Stiftung Tierärztliche Hochschule Hannover
University of Veterinary Medicine Hannover, Foundation

Probiotika und Funktionsparameter der intestinalen Barriere

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24. Hülsenberger Gespräche
06. – 08. Juni 2012

Gliederung

- Einleitung
- Probiotika und zootechnische Parameter
- Mögliche Wirkmechanismen
  - Allgemeine Konzepte
  - Darmbarriere
  - Infektionsmodell
- Zusammenfassung und Ausblick

Probiotika: Definition

Probiotika sind definierte lebende Mikroorganismen, die in ausreichender Menge in aktiver Form in den Darm gelangen und hierbei positive gesundheitliche Wirkungen erzielen (BgVV 1999).

„University and feed industry researchers have been extremely sceptical of the concept sometimes placing it in the category as snake oil or fufu dust“ (Pollmann 1986)

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Leistungsdaten – Einfluss auf die Ferkelaufzucht (Jadamus, Vahjen und Simon 1999)

	Kontrolle [n=85]	*B. cereus var. toyoi* [n=91]	Differenz zur Kontrolle
Futteraufnahme [g/Tag]	860 ± 102	901 ± 137	+ 4,8 %
Zunahme [g/Tag]	488 ± 56	524 ± 87	+ 7,4 %
Futteraufwand [:1]	1,76 ± 0,14	1,72 ± 0,09	- 2,4 %
Durchfall ≥ 2 Tage [%]	17,6	2,2	-15,4

Tiere: 176 Ferkel aus 16 Würfen, 29.-70. Lebenstag
Futter: 18,6% XP, 12,7 MJ ME/kg; *B. cereus var. toyoi* 10^9 KbE/kg

Leistungsdaten – Einfluss auf die Leistung von Absetzferkeln (Van Isterdael 1998)

	neg. Kontrolle	pos. Kontrolle [50 mg/kg]	*B. cereus var. toyoi* [10^9 KbE/kg]
Futteraufnahme [g/Tag]	666 (100)	704 (106)	685 (103)
Zunahme [g/Tag]	384^b (100)	431^a (112)	410^a (107)
Futteraufwand [:1]	$1,73^b$ (100)	$1,63^a$ (94)	$1,67^a$ (97)

Tiere: 30 Ferkel/Behandlung (Seghers x Pietrain), 9-25 kg Lebendmasse
Futter: 18,3% XP, 4,7 % XL, 3,7% XF

Einfluss von *Enterococcus faecium* auf die Performance von Ferkeln (Zeyner und Boldt 2006)

Table 3 Mean ± pooled SD litter size, age at weaning, diarrhoea score and DWG of piglets from 54 and 60 VG and PG sows, respectively

Item	VG	PG	±Pooled SD
Litter size			
Viable born piglets (*n* per litter)	11.8[a]	11.9[a]	±2.78
Stillborn piglets (*n* per litter)	1.0[a]	1.0[a]	±1.1
Weaned piglets (*n* per litter)	9.9[a]	10.1[a]	±1.29
Age at weaning (days p.n.)	23.8[a]	24.2[a]	±3.10
Diarrhoea score	1.2[b]	1.5[a]	±0.54
DWG (g/day)	261[a]	244[b]	±43.1

VG = verum group.
PG = placebo group.
DWG = daily weight gain.
[a,b]Means sharing unique superscripts are significantly different at $p < 0.05$.

Diarrhoe-Inzidenz

VG	PG
14,8%	40,0%

p<0,05

Einfluss von *Enterococcus faecium* auf die Performance von Sauen und ihren Ferkeln (Taras et al. 2006)

- Versuchsdesign
  - Behandlung der Sauen von Tag 90 *ante partum* bis Tag 28 *post partum* (17 Wochen)
  - Behandlung der Ferkel von Tag 15 *post natum* bis Tag 56 *post natum* (6 Wochen, Absetzen am Tag 28)

Table 2. Effect of probiotic supplementation on sow weight, born and nursed piglets, as well as piglet losses per litter during lactation

Item	Diet		SEM	*P*-value
	Control Mean	*Enterococcus faecium* Mean		
Sows				
N	10	10		
Parity	1.50	1.70	0.13	0.58
ADFI, kg	4.72	4.66	0.08	0.68
Weight loss during lactation	25.4[1]	20.6	2.09	0.51
Born piglets				
Alive	11.4	10.7	0.60	0.53
Dead	0.70	0.70	0.22	0.85
Added piglets[2]	0.70	0.40	0.22	0.68
Nursed piglets				
D 3	10.60	10.70	0.47	0.68
D 7	10.50	10.30	0.32	0.85
D 14	10.10	9.60	0.34	0.48
D 28	8.90	8.80	0.32	0.91
Piglet losses				
D 0 to 3	1.50	0.40	0.27	0.09
D 0 to 7	1.60	0.80	0.30	0.35
D 0 to 14	2.00	1.50	0.37	0.68
D 0 to 28[3]	2.70	1.80	0.45	0.44

[1]Mean of just 5 sows because of the initial BW exceeding the scale limits.
[2]Adjustment of litter size during the first lactation day with comparable piglets derived from sows, which were excluded from trial but received group-specific diets.
[3]An additional 5 piglets per group were killed on d 14 for digesta and tissue samples.

Taras et al. 2006 J Anim Sci 84, 608-617

Table 3. Effect of probiotic supplementation on ADG, ADFI, and G:F of weaned piglets between d 28 and 56 of age

Item	Diet: Control median (25–75%)[1]	Diet: *Enterococcus faecium* median (25–75%)[1]	*P*-value
Piglets (n)	77	76	
Wk 5			
ADG, g	136 (66 – 264)	97 (28 – 250)	0.16
ADFI, g	237 (181 – 367)	235 (194 – 302)	0.70
G:F, g/kg	578 (297 – 1059)	432 (144 – 918)	0.19
Wk 6			
ADG, g	393 (246 – 489)	337 (265 – 413)	0.11
ADFI, g	577 (448 – 681)	533 (434 – 603)	0.33
G:F, g/kg	697 (584 – 806)	706 (503 – 784)	0.67
Wk 7			
ADG, g	529 (422 – 650)	523 (433 – 613)	0.69
ADFI, g	861 (719 – 978)	832 (730 – 902)	0.39
G:F, g/kg	633 (532 – 706)	623 (574 – 707)	0.80
Wk 8			
ADG, g	637 (490 – 767)	658 (552 – 765)	0.28
ADFI, g	1191 (877 – 1269)	1096 (971 – 1322)	0.67
G:F, g/kg	577 (505 – 608)	607 (514 – 730)	0.11
Postweaning period, wk 5 to 8			
ADG, g	423 (370 – 503)	422 (360 – 495)	0.78
ADFI, g	694 (618 – 783)	689 (621 – 775)	0.61
G:F, g/kg	616 (578 – 655)	620 (546 – 681)	0.95

[1]Medians are calculated from weekly feed intake and BW gain of 36 and 38 pens with 2.19 ± 0.07 and 2.03 ± 0.05 pigs per pen in control and probiotic group, respectively. Supplied in parenthesis the values for the 25 and 75% quartile.

Taras et al. 2006 J Anim Sci 84, 608-617

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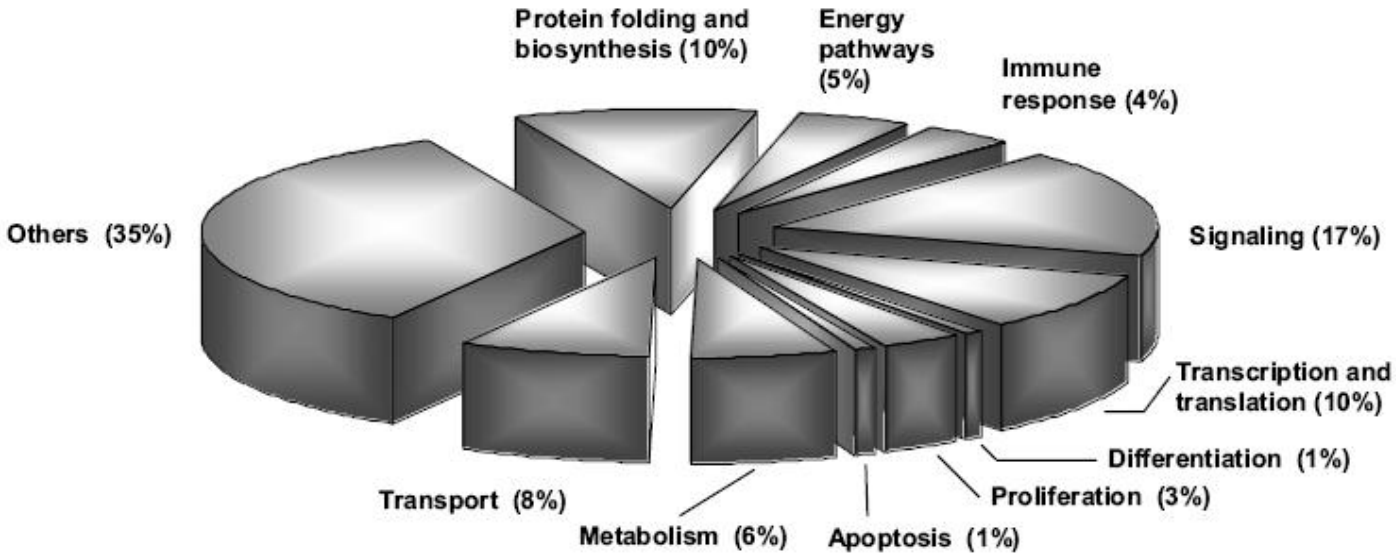
Genexpressionsprofile von Caco-2 Zellen nach 6-stündiger Inkubation mit E. coli Nissle

How do probiotics act?



