



UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI MEDICINA VETERINARIA
E SCIENZE ANIMALI

Bacillus coagulans supplementation in female Holstein Friesian calves' diet

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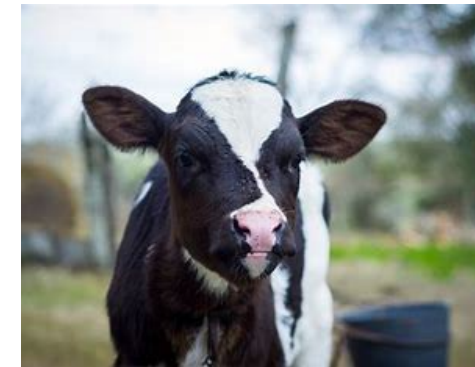
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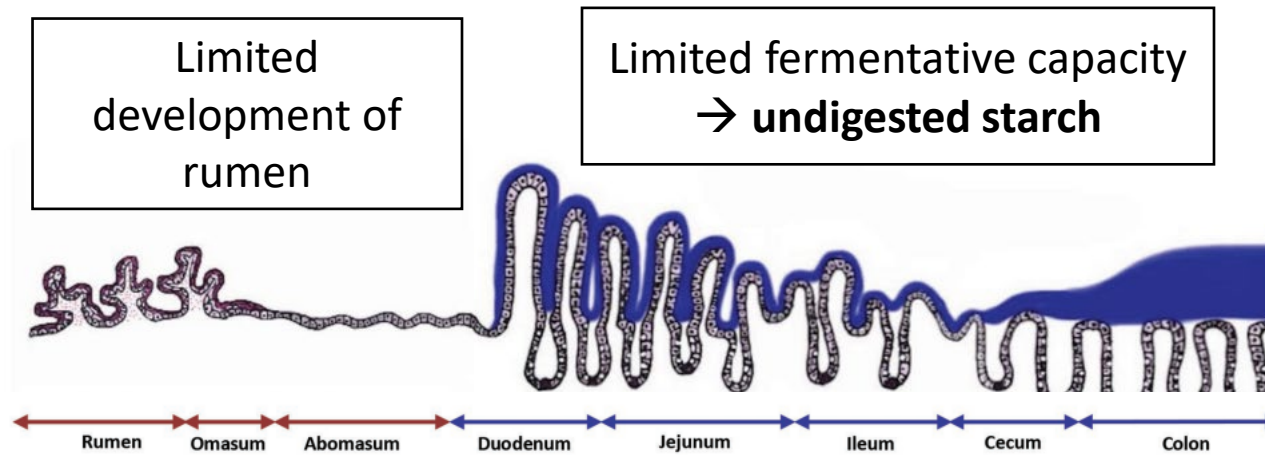
³ DVM freelance

⁴ Istituto di Biologia e Tecnologia Agraria, Consiglio Nazionale della Ricerca (IBBA-CNR), Via Einstein, 26900, Lodi, Italy

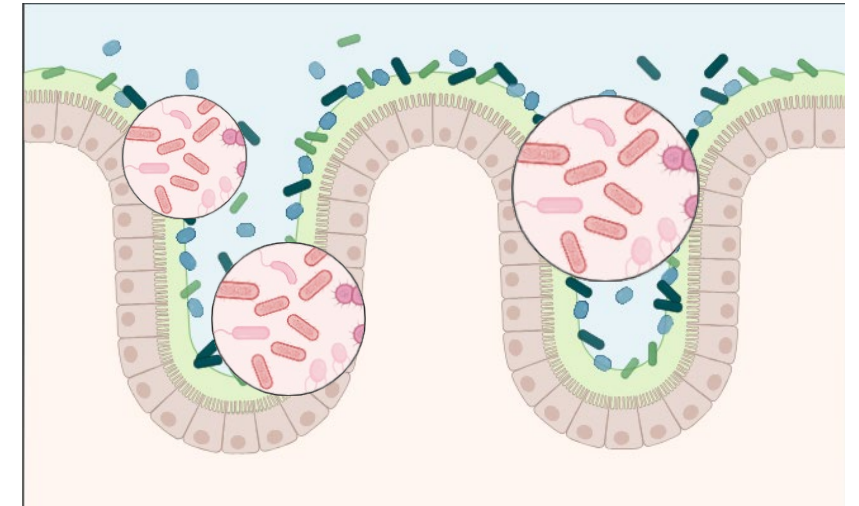
⁵ Istituto di Biotecnologie Mediche, Consiglio Nazionale della Ricerca, (ITB-CNR), Segrate, Milan, Italy.



