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Lactulose:
Health benefits in the gastro-intestinal tract

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Overview

Introduction

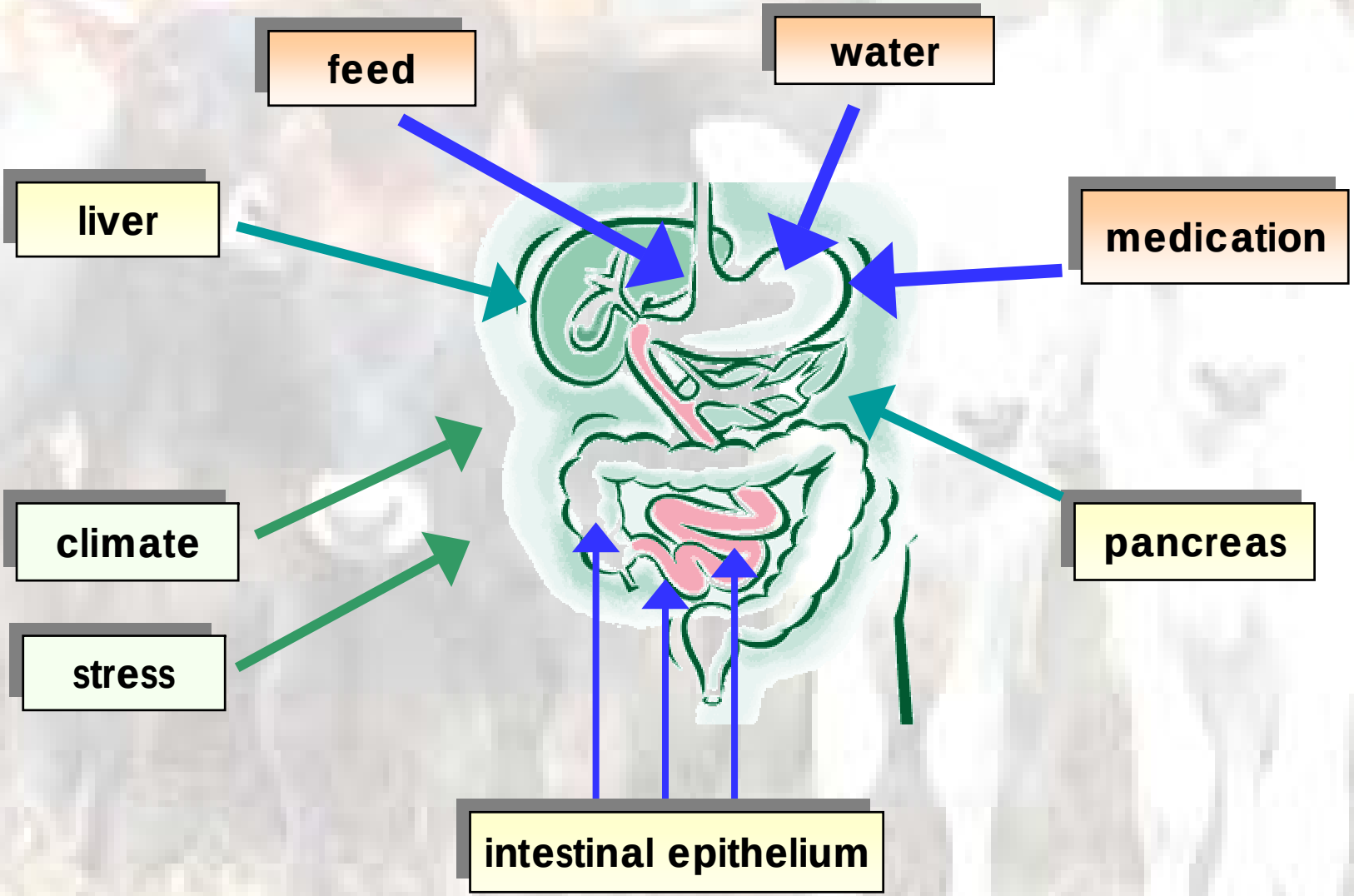
- Influencing factors on Eubiosis in GIT
- Prebiotica and Probiotica
- Effect of Lactulose
- Modulation of the immune system

Experimental design

Result & Discussion

- Monitoring of feed intake, and average daily weight gain (ADG)
- Histology
  - change in the GIT villi morphology
- mRNA Gene Expression studies
  - anti- and pro-inflammatory markers in the intestine
  - proliferation- and apoptosis-marker in the intestine

Factors which influence the GIT

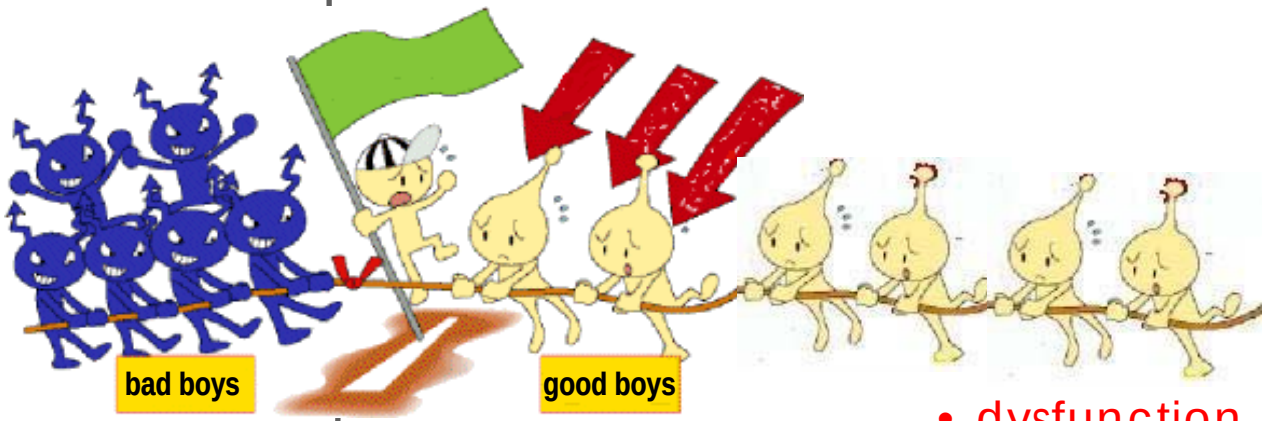


Eubiosis vs. Dysbiosis in GIT

Eubiosis

- benefit for digestion
- prevention of pathogenic bacterial colonization and adhesion
- synthesis of vitamins
- stimulation of the immune system

balanced



Prebiotics and Probiotics

unbalanced

- dysfunction
- loss of efficiency
- loss of resistance

Dysbiosis

- predisposition for disease
- disease due to infection & toxins

Probiotics - definition

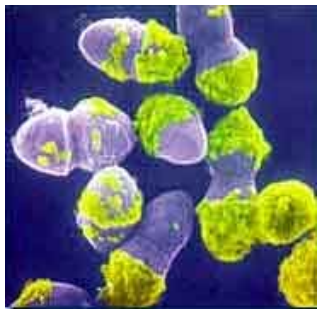
" Probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance. "

Fuller 1989

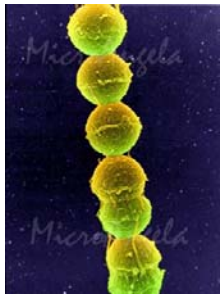
Bifidobacterium
(bifidum)

Lactobacillus
(casei)

Bacillus
(subtilis)



Enterococcus
(faecium)



Strepto -
coccus
(lactis)

Saccaromyces
(boulardii)