(Ukena et al. 2005)

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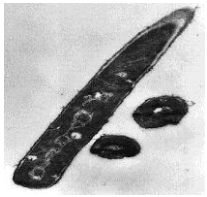
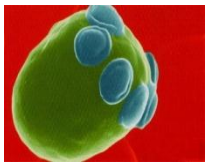
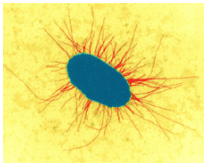
Darmbarriere: Effekte bei gesunden Schweinen

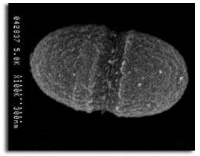
- Besiedlung/Kolonisation
- Schleimhautarchitektur
- Mucosale Barriere-Funktionen
- Enterisches Nervensystem

Applied probiotics







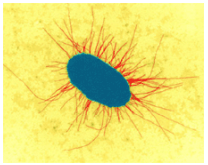


- *Bacillus cereus* var. toyoi (ToyoCerin®, growth promotor in livestock farming)
- *Saccharomyces boulardii* (Perenterol®, anti-diarrhoeal activity)
- *Escherichia coli* Nissle 1917 (Mutaflor®, anti-diarrhoeal and anti-inflammatory activity)
- *Enterococcus faecium*

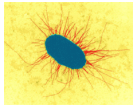
Nachweis von *EcN* in Schweinebeständen in Deutschland (Kleta et al. 2002)

"Screening of five epidemiologically unlinked swine farms and two wild boar groups showed one farm positive for E. coli Nissle 1917."

"A feeding experiment with four piglets showed viable E. coli Nissle 1917 in the intestine of three animals."



Kolonisation von *EcN* im Darm



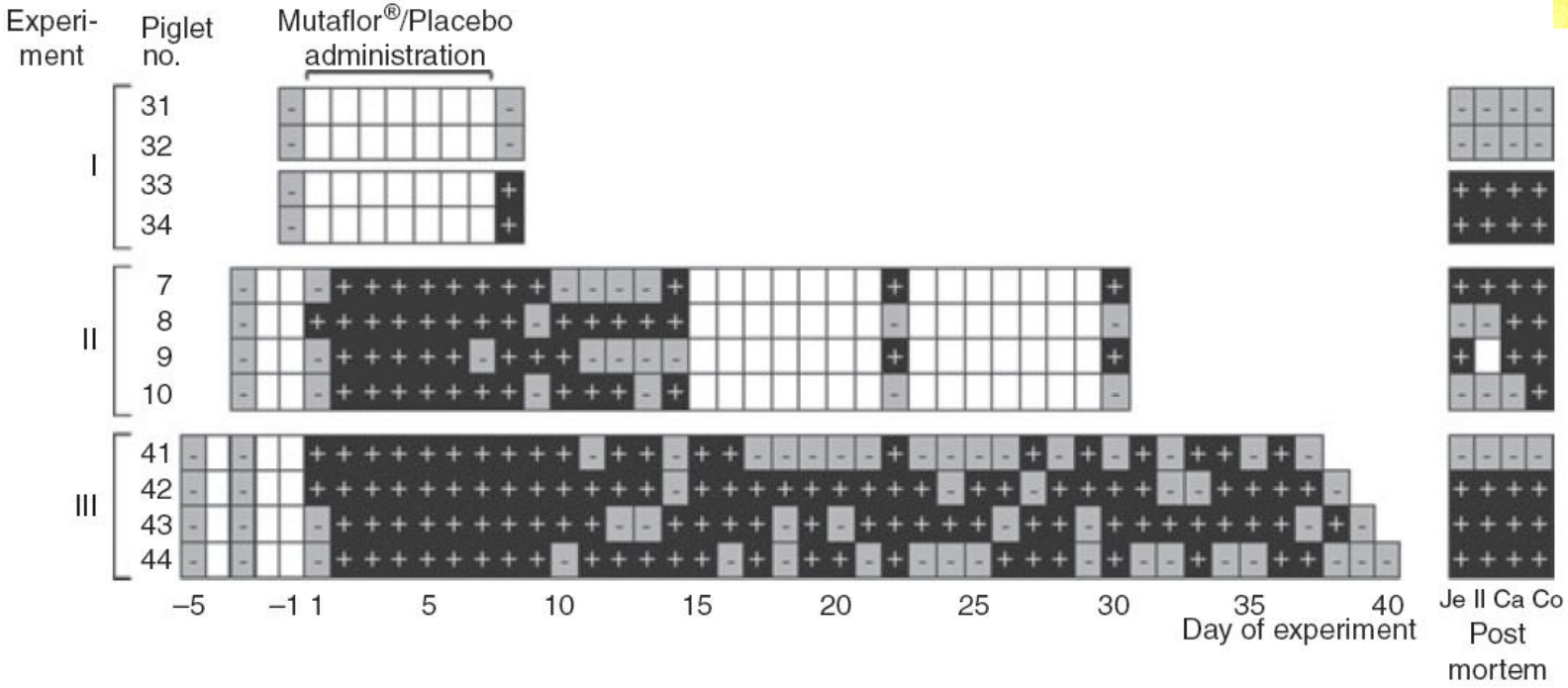
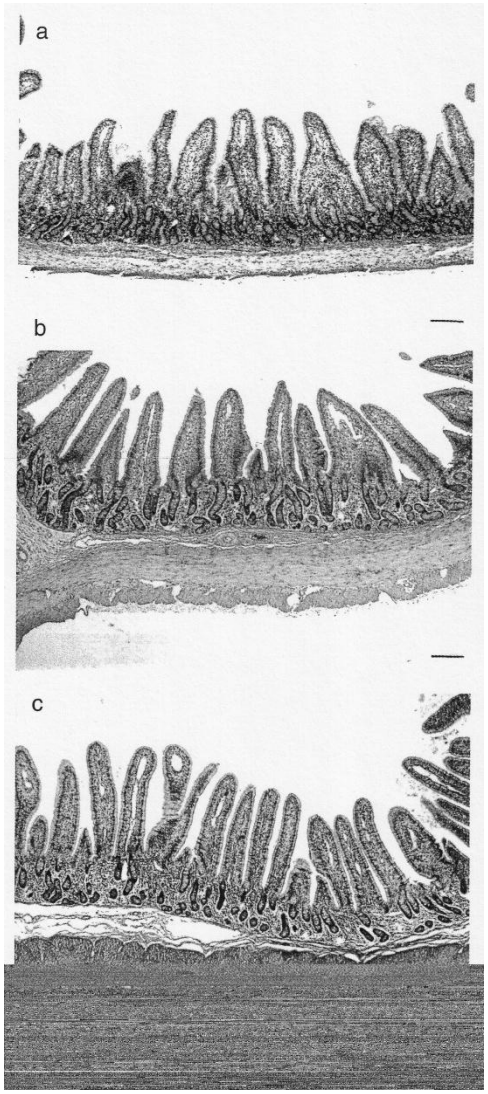
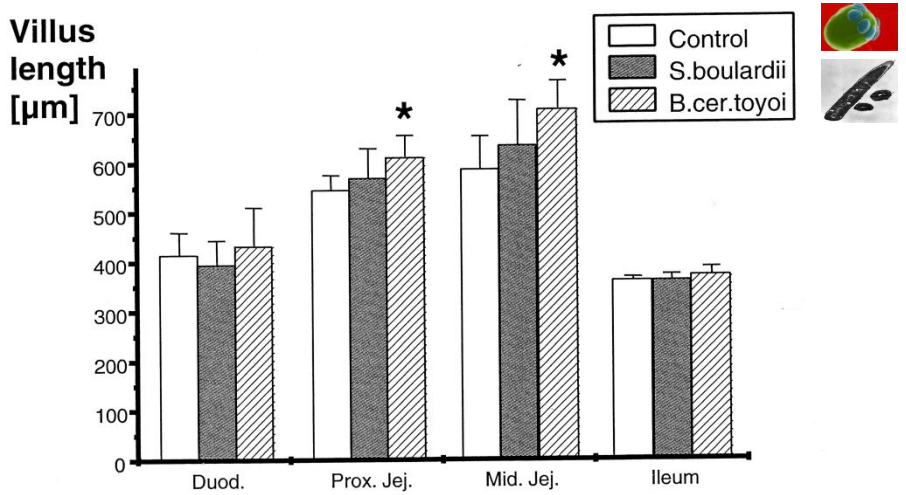


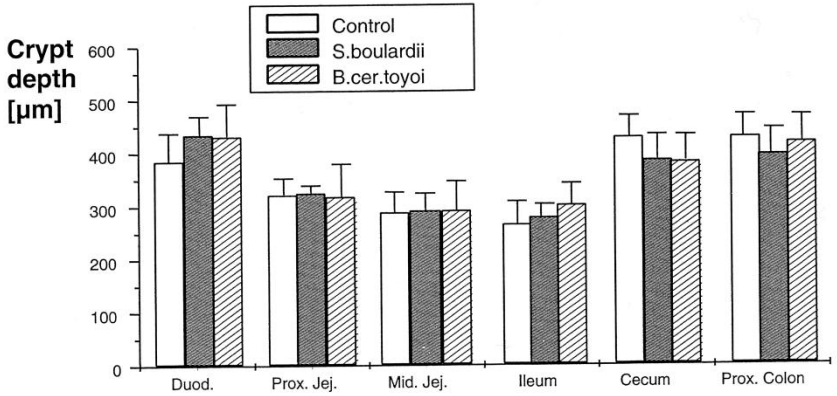
Figure 2 Detection of EcN in faecal and swab specimens from piglets after Mutaflor® feeding (results of the principles of experiments I, II and III). Piglets were daily inoculated *per os* with 3·75–37·5 × 10^8 CFU per kg body weight per day Mutaflor® (piglets 33 and 34, as well as all piglets in experiments II and III) or placebo (piglets 31 and 32) from days 1 to 7. By multiplex PCR of Gassner suspensions of EcN detection was performed in the respective samples. Postmortem samples derived from jejunum (Je), ileum (Il), caecum (Ca) and colon (Co). (+), sample positive for EcN-DNA; (–), sample negative for EcN-DNA; (□), no sample available.