Background



Local and systemic inflammatory response, increased intestinal permeability



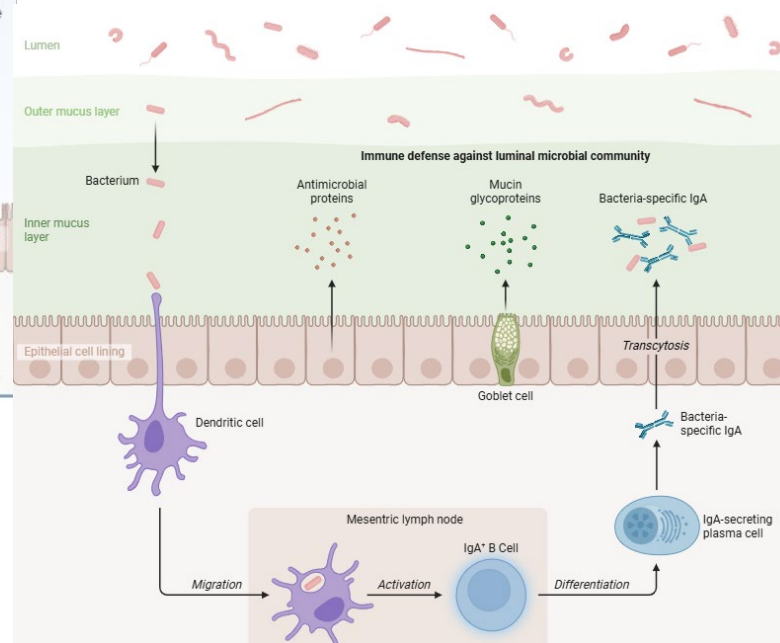
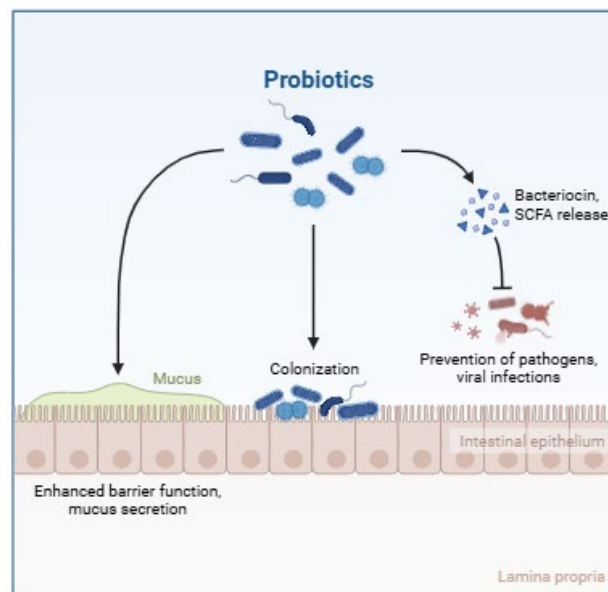
Pre-weaning calves are susceptible to diarrheal disorders

Background

Necessity to minimize antibiotic usage and AMR

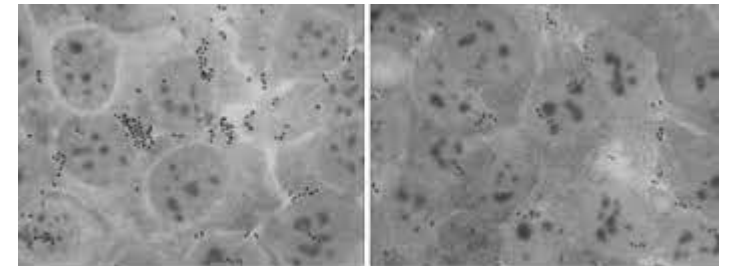
Probiotics (LAB)

Continuous research: *Bacillus coagulans* strains?