Subject to following conditions

- resistant against stomach acids, GIT enzymes and bile acids
- populating the colon
- effectiveness and security have to be proven under the recommended allowance (dose rate, kind of allowance) for each target group

Prebiotics -definition

FAO/WHO, 2001 & DGE, Nourishing Report, 2000

" Prebiotics are non-digestible food ingredients which beneficially affect the hosts health, by selective stimulation of growth of one or a limited number of bacteria in the colon, and thus improves host health. "

- are principally oligo-saccharides
- they mainly stimulate the growth of *bifidobacteria* (= bifidogenic factors)
- must be intact upon transfer to the colon
- must be stable under acidic condition and small gut secretions

Feed-additives in animal nutrition

=> Pre- and probiotics represent an attractive and natural alternative to improve health

Since January 2006:

ban of antibiotics for use as performance accelerators

- **Salinomycin-Natrium**
- **Avilamycin**
- **Monensin-Natrium**
- **Flavomycin**

Farm animal pre- and probiotics:

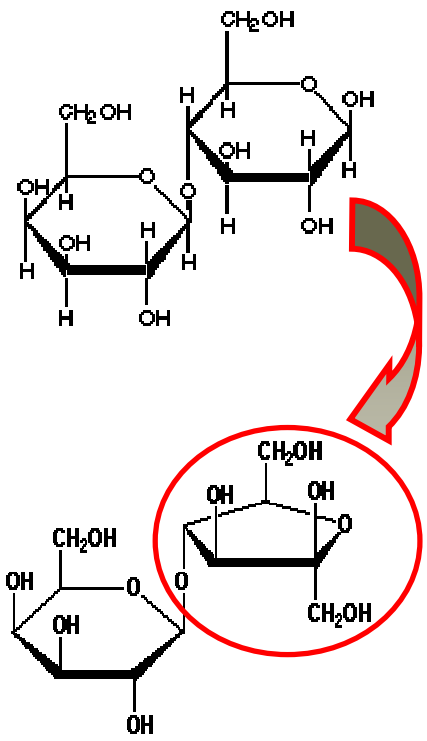
- Improve GIT health
- Improve resistance to disease
- Improve animal health
- Improve feed conversion ratio
- Promote higher growth rate
- Decrease rearing losses

Lactulose

Lactose

4-O-ß-D-galactopyranosyl-D-glucose

Gal-Glu



Lobry de Bruyn
Alberda von Ekenstein
Isomerisation

Lactulose

4-O-ß-D-galactopyranosyl-D-fructose

Gal-Fru

Application for human health benefits

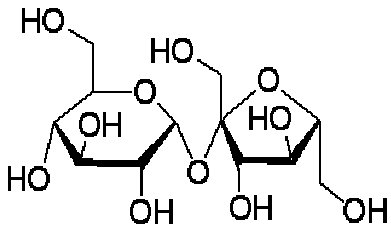
- **constipation** (10-20g)
- **liver dysfunction, liver cirrhosis,** portal-systemic encephalopathy (20-30g)
- **colon cancer** (antitumor activity of butyrat, increasing concentration of calcium and magnesium reduce damaging effects on colonocytes)
- **disorder of lipid metabolism** (propionat inhibit HMG-CoA = lower cholesterin and triglycerides)
- **diabetes mellitus,** without causing a significant rise in blood sugar levels

=> Lactulose can reduce Glycaemic Index !

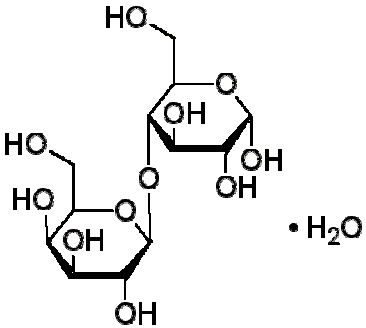
GI = Glycaemic index

- GI is a ranking system for carbohydrates based food on their immediate effect on the blood glucose level.
- GI values can be interpreted intuitively as percentages on an absolute scale.

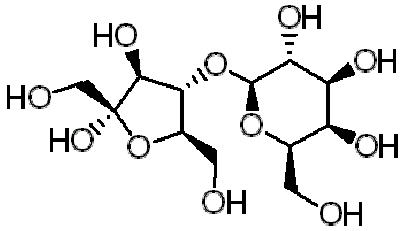
Classification	GI range	Example
Low GI	< 55	fruit, vegetables, yoghurt, fish
Medium GI	56 – 69	sucrose, rice, noodles
High GI	> 70	backed potatoes, honey, bananas, beer