(Barth et al. 2009)

Wirkungen von *S. boulardii* und *Bac. cer. toyoi* auf Zottenlänge und Kryptentiefe





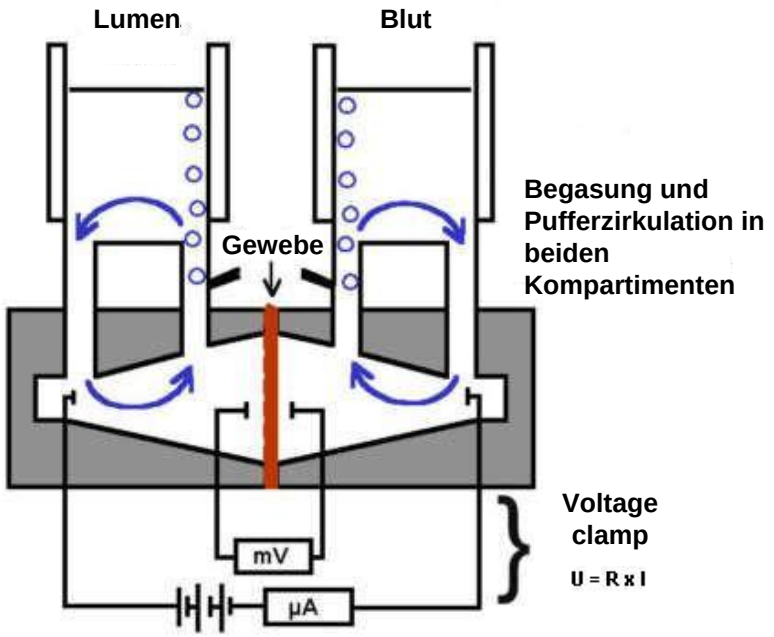


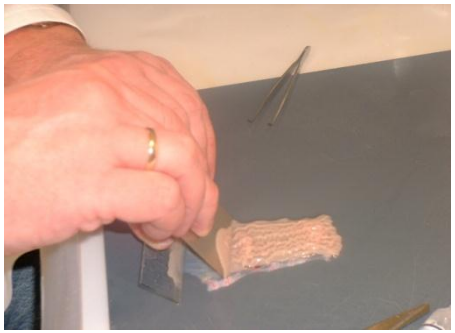
(Baum et al. 2002)

Mucosale Barriere-Funktion: Ussing-Kammer-Technik

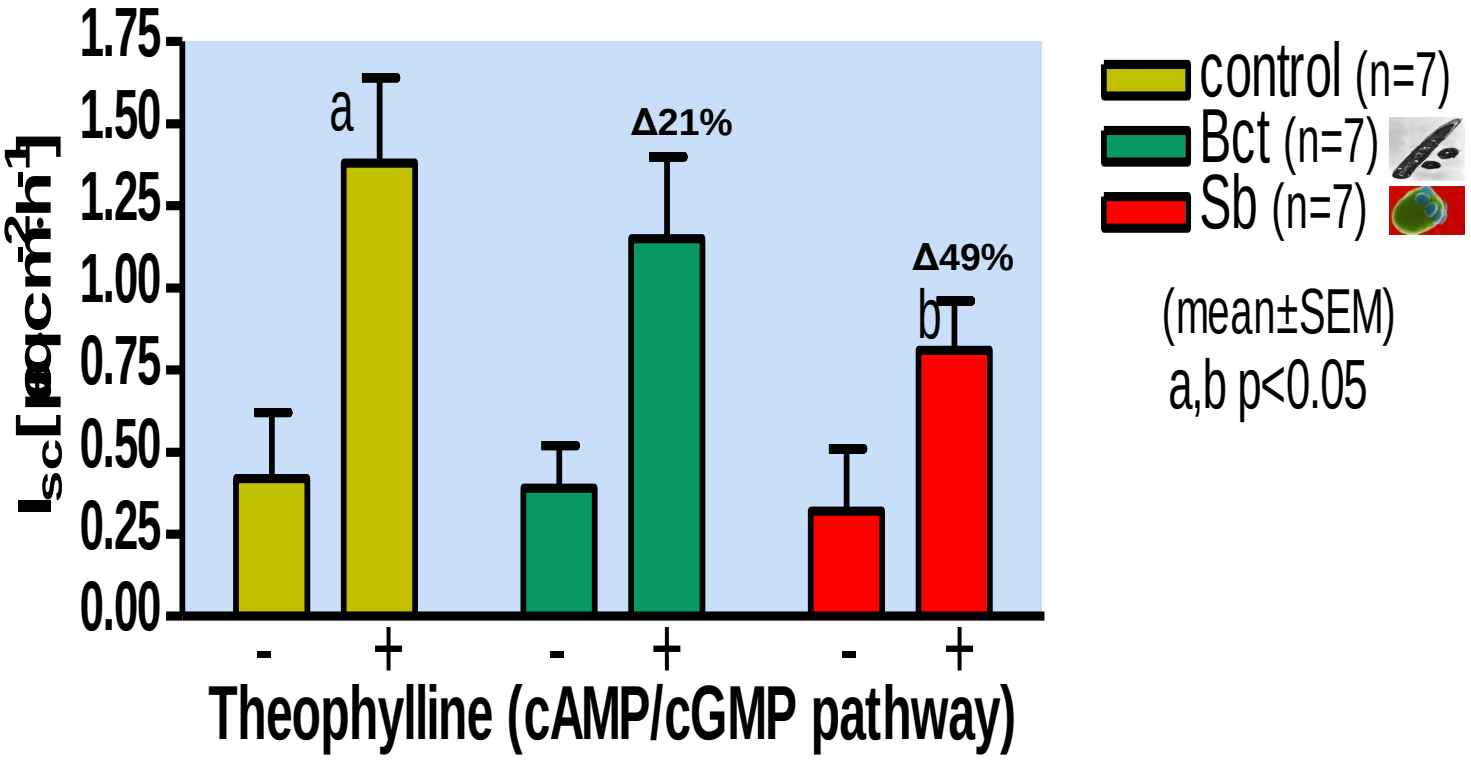
<u>Gewebeparameter</u>

- Sekretorische Kapazität
- Second-messenger-Wege
- Parazelluläre Permeabilität
- Nährstofftransport
- Enterisches Nervensystem



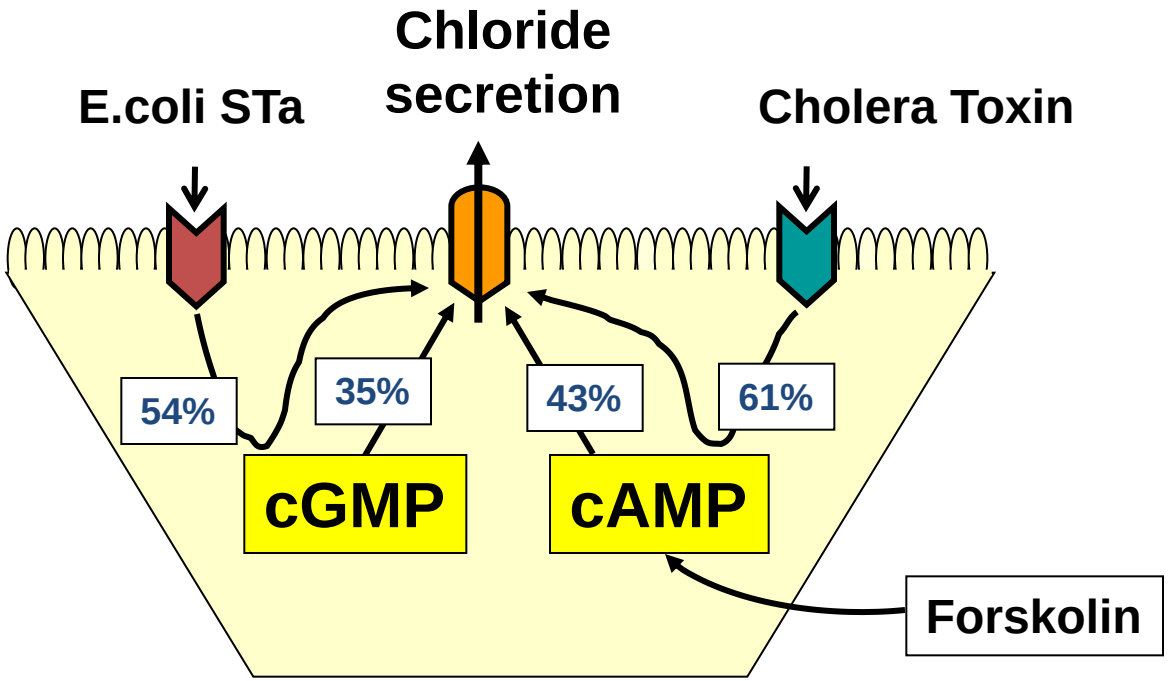


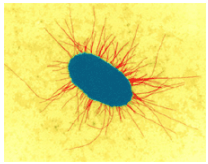
Abnahme der Theophyllin-induzierten Stromantwort