Bacillus coagulans

- *Bacillus coagulans* is a **spore forming, Gram positive, facultative anaerobic**, motile rod able to grow over a **wide range of temperatures** (30–57°C) and pH values (4–10.5).
- **Functional** resistance to gastric pH, digestive enzymes and intestinal environmental conditions
- **Technological aspects** > resistance to technological stresses during production and storage processes



Adhesion test
performed at pH 5

Adhesion test
performed at pH 7



To evaluate the **effects** of an oral administration of ***Bacillus coagulans DSM 32016 (Technospore®)*** on Italian Holstein Friesian female calves health during pre-weaning phase



Experimental design

Azienda Agricola Ceradello- Crotta Adda(CR)



Trial started from the 7th day of life and lasted 56 days, individual housing (2.2 x 1.5 m)

Treatment	Diets (0-56 d)
Control (CTR)	• MRP • Starter feed
Treatment(T)	• MRP + 10⁹ CFU/kg • Starter + 10⁹ CFU/kg
Number of treatments: 2 Replicates per treatment: 10 Total number of replicates: 20	



Colostrum samples were collected at 6-12h after birth for IgG content (ELISA test)



96% of the samples in this study contained >50 g/l IgG, which is considered to be indicative of high-quality colostrum



Basal diet

Starter feed

% as fed	Chemical analyses
DM, %	89.00 ± 1.20
CP, %	16.74 ± 1.75
EE, %	4.00 ± 0.90
CF, %	9.81 ± 1.37
Ash, %	7.57 ± 1.49

n= 5 samples



Milk replacer

% as fed	Chemical analyses
DM, %	96.50 ± 1.70
CP, %	22.50 ± 2.10
EE, %	18.00 ± 1.87
Ash, %	7.7 ± 1.05

n=5 samples

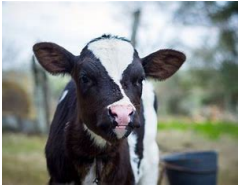
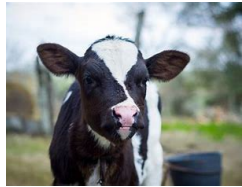


Sampling and methodologies

Day 0

Day 28

Day 56



Growth performance, biometric measurements and daily fecal score



Fecal microbiota (α and β diversity, taxa composition) and functional metabolic prediction



**Plasma BHB, Glucose, NEFA and IgG
Serum T-AOC**



Results: growth performance

Parameters	CTR	T	SEM	Time	Treatment	Time*Treatment
BW (kg)						
0 d	41.11	41.30	1.28	<.0001	<0.01	0.021
28 d	49.75	52.85				
56 d	67.90 ^b	75.55 ^a				

ADG (kg/d)						
0-28 d	0.308	0.412	0.051	<.0001	<0.01	0.86
28-56 d	0.648	0.811				
0-56 d	0.478	0.612				

Parameters	CTR	T	SEM	Time	Treatment	Time*Treatment
ADDMI (kg/d)						
0-28 d	1.105	1.094	0.088	<.0001	0.240	0.53
28-56 d	1.724	1.914				
0-56 d	1.415	1.504				
FCR						
0-28 d	4.23	3.06	0.33	0.03	0.04	0.55
28-56 d	2.93	2.53				
0-56 d	3.02	2.51				

a, b p<0.05. BW: body weight; ADG: average daily gain; ADDMI: average daily dry matter intake; FCR: feed conversion ratio.



Results: Biometric measurements

Parameters (cm)	CTR	T	SEM	Time	Treatment	Time*Treatment
Body length						
0 d	65.50	67.70	1.16	<.0001	0.16	0.79
28 d	72.20	73.60				
56 d	79.90	80.50				
Heart girth						
0 d	78.90	78.70	0.98	<.0001	0.24	0.48
28 d	86.10	87.00				
56 d	97.80	100.00				
Withers height						
0 d	77.20	77.50	0.93	<.0001	0.06	0.44
28 d	82.10	83.60				
56 d	87.20	89.90				
Hip width						
0 d	18.10	17.80	0.38	<.0001	0.35	0.13
28 d	19.60	19.60				
56 d	20.90	22.10				