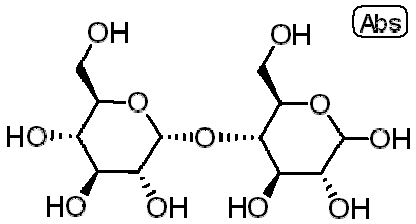
Sucrose
GI: 58-65
Glu-Fru



Lactose
GI: 43-48
Gal-Glu



Lactulose
GI: ???
Gal-Fru

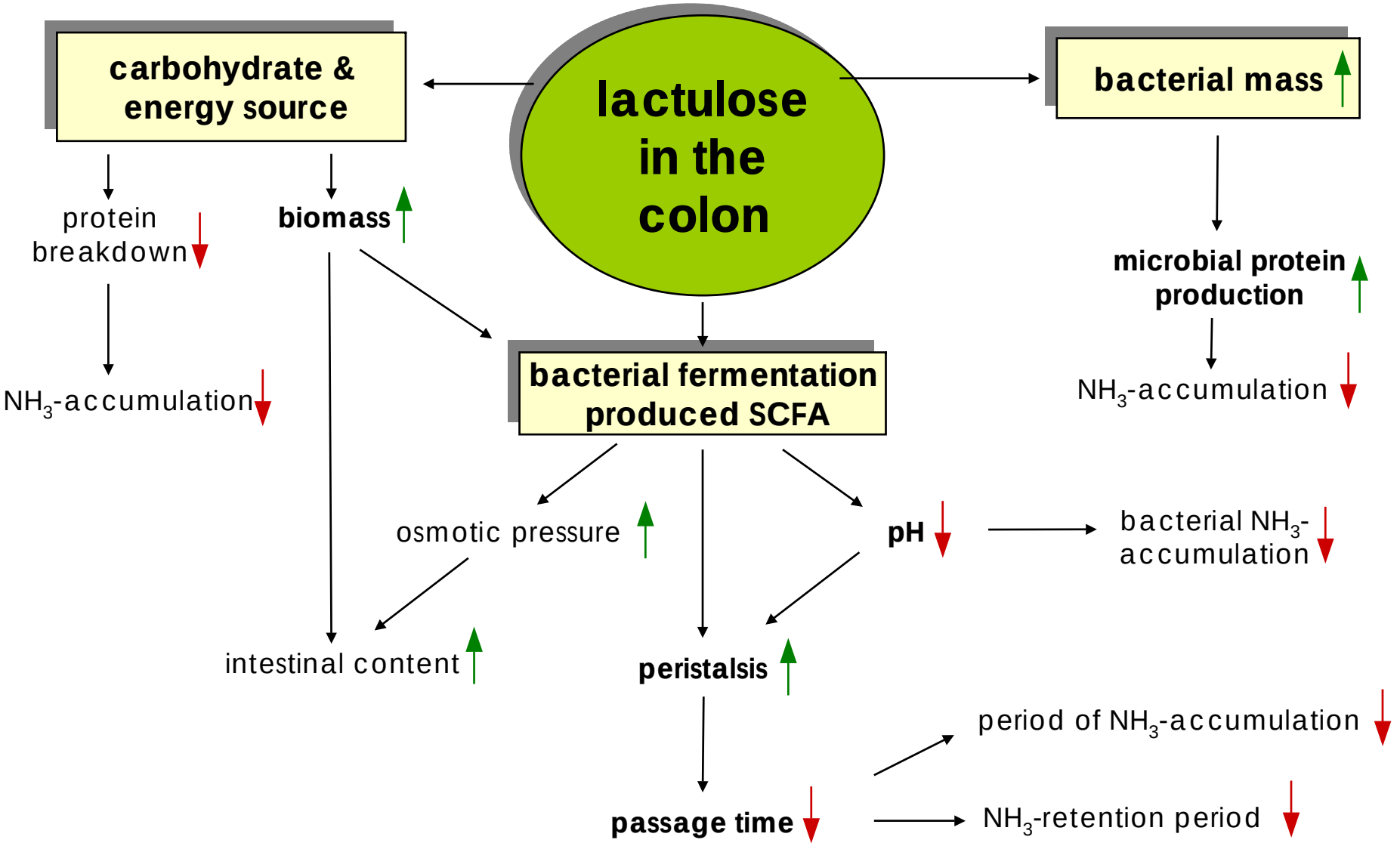


Maltose
GI: 105
Glu-Glu

Glucose: GI 100 **Fructose: GI 12-24** **Galactose: < GI 10 [???]**

Effect of lactulose

lactulose is resistant to digestion in the stomach and small intestine (no corresponding disaccharidase)



Microbiological interactions in the intestine

Gut Associated Lymphatic Tissue (GALT)

Peyer's patches with B & T cells (lymph follicle & lymphocytes, dentritic cells, plasma-cells and T cells of lamina propria, macrop

Problem => Disease:

pathogens and their toxins adhe
damage it

Competition for nutrition:

probiotics compete with pathog

Competitive exclusion:

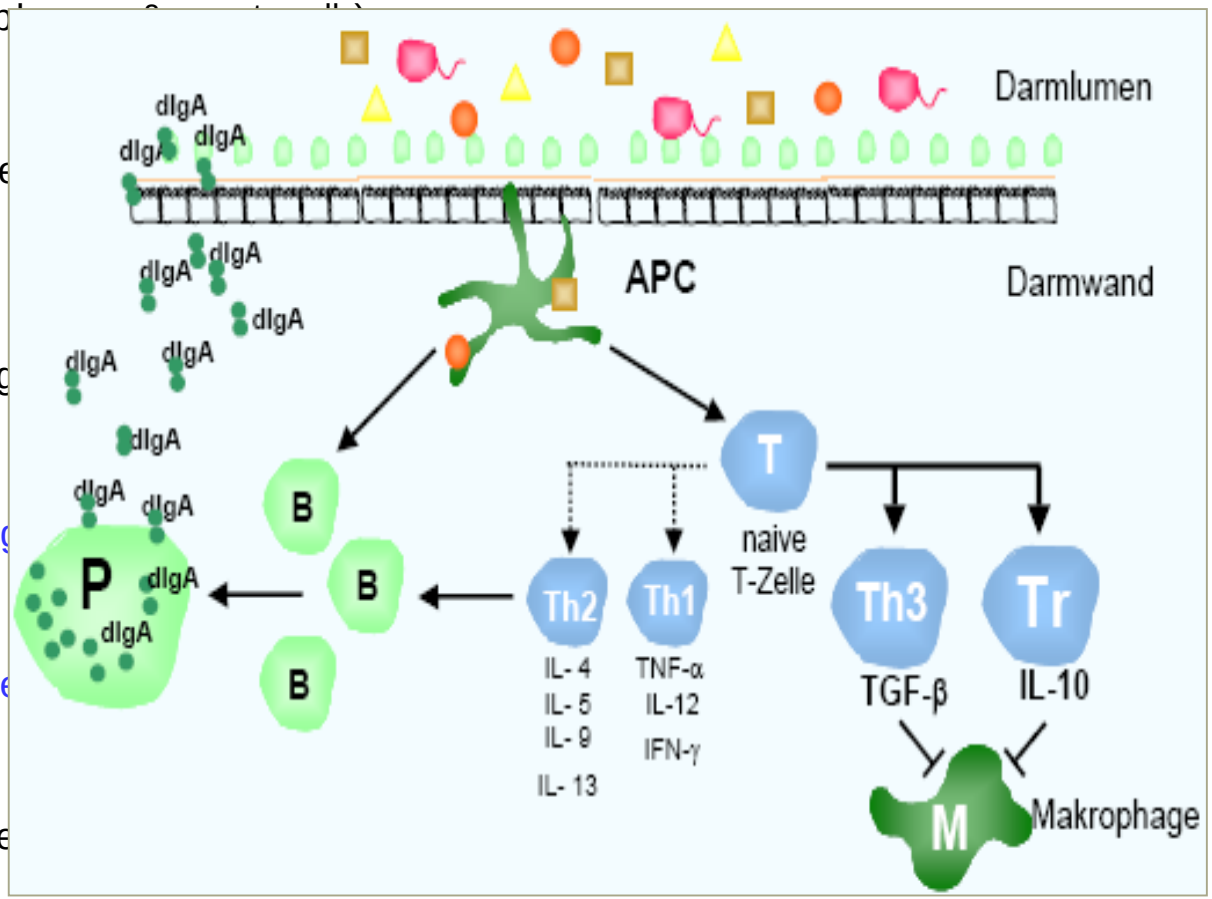
probiotics block intestine pathog

Masking:

as probiotics occupy intestinal re

Aggregation:

probiotics hamper the attachme



=> The immune response is stimulated and the activity of host antibodies increased.

Experimental design

Bayerische Landesanstalt für Landwirtschaft (LFL), Grub

Dr. W. Preißinger
Institut für Tierernährung und Futterwirtschaft

42 Simmental calves

21 female & 21 male

Control	L1	L3
no lactulose	1% lactulose	3% lactulose

milk replacer (MR)
+
10^9 CFU *Enterococcus faecium* per kg MR
(Milkibeef® TOP)
+
500 g hay/d

19 weeks

MONITORING

- animal health
- food intake
- weight gain
- blood analysis (WBC)

Experimental overview

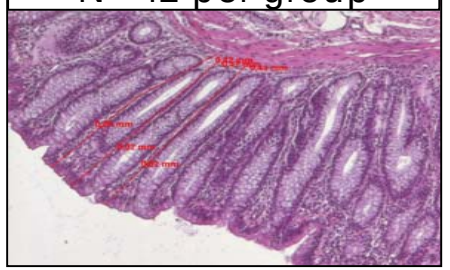
Tissue
jejunum, ileum, caecum, colon, mes. lymph node, spleen, ...