(Winckler et al. 1998)

Abnahme der second-messenger-induzierten Stromantwort durch EcN



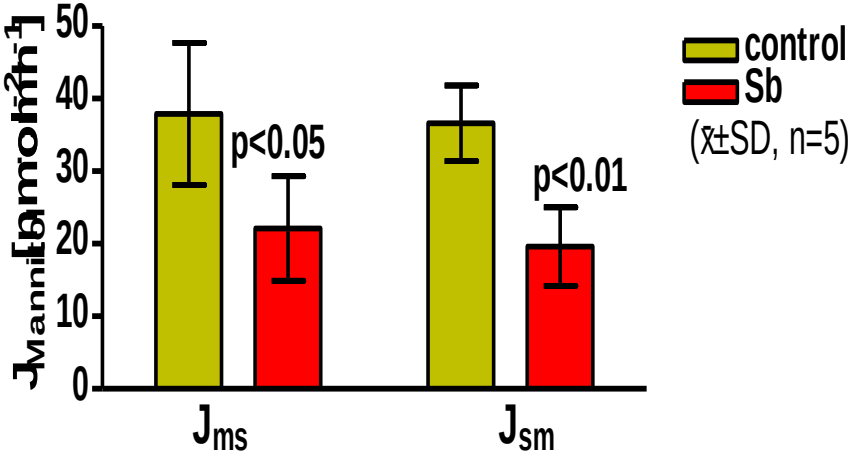


14 d *EcN* application;
response in % of control

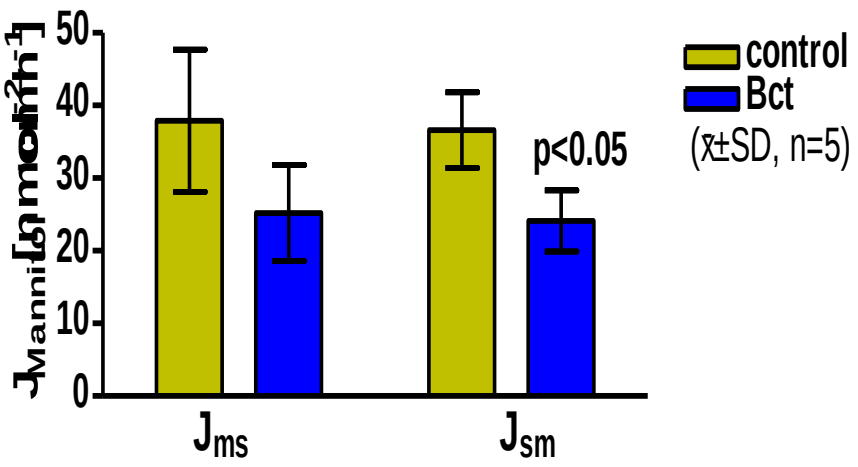
(Breves et al. unpublished)

Reduktion der Mannitol-Fluxraten (J_{ms} und J_{sm}) im Jejunum

Sb



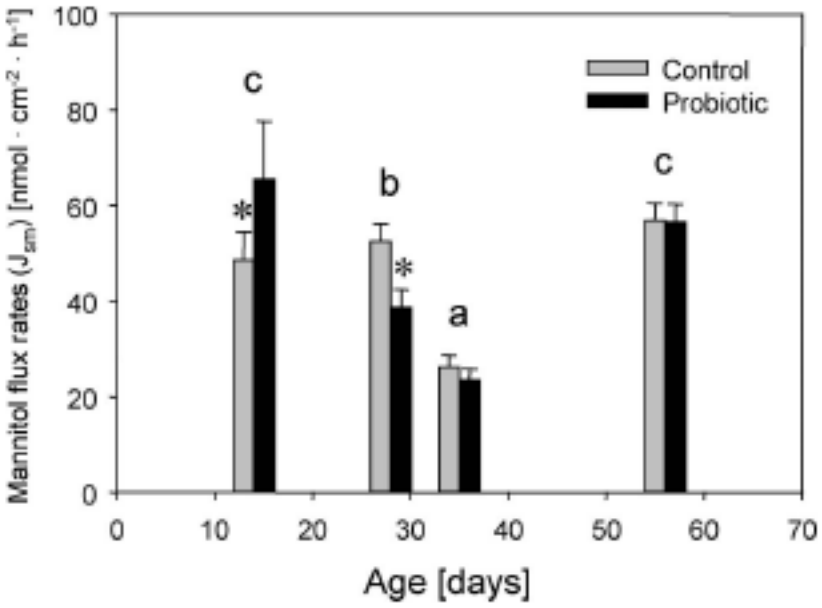
Bct

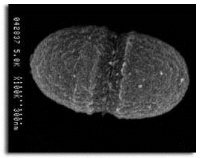


(Winckler et al. 1998)

Effekte von *E. faecium* auf die Mannitol-Fluxraten (J_{sm}) im Jejunum vom Schwein

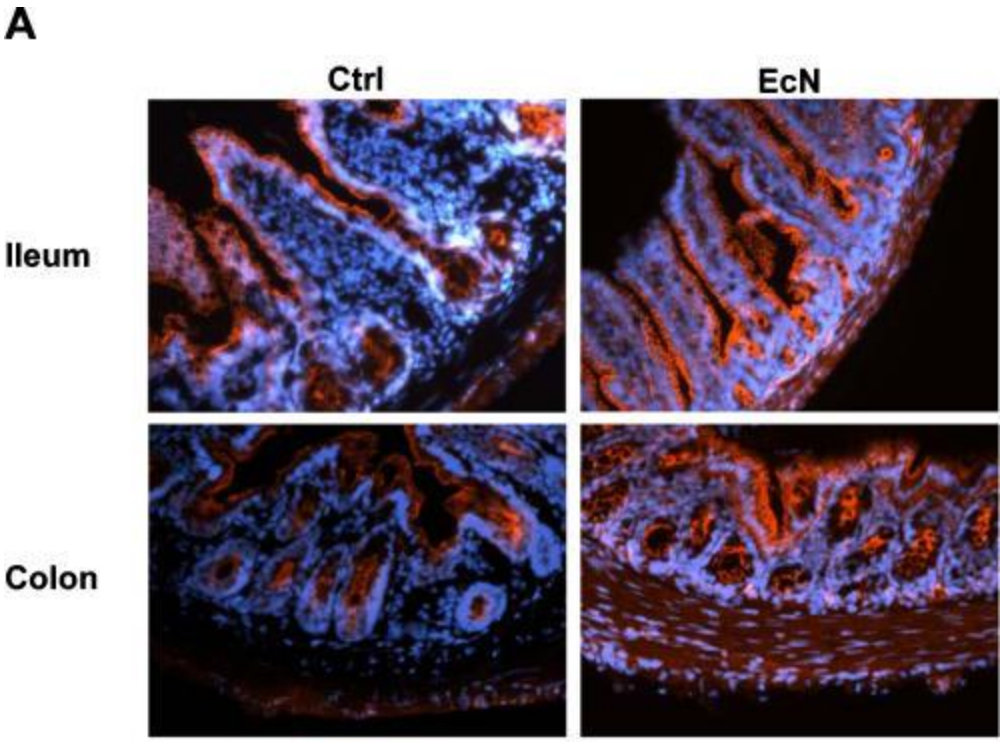
(means±SEM, N=5)



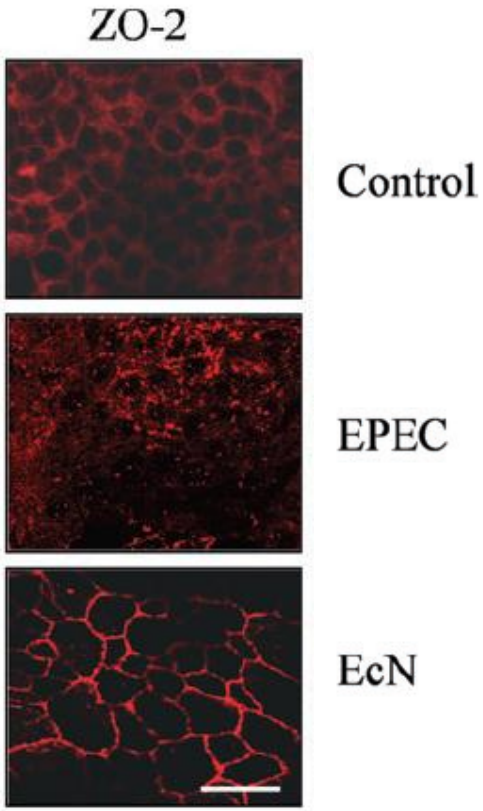


(Lodemann et al. 2006)