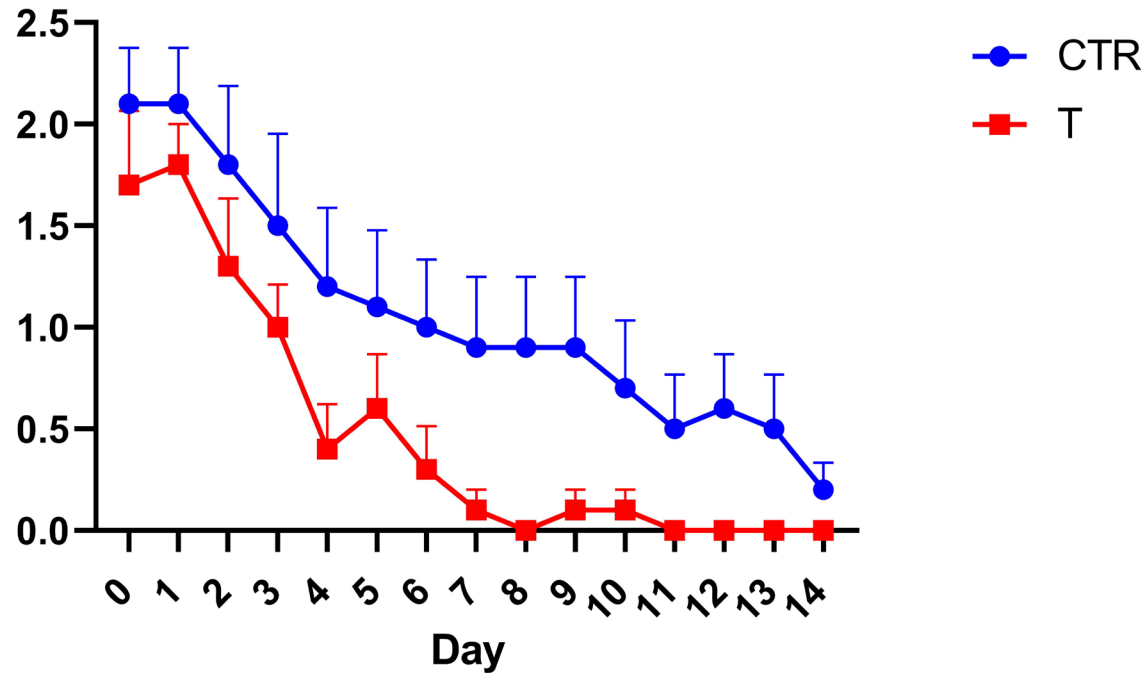
Positive effects on performances but
no effects on biometric measurements



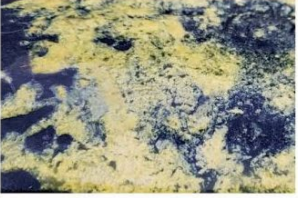
Enhanced enzymatic activity, better
nutrient absorption and digestion,
increased immunity...



Results: fecal score



Better fecal score in T group during the first 14 d of trial
Treatment effect $p < 0.01$

