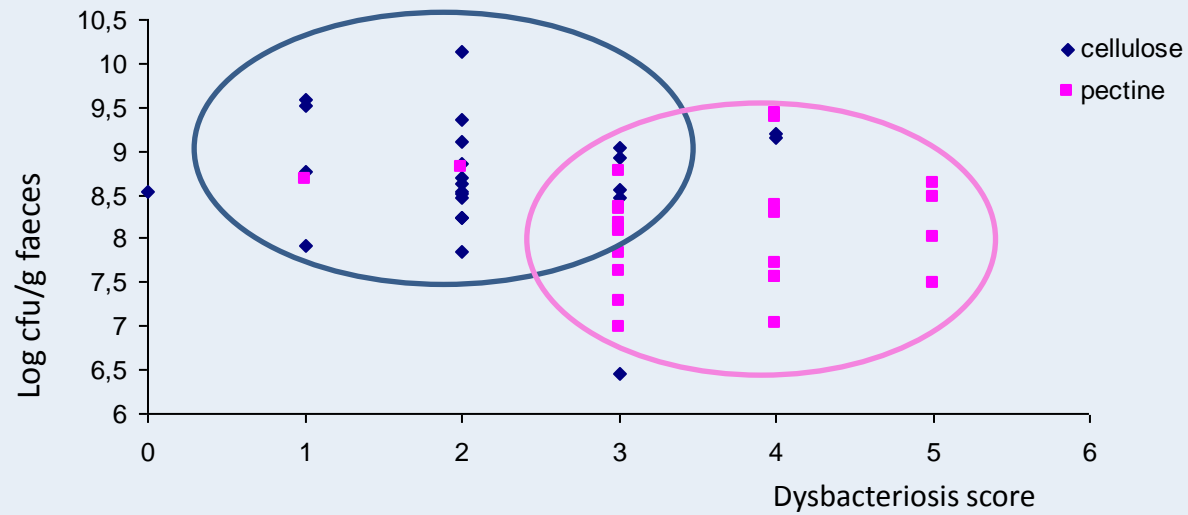
day 22

ButCoA-AcCoA-transferase



day 22

CoA-transferase



Energy source for colonocytes

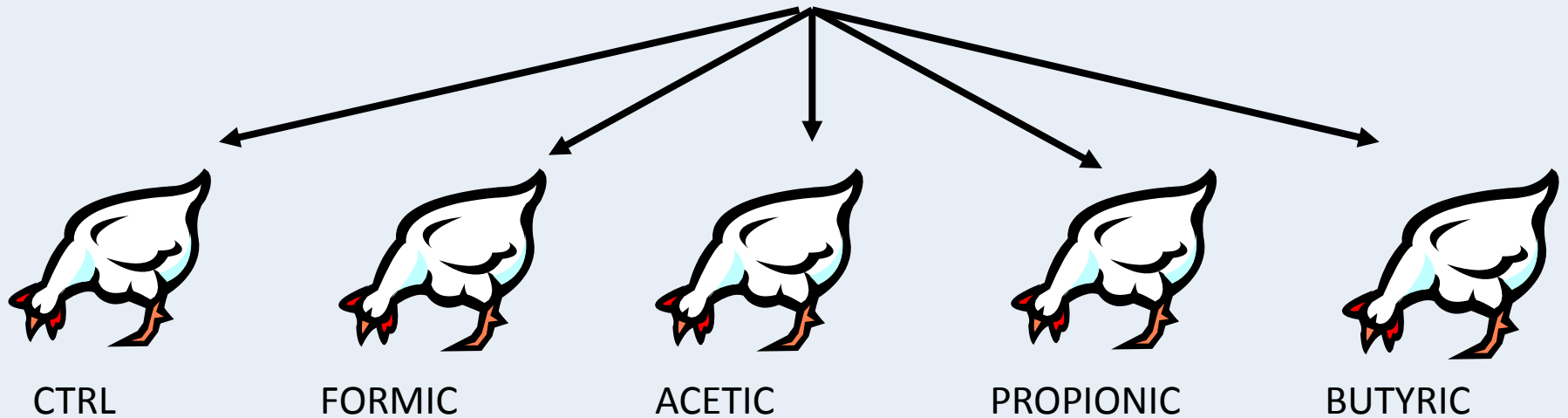
Anti-inflammatory properties

Anticarcinogenic potential

Butyric acid

What is the effect of SCFA on *Salmonella*?