Einfluss von *EcN* (6 Tage) auf die Expression von ZO-1 Protein bei Mäusen

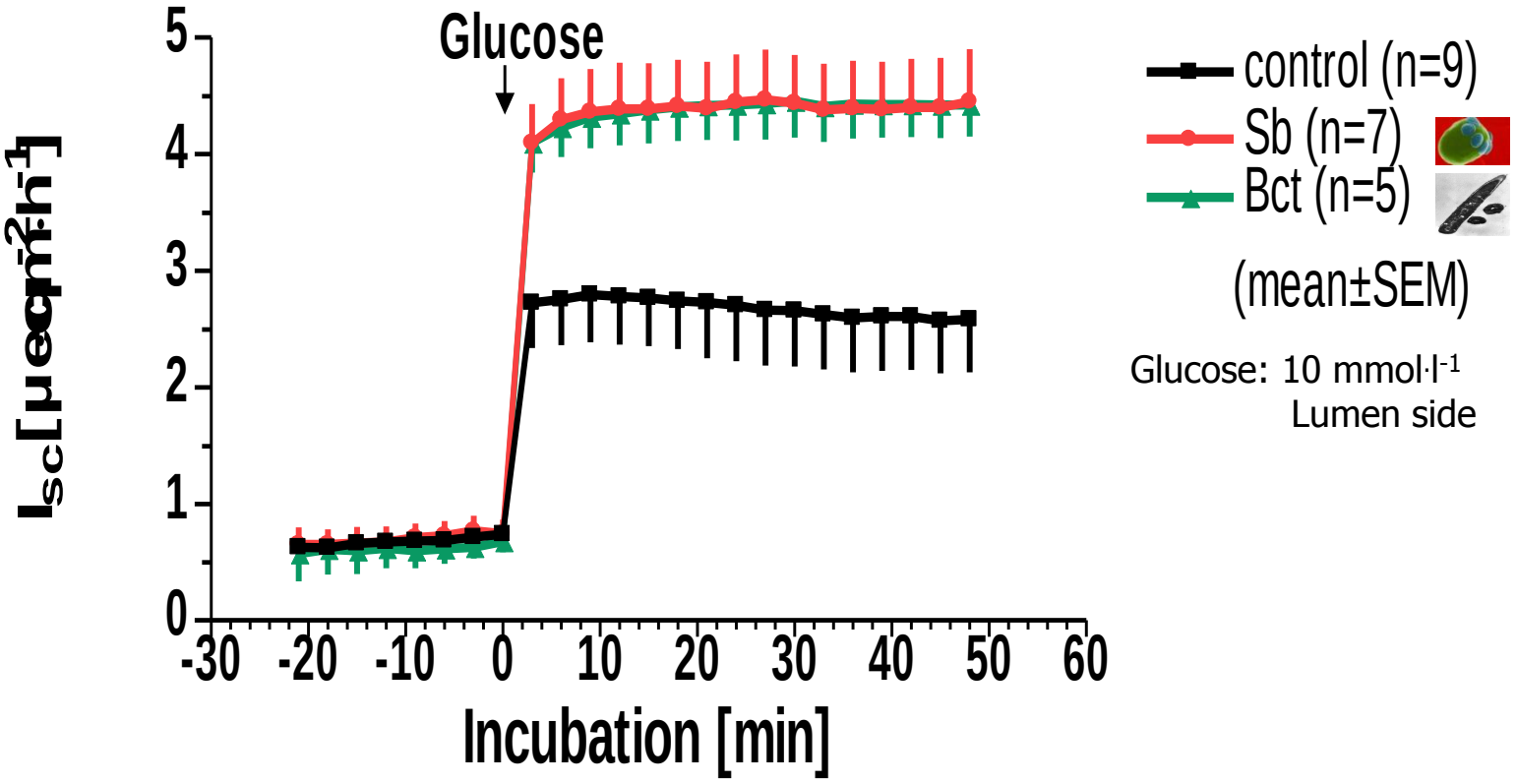


(Ukena et al. 2007)



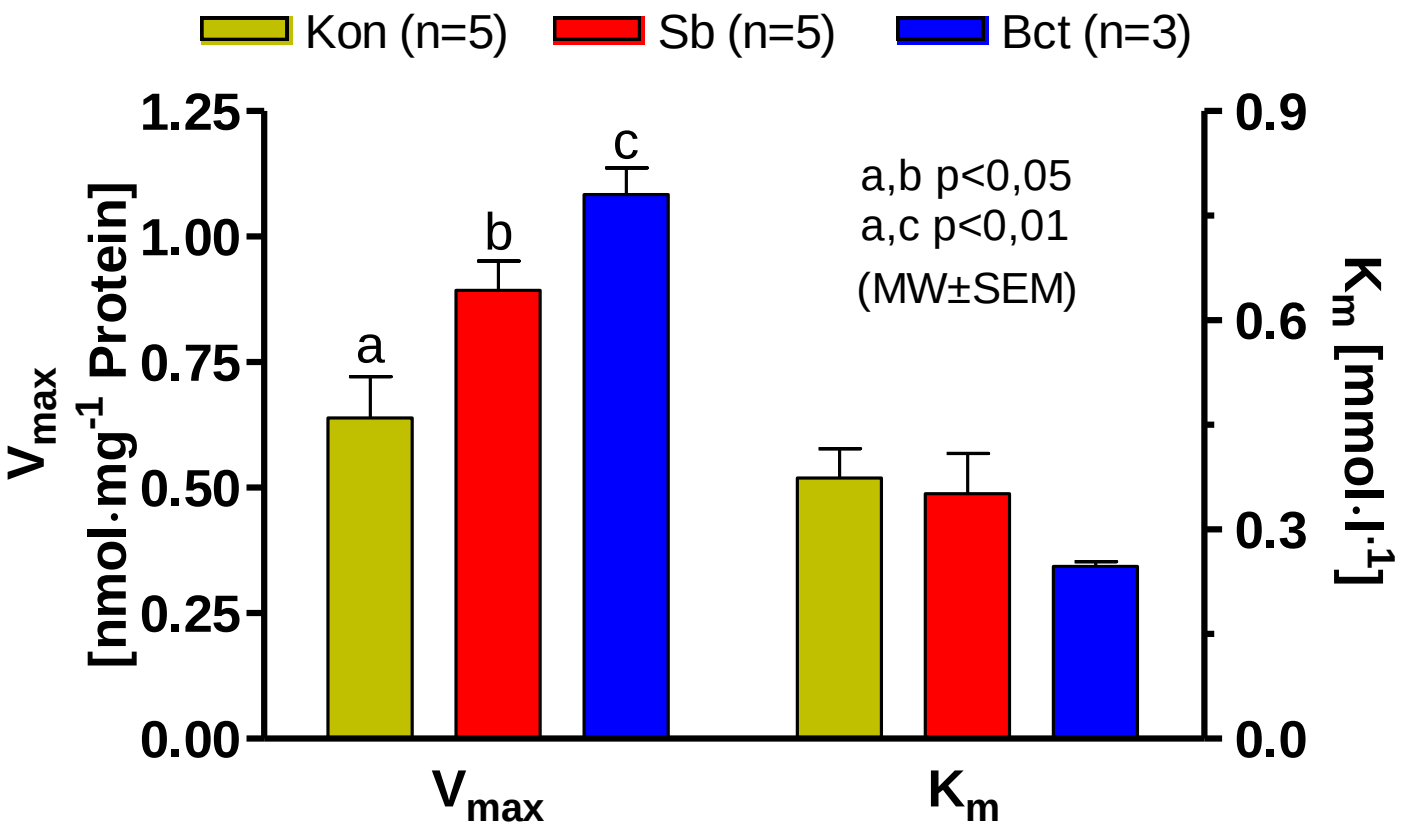
(Zyrek et al. 2007)