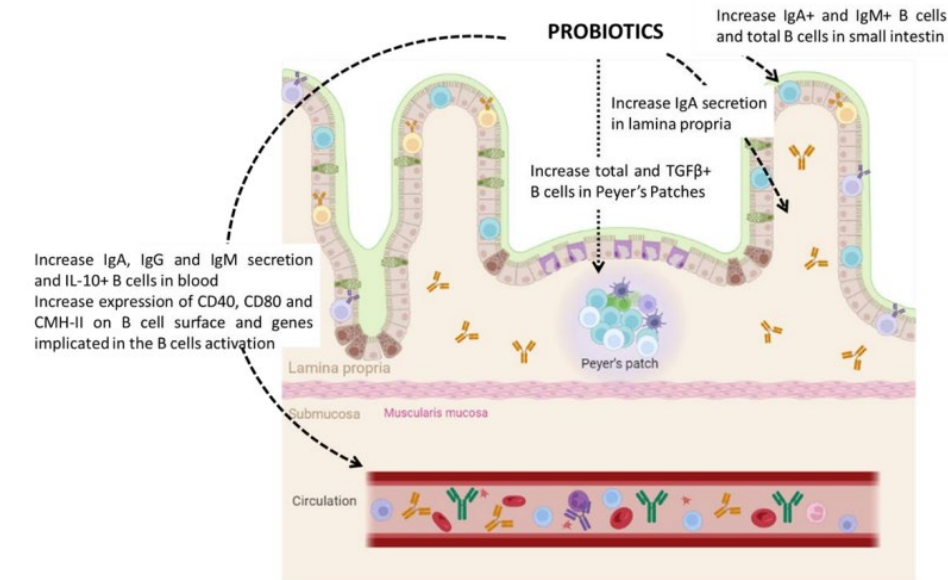
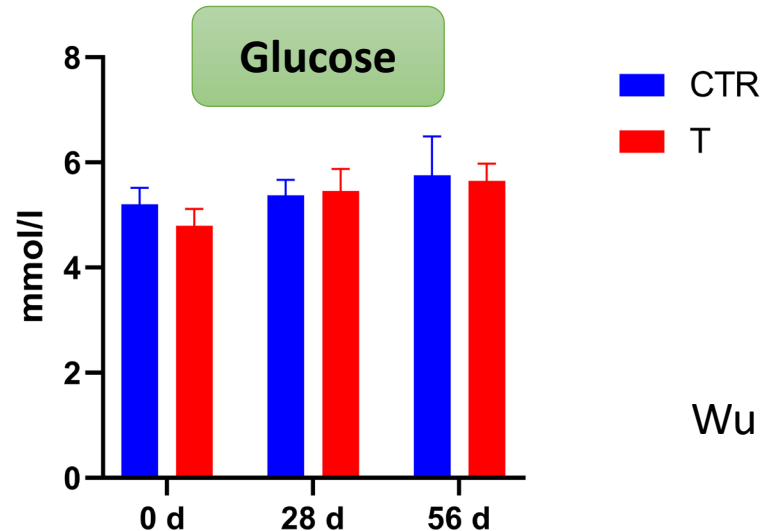
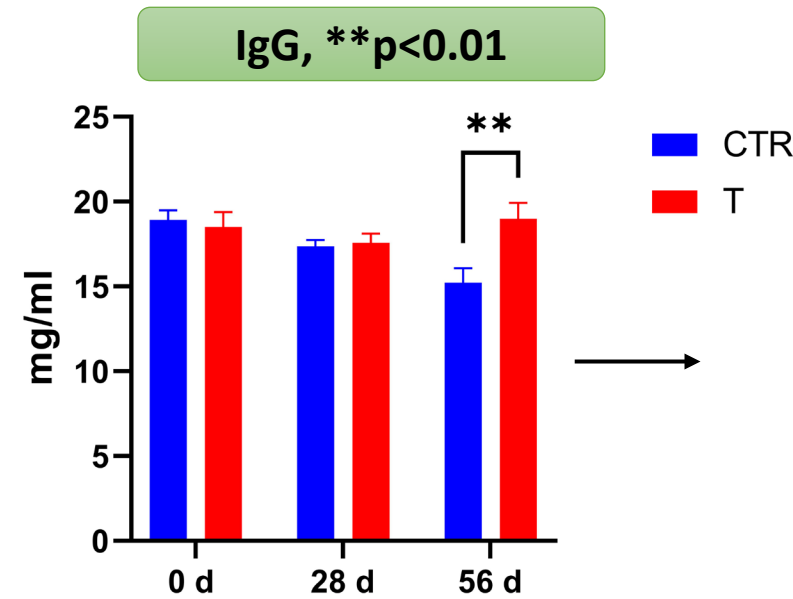
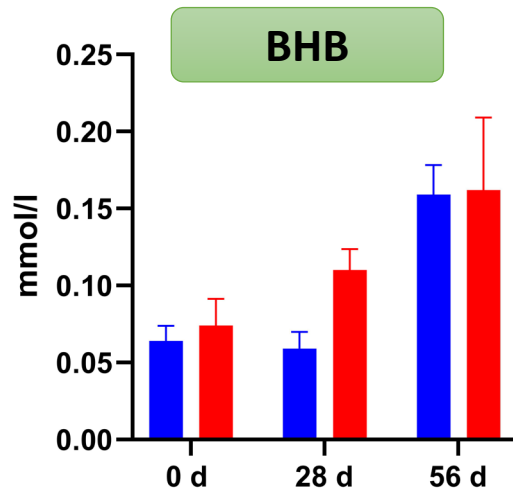
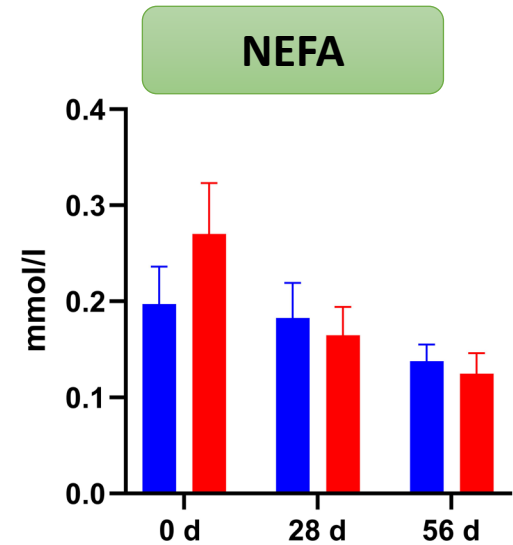
0		NORMAL - firm but not hard - original form is distorted slightly after dropping to the floor and settling
1		SOFT - does not hold form - piles but spreads slightly
2		RUNNY spreads readily
3		WATERY - liquid consistency - splatters