Histology
jejunum, ileum, caecum, colon
peyer´s patches

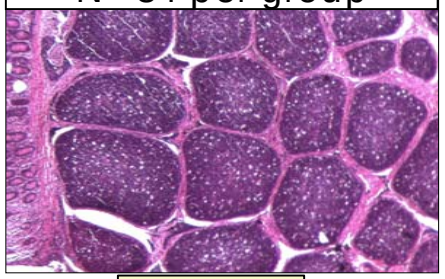
WBC

N= 42 per group

N= 42 per group



N= 84 per group



RNA extraction & RNA quality control

Quantitave one-step real-time RT-PCR (qRT-PCR)

pro- & anti-inflammatory: IL-1ß, TNF-α, IL-8, IL-10, TGF-ß, IgA FcR
diverse CD markers: CD4, CD8, CD25, CD69
proliferation/apoptosis: Caspase 3, EGFR, Bax, Bcl-xl
reference genes: Ubiquitin, ß-Actin, GAPDH

Statistics
one/two way ANOVA

Results and Discussion

- **Average daily food and nutrition intake**
- **Average daily weight gain**
- **Histology**
- **RNA quality**
- **Expression analyses**

Average daily food and nutrition intake

Table 3 *Average daily food and nutritient intake*

Intake†	Experimental group L0	L1	L3	Pooled s.e.	Significance of group differences L0 v. L1	L0 v. L3	L1 v. L3
MR intake							
MR (g DM)	2080	2019	2199	45		*	**
male	1958	1934	2133	73			
female	2201	2104	2264	26	*		**
Energy (MJ ME)	35	34	37	1		*	**
Crude protein (g)	455	452	514	10		***	***
Ether extract (g)	410	398	433	9		*	**
Total intake							
Hay (g DM)	205	207	211				
Total food (kg DM)	2.3	2.2	2.4				
Energy (MJ ME)	37	36	39				
Crude protein (g)	477	475	545				
Crude fibre (g)	68	69	70				
Ether extract (g)	409	398	437				

CP +13%

† The milk replacer (MR) intake data show the mean values ± s.e. For the total intake, no s.e or significance values could be calculated because hay was offered to entire feeding groups (means are different between treatment groups as shown).

Fleige et al., (2007) Animal 1(3): 367-373

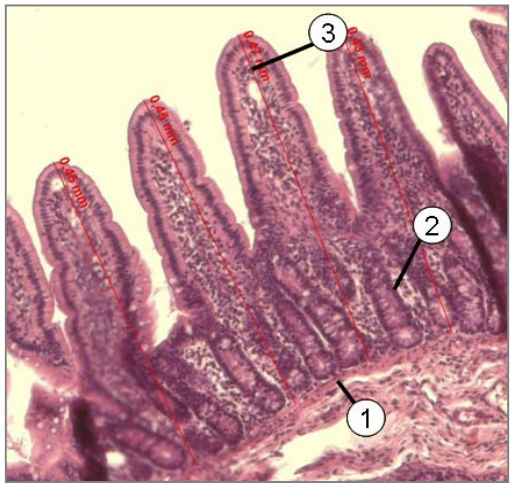
Average daily weight gain

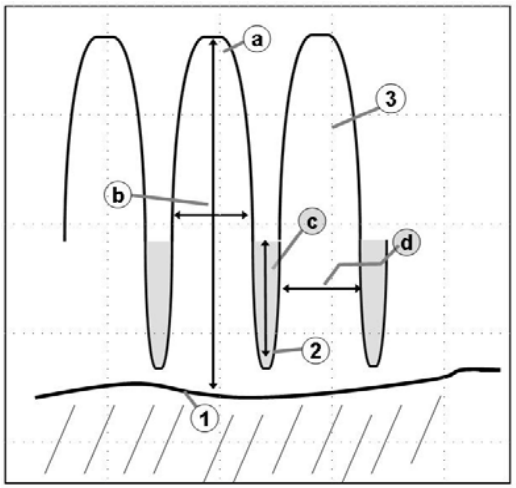
Male:
kg /d Control: 1.260 ± 0.274
kg/d 1% Lactulose: 1.223 ± 0.355 **(- 2,2%)**
kg/d 3% Lactulose: 1.377 ± 0.149 **(+ 9,3%)**

Female:
kg /d Control: 1.316 ± 0.207
kg/d 1% Lactulose: 1.329 ± 0.158 (+ 0,9%)
kg/d 3% Lactulose: 1.323 ± 0.163 (+ 0,5%)

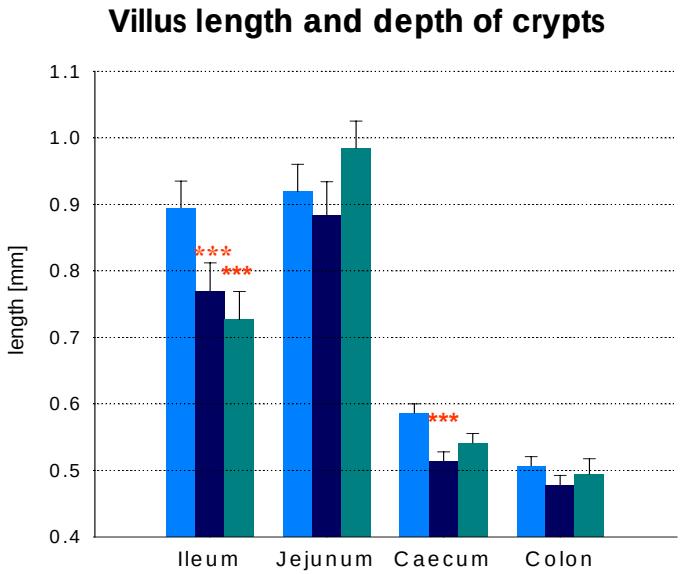
Histology results (1)

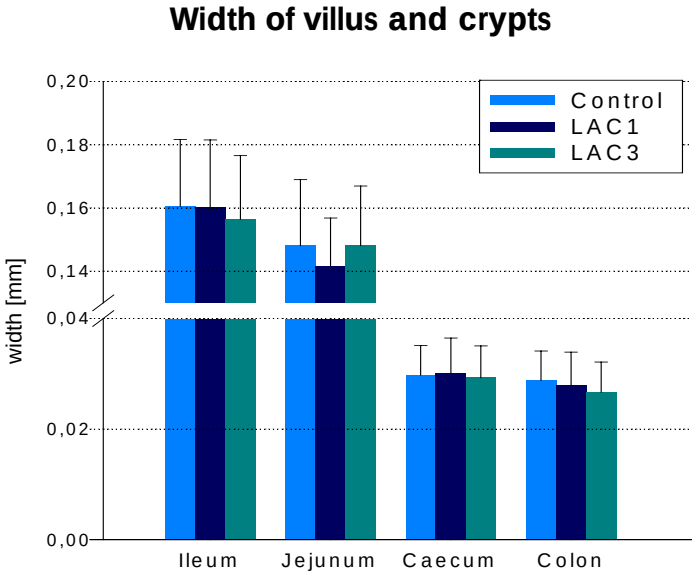
Fleige et al., (2007) Animal 1(3): 367-373





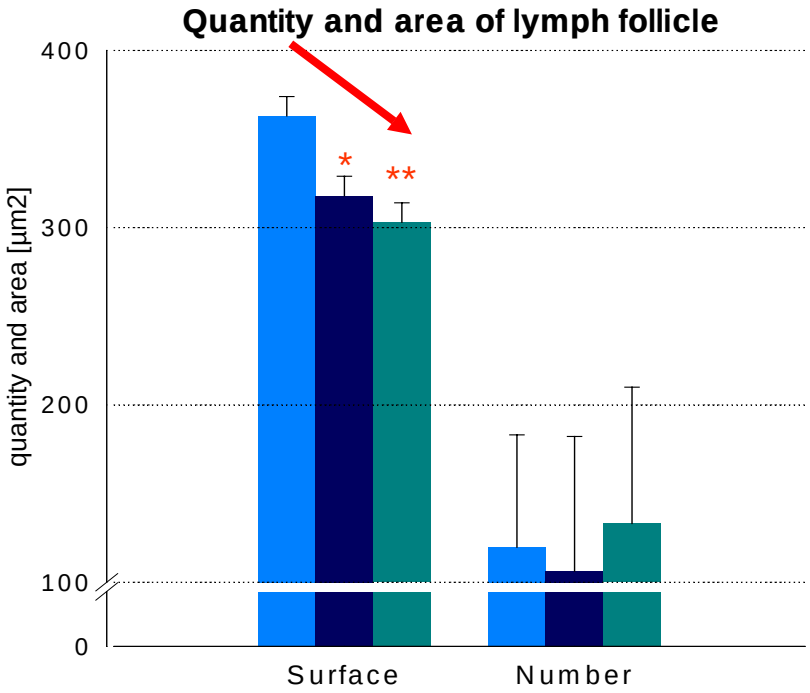
1. Lamina muscularis mucosae
2. Crypt of Lieberkuhn
3. Villus