Sodium-coupled glucose transport is stimulated in jejunum



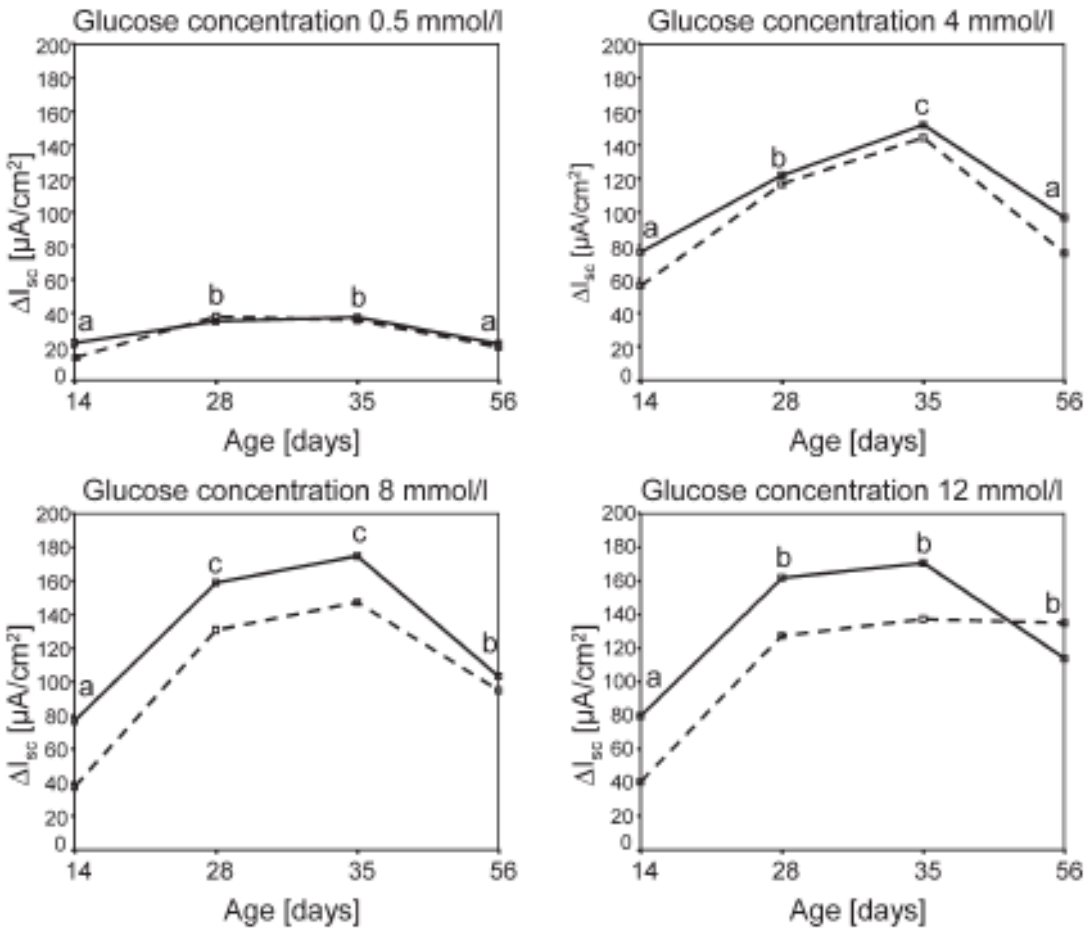
(Breves et al. 2000)

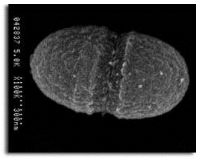
Kinetische Eigenschaften des Na^+-abhängigen Glukose-Transportes über die jejunale Bürstensaummembran nach Sb- bzw. Bct-Applikation



Breves et al. 2000

Effekte von *E. faecium* auf den jejunalen Glukose-Transport beim Schwein



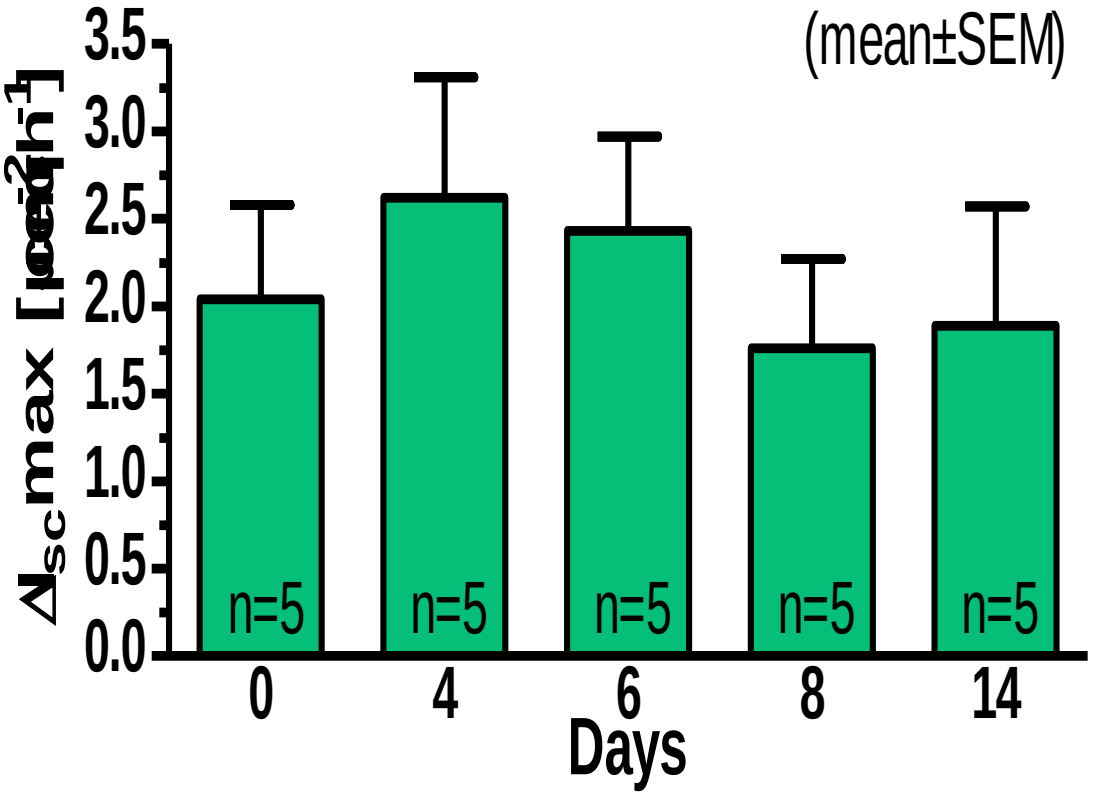


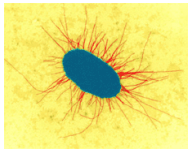
E. faecium

Kontrolle

(Lodemann et al. 2006)

No effect of EcN on sodium-coupled glucose absorption in jejunum

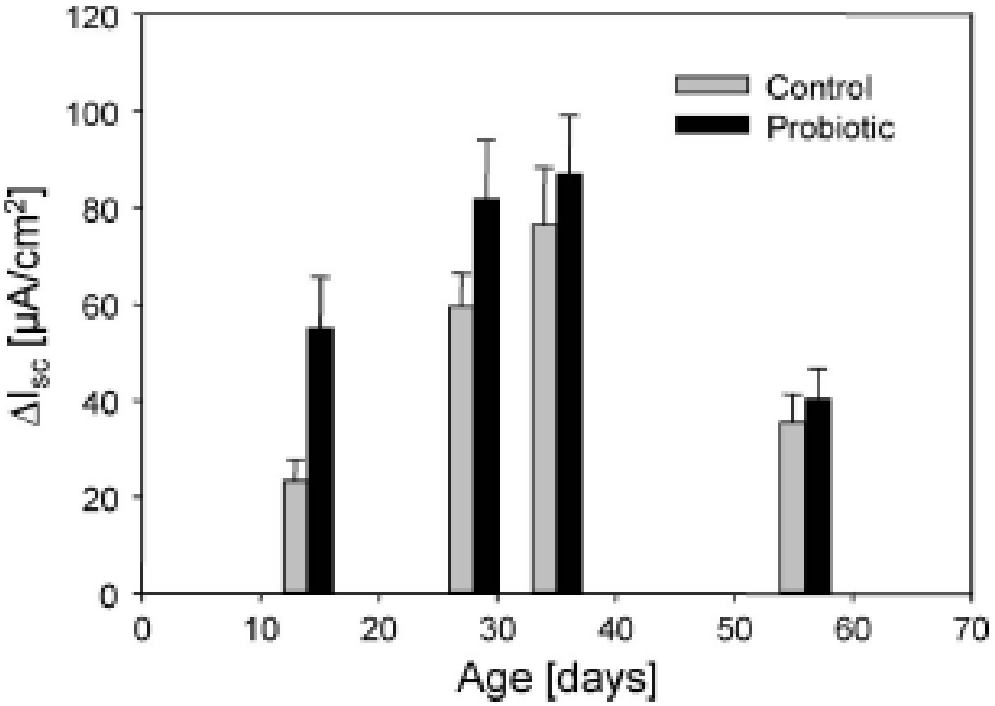


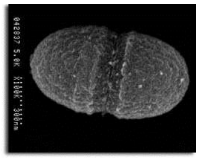


(Breves et al. unpublished)

Effekte von *E. faecium* auf den jejunalen Glutamin-Transport beim Schwein

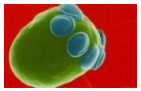
(means ± SEM, N=5)





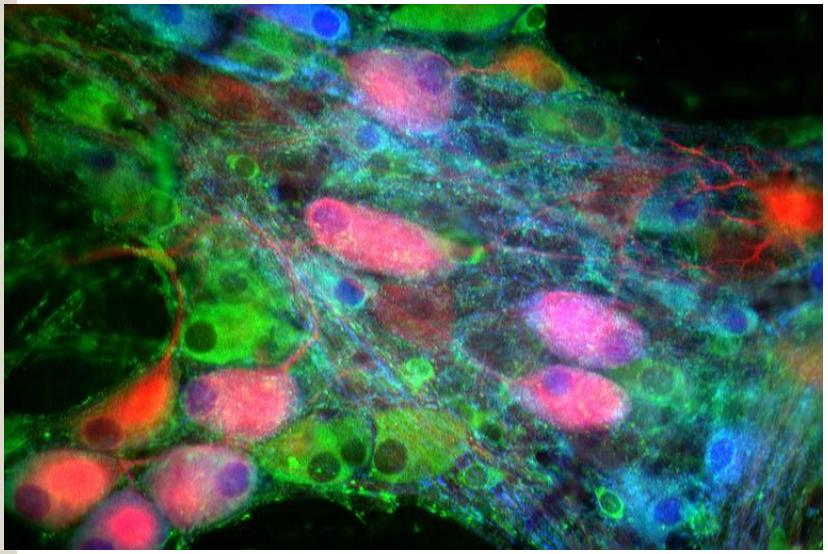
(Lodemann et al. 2006)