Lan et al, 2016. Journal of Animal Physiology and Animal Nutrition, 100(6), 1130-1138.

Renaud et al, 2020. J Dairy Sci. 2020 Nov;103(11):10709-10714.



Results: plasma metabolites and IgG



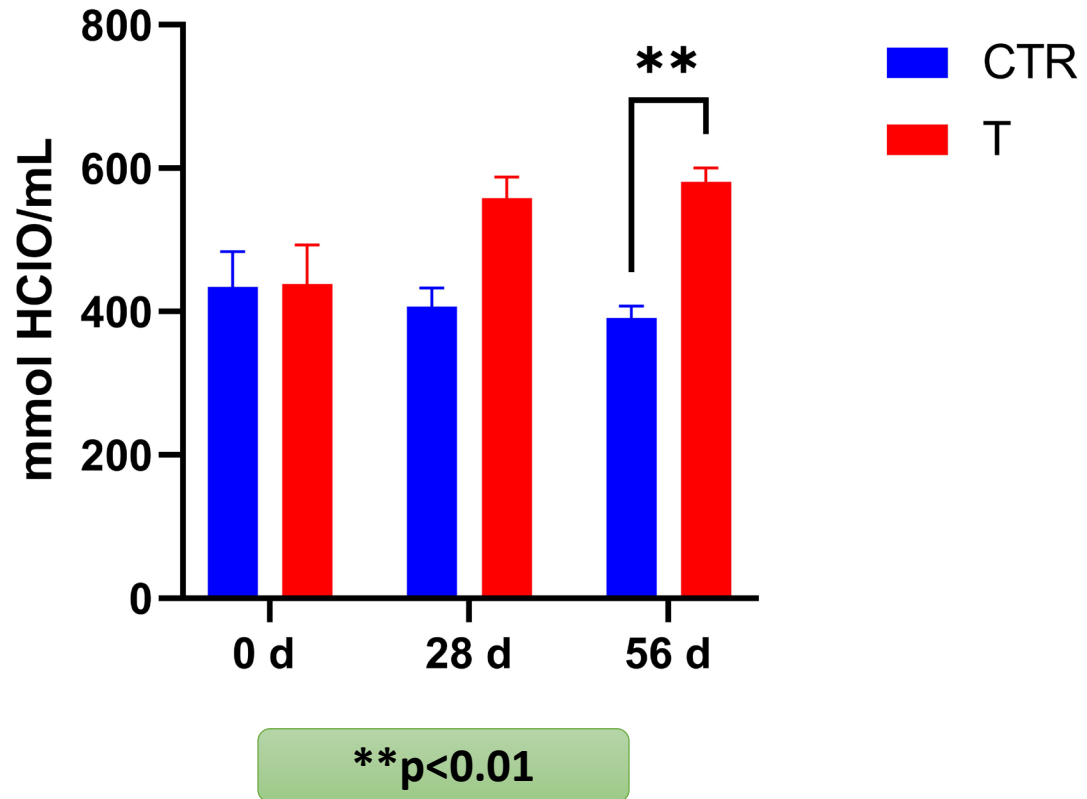
Rousseaux et al, 2023 Nutrients, 15(2), 269.