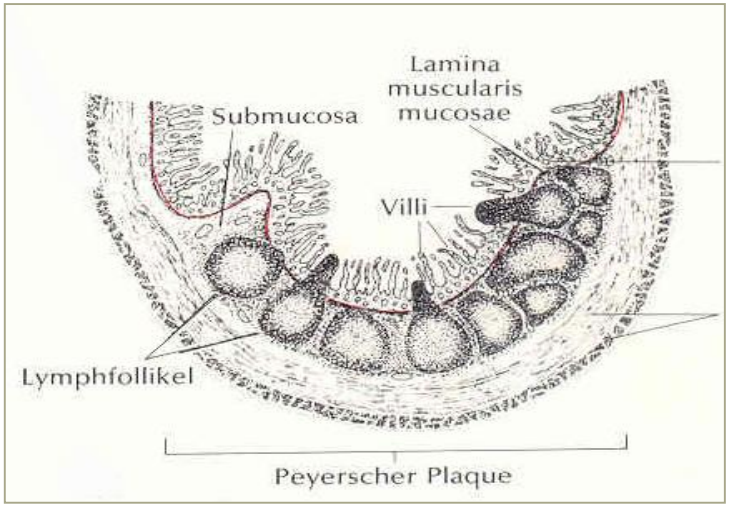


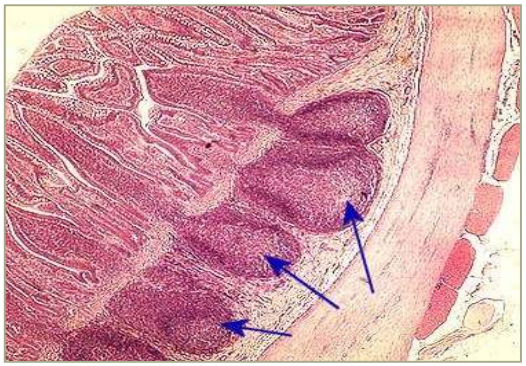


Histology results (2)

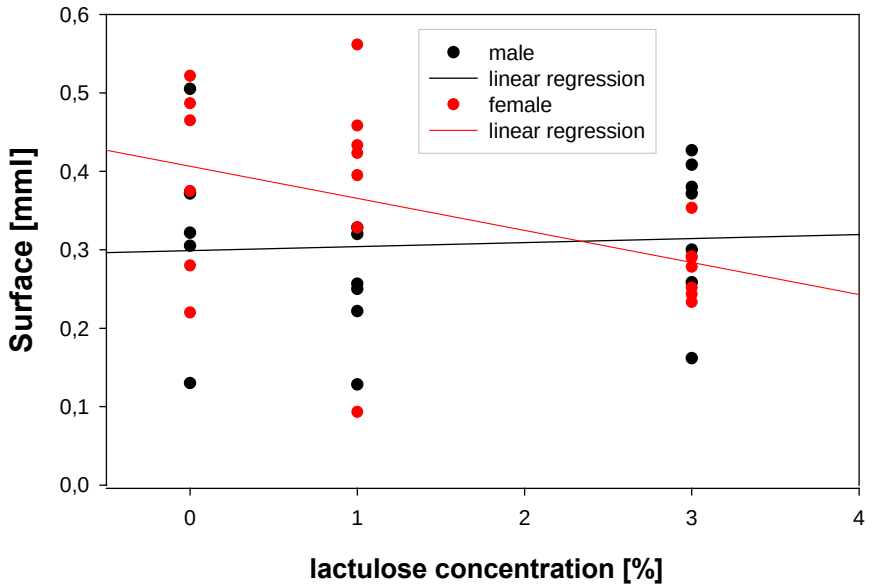
Fleige et al., (2007) Animal 1(3): 367-373