Five groups of 20 chickens :



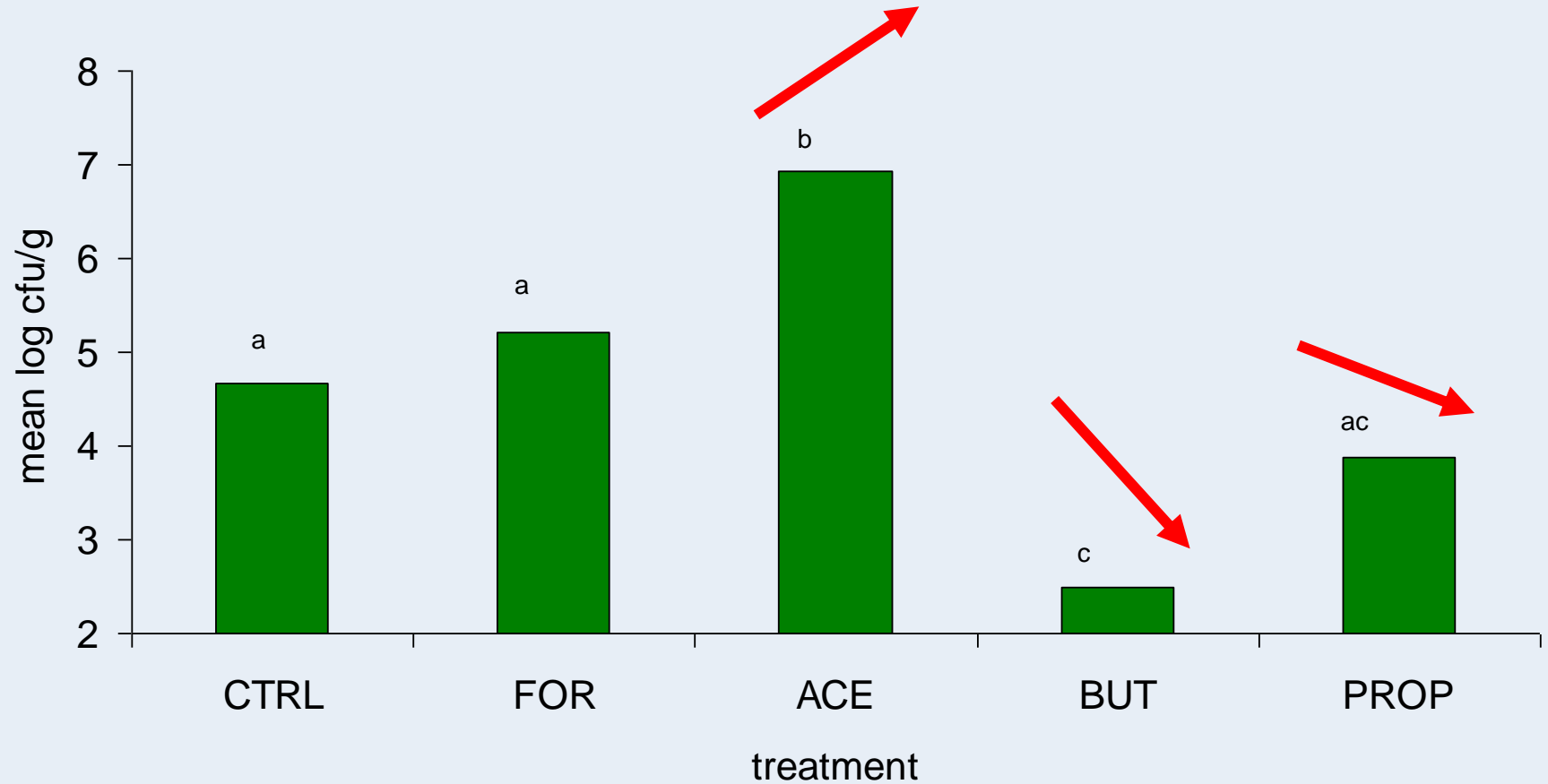
ALL ACIDS IN COATED FORM

Infection at day 5 with $5 \cdot 10^3$ cfu *S. Enteritidis*

Euthanasia at day 8

Bacteriological analysis of caeca, liver and spleen : titration on BGA

Caecal colonization of *Salmonella*



Thank you for your attention



Faculty of Veterinary Medicine
Ghent University