Probiotic effect on ENS neurochemical code: *S. boulardii* reduces Calbindin positive neurons



Myenteric plexus
control

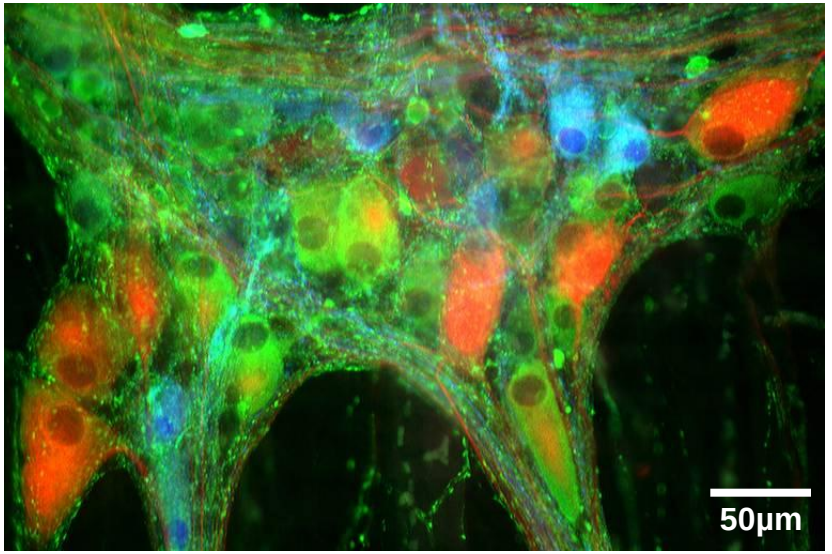
Myenteric plexus
S. boulardii



ChAT

160 kD

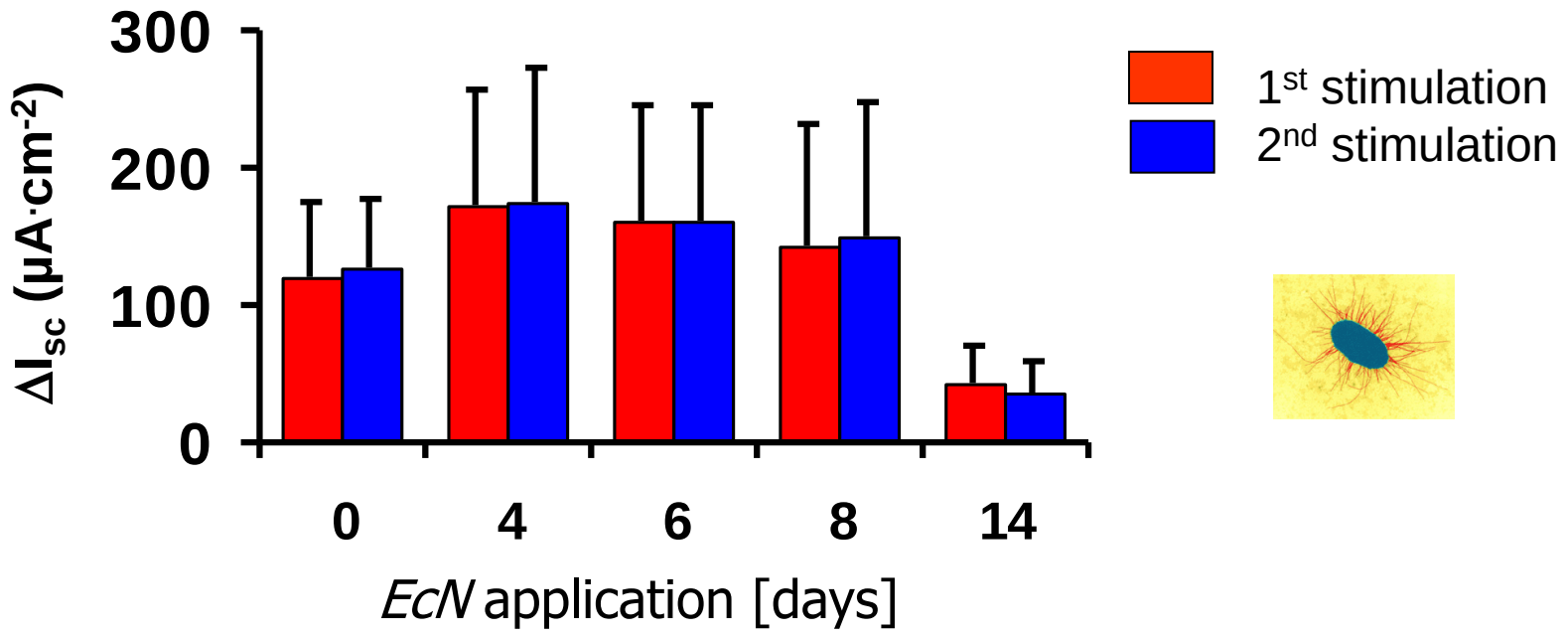
Calb



(Kamm et al. 2004)

ENS mediated secretory response in distal jejunum is reduced after 14 d *EcN*

Electric field stimulation: 16 V, 1 ms, 10 Hz, 10 s;
mucosa/plexus submucosus preparation



(Breves et al. unpublished)

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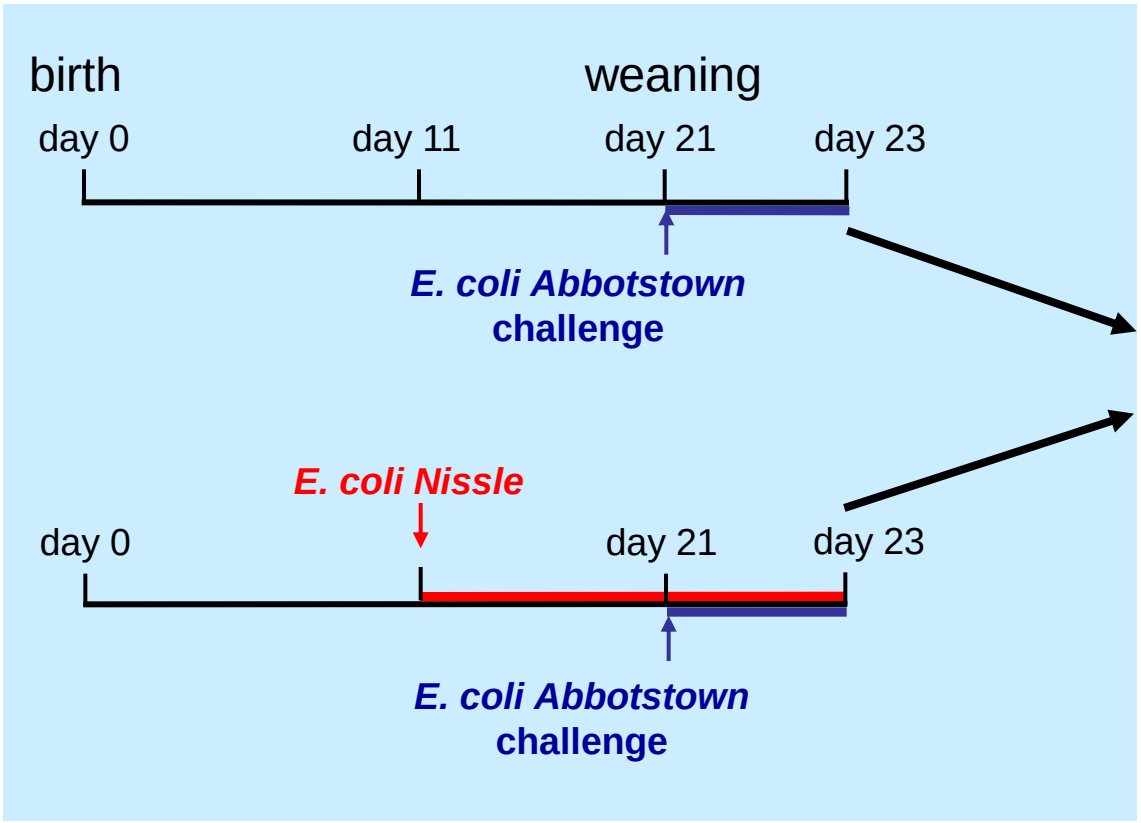
  - Infektionsmodell
- Zusammenfassung und Ausblick