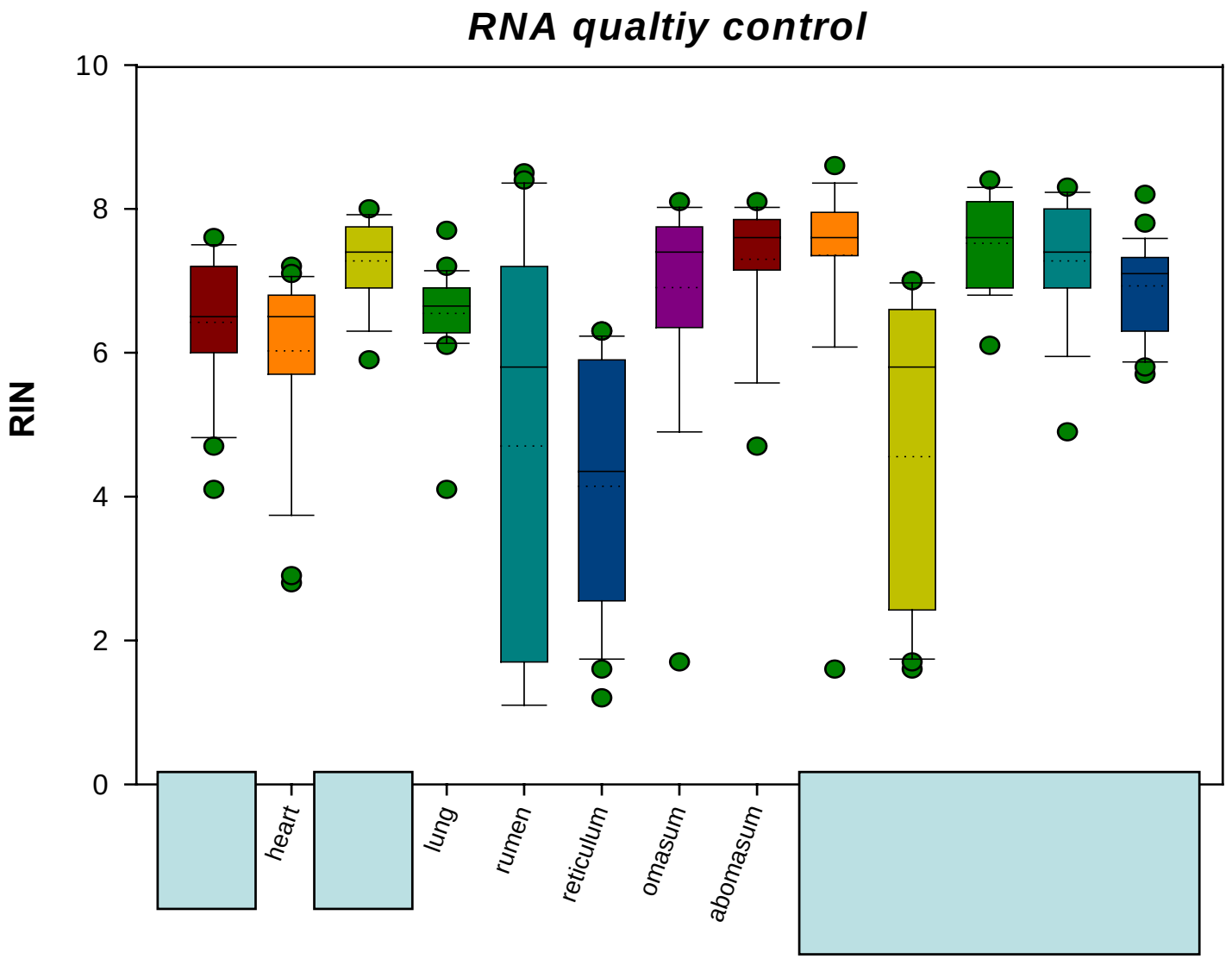




significant change of lymph follicle surface area in female calves



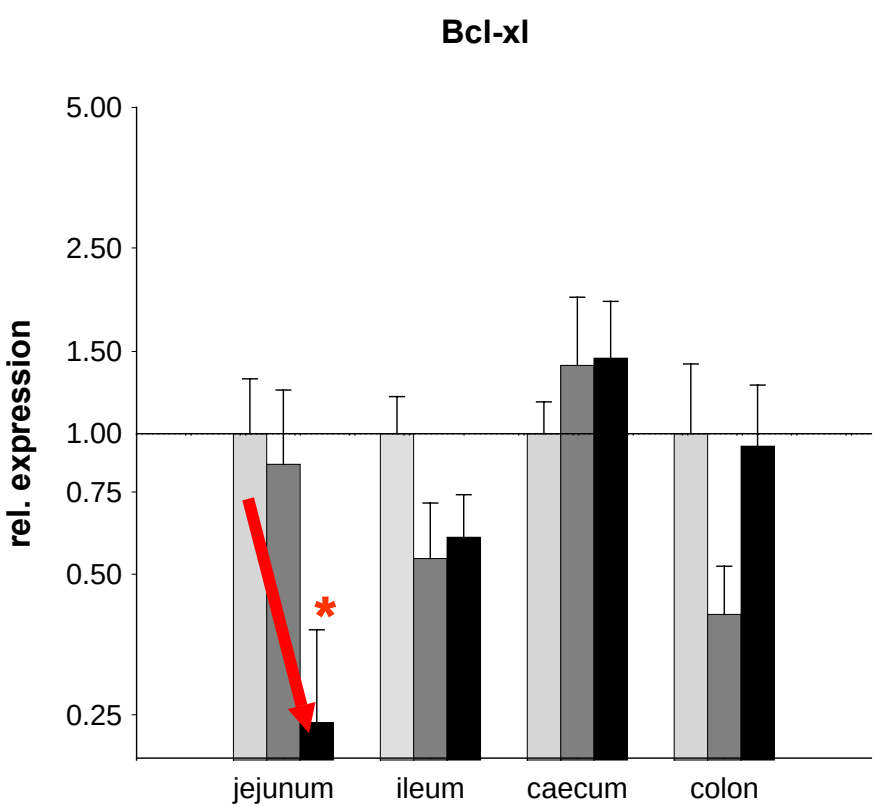
RNA quality – tested in the Agilent Bioanalyzer 2100



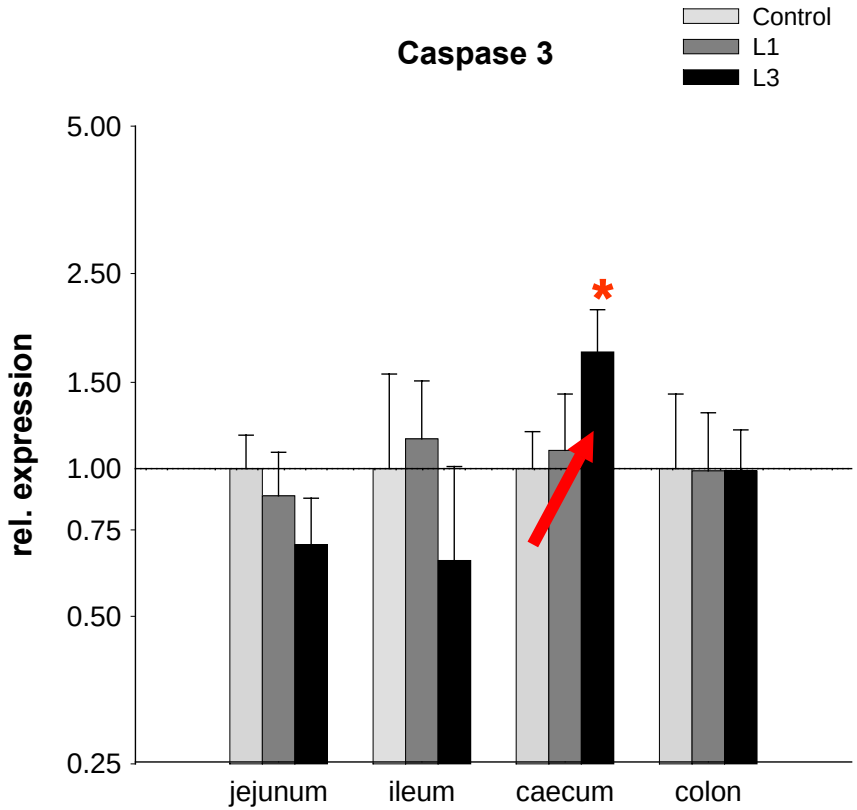
Fleige & Pfaffl, RNA integrity and the effect on the real-time qRT-PCR performance. MAM 2006

Apoptotic factors

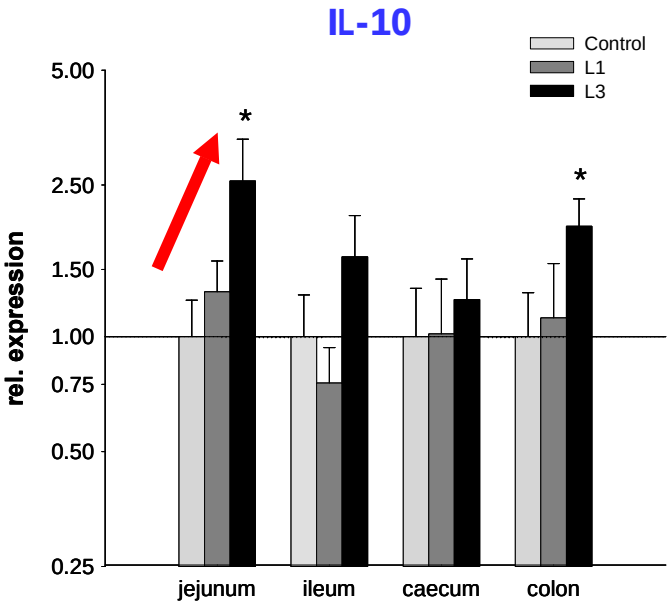
Anti-apoptotic factor

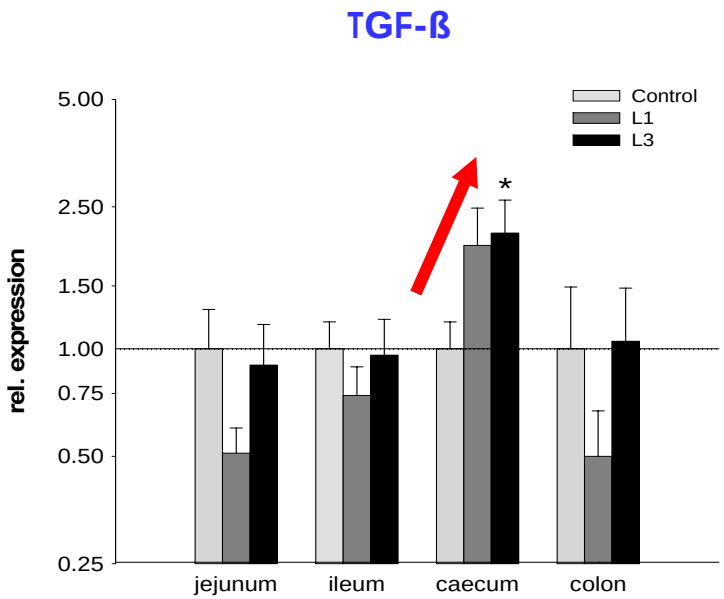


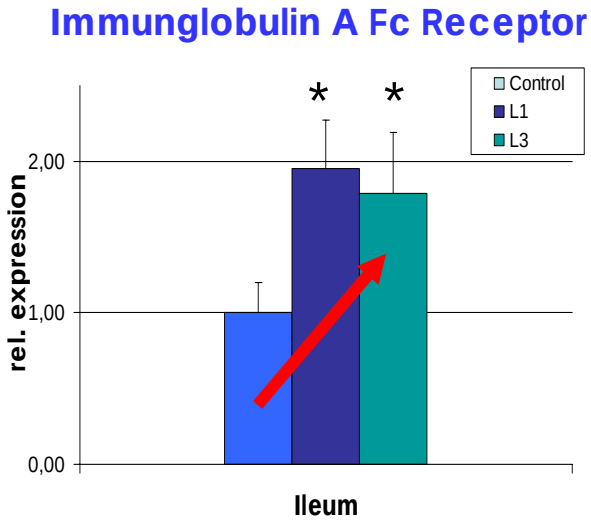
Apoptotic factor

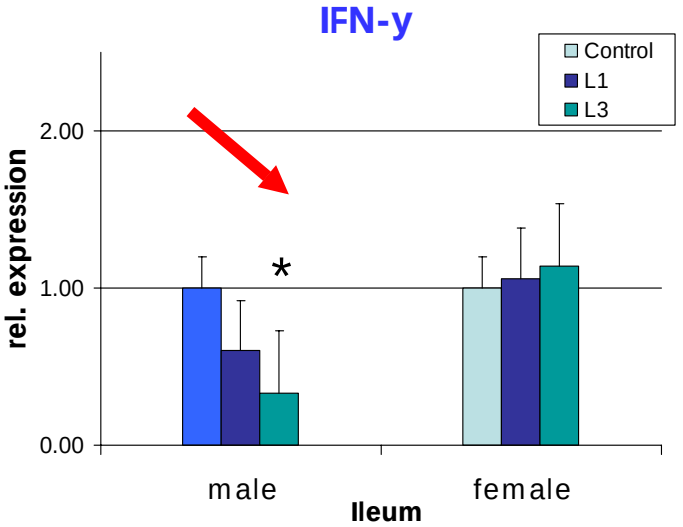


Inflammatory marker genes









Regulation of pro- and anti-inflammatory markers

1% Lactulose

↑ IgA FcR in ileum

↓ Surface of lymph follicles

3% Lactulose

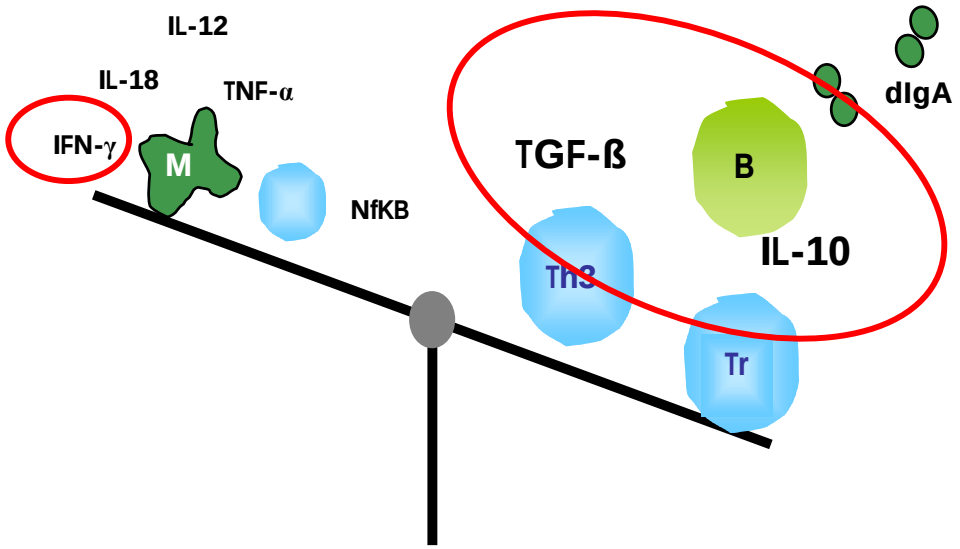
↑ IL-10 in the ileum and colon

↑ TGF-ß in the caecum

↓ IFN-γ in ileum

↓ Bcl-xl in jejunum

↓ Surface of lymph follicles



=> Promote GIT Eubiosis

Discussion

Prebiotics are proven to

- increase apoptotic rate in the intestine
- decrease proliferate activity, villi renewal rate

=> exert a protective effect in carcinogenesis (Hughes and Rowland, 2001)

Ileum

- Lactulose reduce cell proliferation after supplementation for some days (Kien et al., 1999)
- Higher lactulose in ileum than in jejunum (Kamphues et al., 2003; Branner et al. 2004) might be a reason for the effect on villus heights only in the ileum

Peyer plates

- constant number of lymph follicles (own findings)
- Lower total Peyer Plates area => lower activation of lymphatic system

Caecum

- increased apoptotic rate due to the production of SCFA
- morphological effect of butyrat, acetate and propionate (Mandal et al. 2001)

Conclusion

The results indicate that prebiotic lactulose treatment in combination with *E. faecium:*

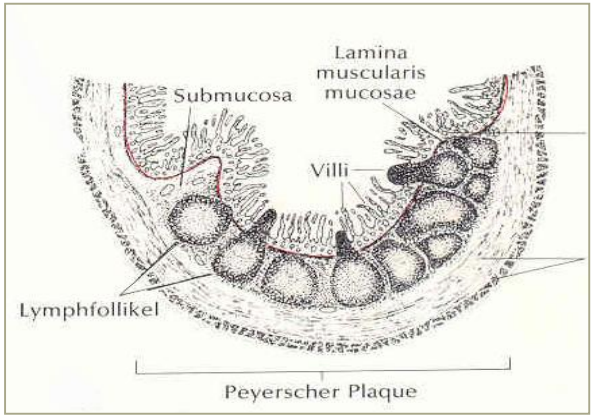
- affects the morphology of the small and large intestine
- affects gene expression of immunological marker genes
- reduced the GALT activation via the Peyer´s patches in the ileum
- has an immune modulatory effect on lymphocyte content and composition of T cell subsets in different immune compartments (results not shown)

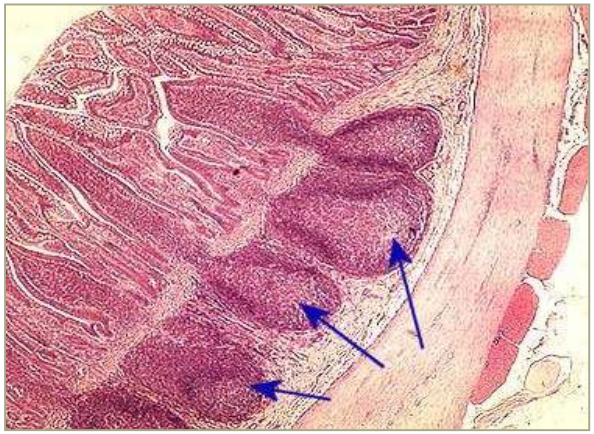
- Effects are obviously sex-specific:
  - male calves tended to have higher body weight
  - female calves tended do have more changes in GIT morphology