Einsatz von Probiotika im Durchfallmodell

Pig infection model

Experimental design

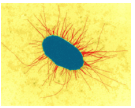


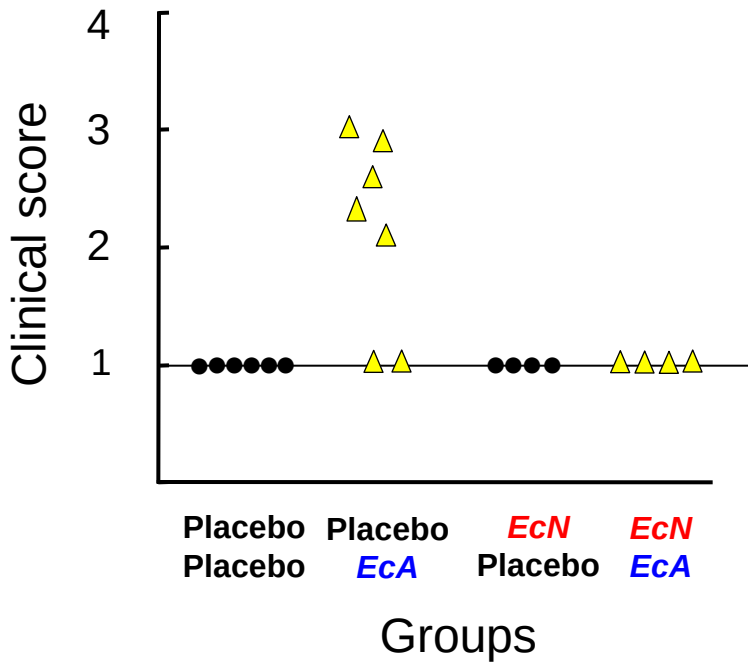
Charakterization

- Clinical score
- Histology
- Secretory capacity

Clinical score

Faeces consistency

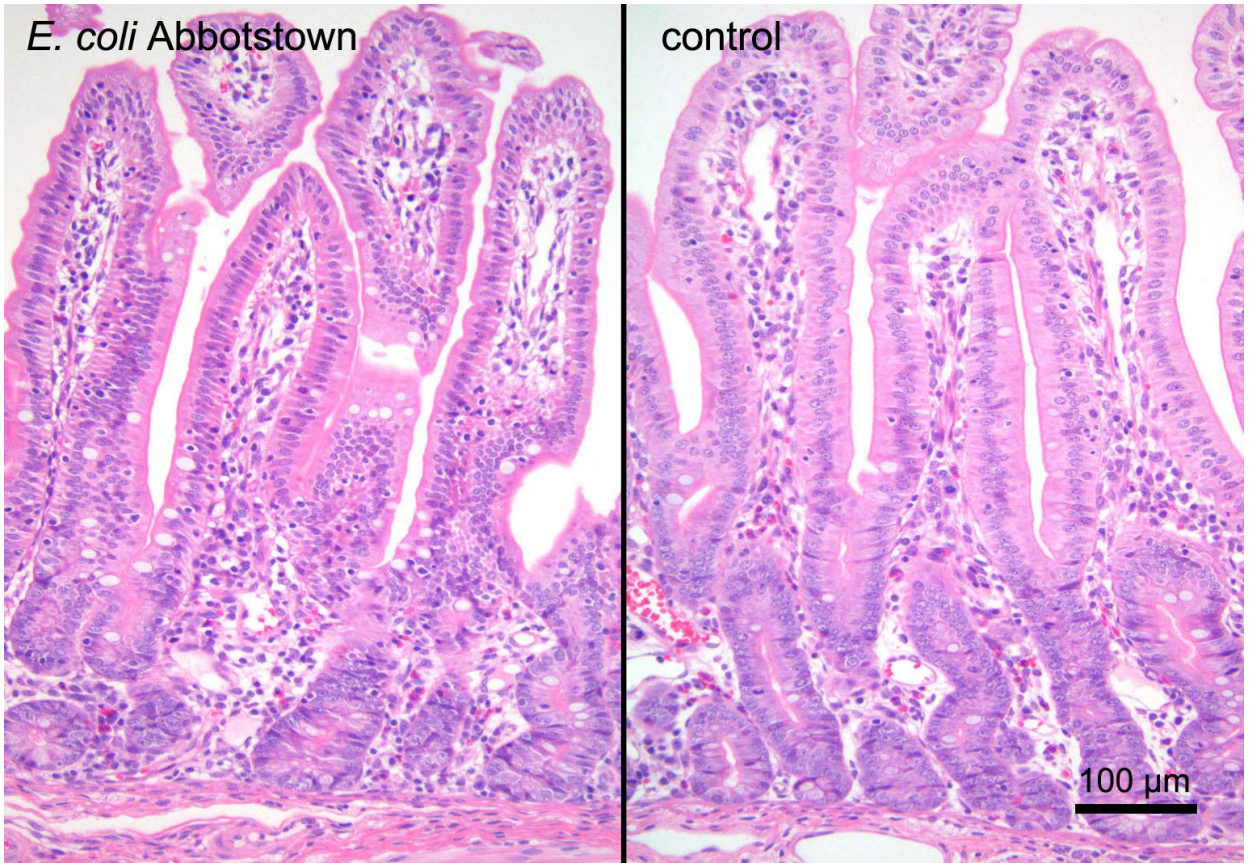




(Schröder et al. 2006)

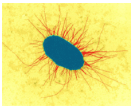
Histology

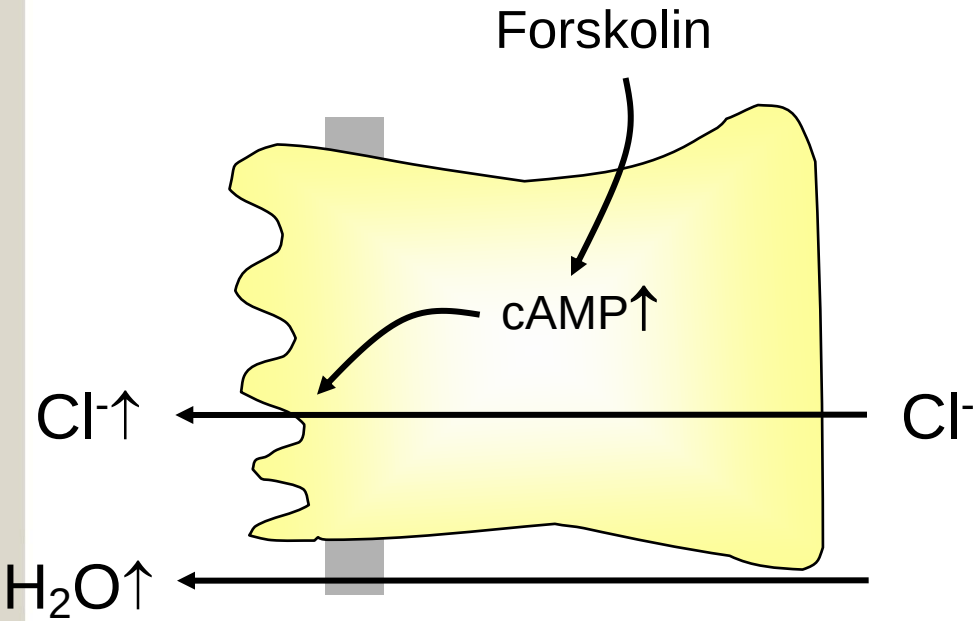
No obvious effects on gut morphology



(Schröder et al. 2006)

Secretory capacity





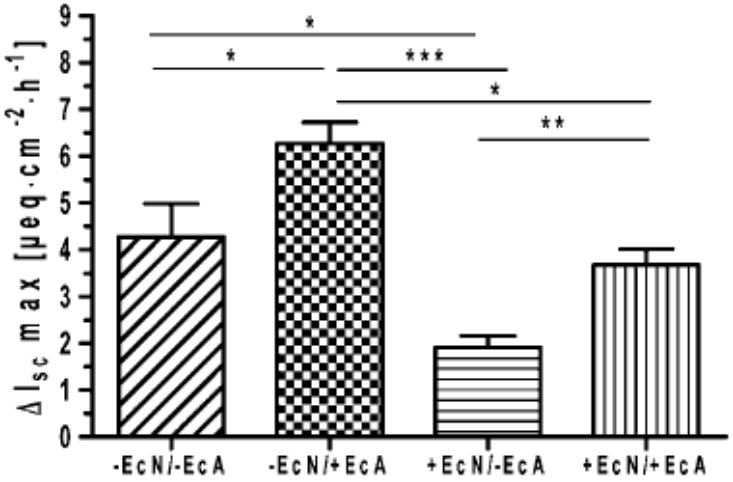


Fig 2. Maximum responses of short-circuit currents (ΔI_{sc}max; μeq·cm^{-2}·hr^{-1}) in pig midjejunum as induced by forskolin and as affected by control conditions (–EcN/–EcA) and in the absence and presence of EcN and/or EcA (for number of animals see Table 1). Means ± SE.

(Schröder et al. 2006)

Zusammenfassung und Ausblick

- Die Wirksamkeit von Probiotika als Ersatz für antibiotische Leistungsförderer ist als nicht eindeutig zu beurteilen.
- Probiotika können Struktur und Funktion der intestinalen Barriere beeinflussen .
- Hinweise auf die daran beteiligten Second-messenger-Systeme liegen vor.
- Im Infektionsmodell können Probiotika an der Prävention klinischer Symptome beteiligt sein.
- Forschungsbedarf besteht weiterhin in der Klärung zellulärer Mechanismen von Probiotika (s. Hülsenberger Gespräche 2004).