=> Lactulose has modulatory effects on immune relevant tissues

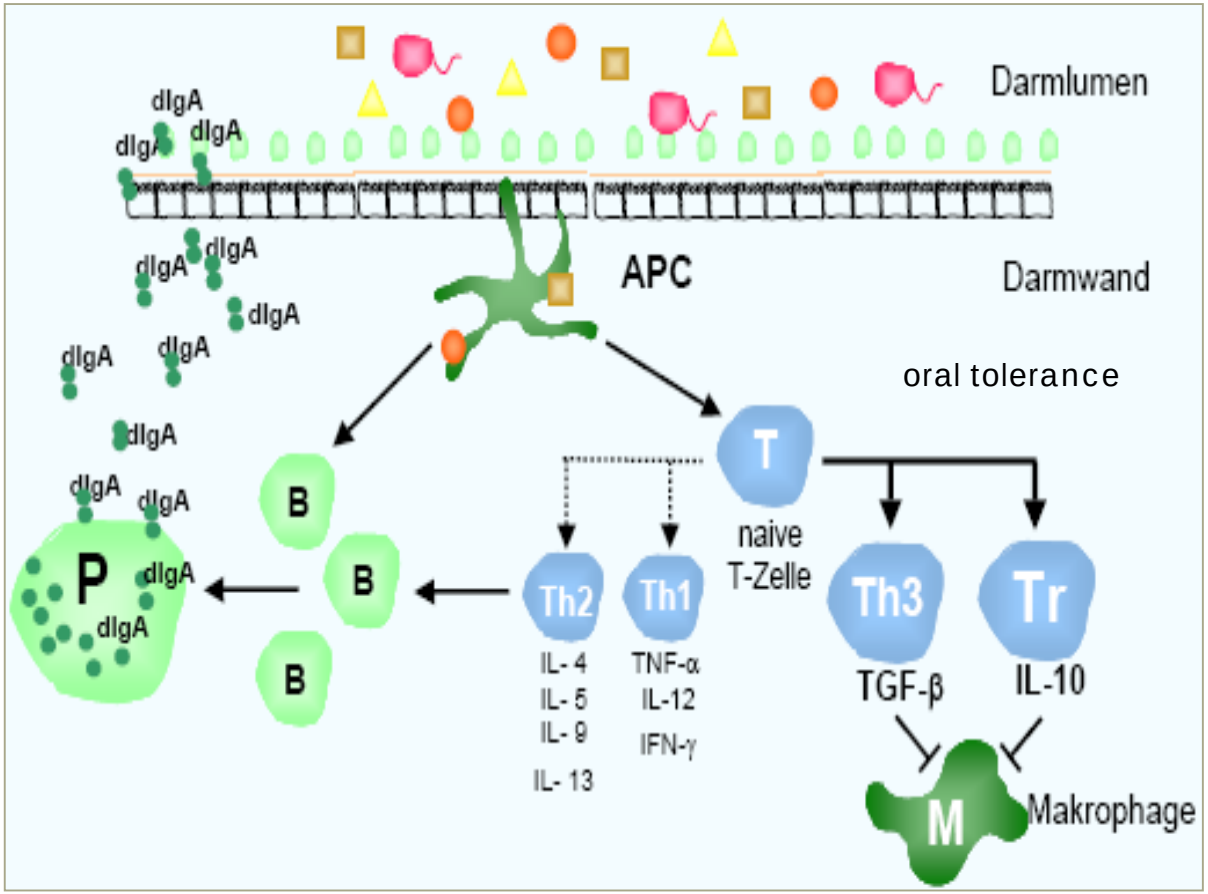
Questions ?

Immune response in the GIT





healthy intestine



Feed intake & weight gain

- **In recent years probiotics have shown a beneficial effect on calves and other animals**
- **During the last decade diverse effects have been found for feed intake, weight gain, decreased faecal coliform count and reduced demand for antibiotic treatment (Fuller, 2005)**

laboratory animals	probiotics		daily weight gain	feed conversation	author
calves	*Tojocerin*		+8.6 %	+7.4 %	LETTNER, 1987
	B. cereus-toyoi		+7.1 %	+6.4 %	ROTH & KIRCHGESSNER, 1988
	Bacillus cereus	10^{7}	+7.6 %		GEDEK et al., 1992
		10^{8}	+8.1 %		GEDEK et al., 1993
		10^{9}	+6.1 %		ROTH et al., 1993
	B. cereus		+3.9 %	+2.2 %	KLEIN, 1995
	B. cereus		+5.6 %	+4.8 %	
	Enterococcus faecium cernelle 68		not significant		DAENICKE & FLACHOWSKY, 1997
	B. subtilis		not significant		JENNY et al., 1991
bull	*B. cereus*		+4.5 %	+3.9 %	
young bull	*Streptococcus faecium SF* 68		+6.5 %		WAGNER & LANGFRIED, 1999
Calves	*E. faecium* + 3% lactulose		+4.9% **=> Not significant**		own investigations

Alteration of proportion of T cells

- **CD4 T cells**
  - T helper cells
  - important in the activation of B cells, T cells and cells of the innate immune system
- **CD8 T cells**
  - T suppressor cells
  - play a role in oral tolerance (antigens encountered in the gut)
  - reduce proliferation of CD4 T cells (Vukmanovic-Stejic et al., 2001)
  - produce IL-4, IL-10 and TGF-ß
- **CD25** (α chain of the IL-2 Receptor)
  - expressed on activated T cells (CD4), B cells and monocytes
- **CD69**
  - marker of leukocyte activation

			CD marker											
			CD4			**CD8**			**CD25**			**CD69**		
tissue	group	sex	x-fold reg.	SEM	P-value	x-fold reg.	SEM	P-value	x-fold reg.	SEM	P-value	x-fold reg.	SEM	P-value
ileum	LAC 1	male	1·00	0·28		1·67	0·49		1·62	0·57		1·78	0·42	
		female				0·42	0·11		0·89	0·43		0·65	0·12	
	LAC 3	male	0·97	0·34		1·66	0·57		0·50	0·18		1·12	0·20	
		female	0·60	0·21		0·80	0·21		1·39	0·43		1·27	0·46	
mLN	LAC 1	male	1·78	0·39	+	1·27	0·37		0·92	0·40		1·37	0·22	
		female				0·96	0·26		1·58	0·52		1·20	0·28	
	LAC 3	male	1·17	0·37		1·15	0·18					1·07	0·25	
		female	0·75	0·09		0·88	0·20		1·63	0·29		0·95	0·14	
spleen	LAC 1	male	1·70	0·53		0·69	0·22		1·35	0·62		0·44	0·13	
		female	0·66	0·17		0·80	0·31		0·80	0·45		0·75	0·34	
	LAC 3	male	0·90	0·17		1·56	0·43		1·09	0·31				
		female	0·72	0·18		0·55	0·13		0·58	0·36		0·58	0·15	
WBC	LAC 1	male	1·06	0·23		0·68	0·10		1·06	0·27		0·74	0·17	
		female	1·56	0·43					1·53	0·28		2·00	0·39	+
	LAC 3	male	1·07	0·22		0·46	0·16		1·34	0·35		1·34	0·38	
		female	0·83	0·20		1·31	0·24		1·43	0·29		1·63	0·25	

Lactulose enhance oral tolerance

Alteration of proportion of T cells and increased lymphocyte numbers in the GALT (Field et al., 1999) and blood (Kaufhold et al., 2000) due to lactulose