Wu et al, 2021. Front Microbiol. 2021 Jul 14;12:681014.

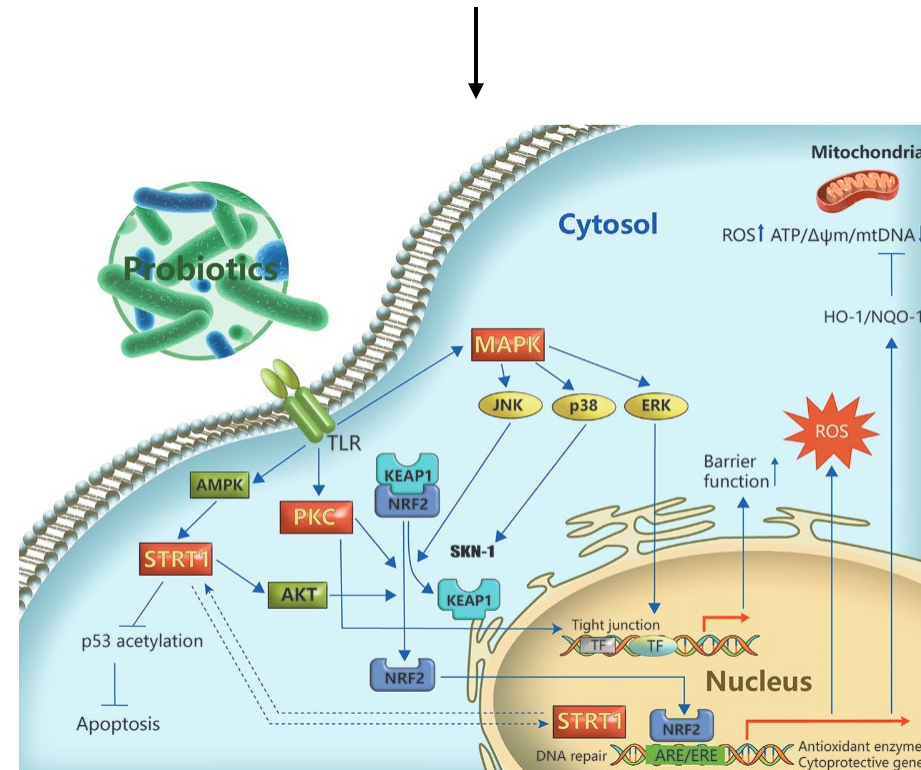
Luise et al, 2022. Front Microbiol.13:801827.



Results: serum T-AOC



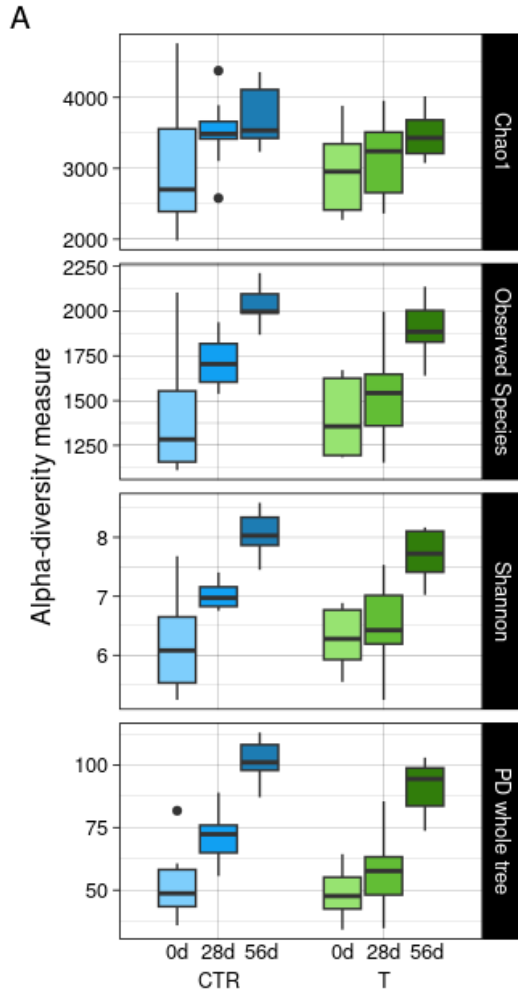
Gradually increase of serum T-AOC



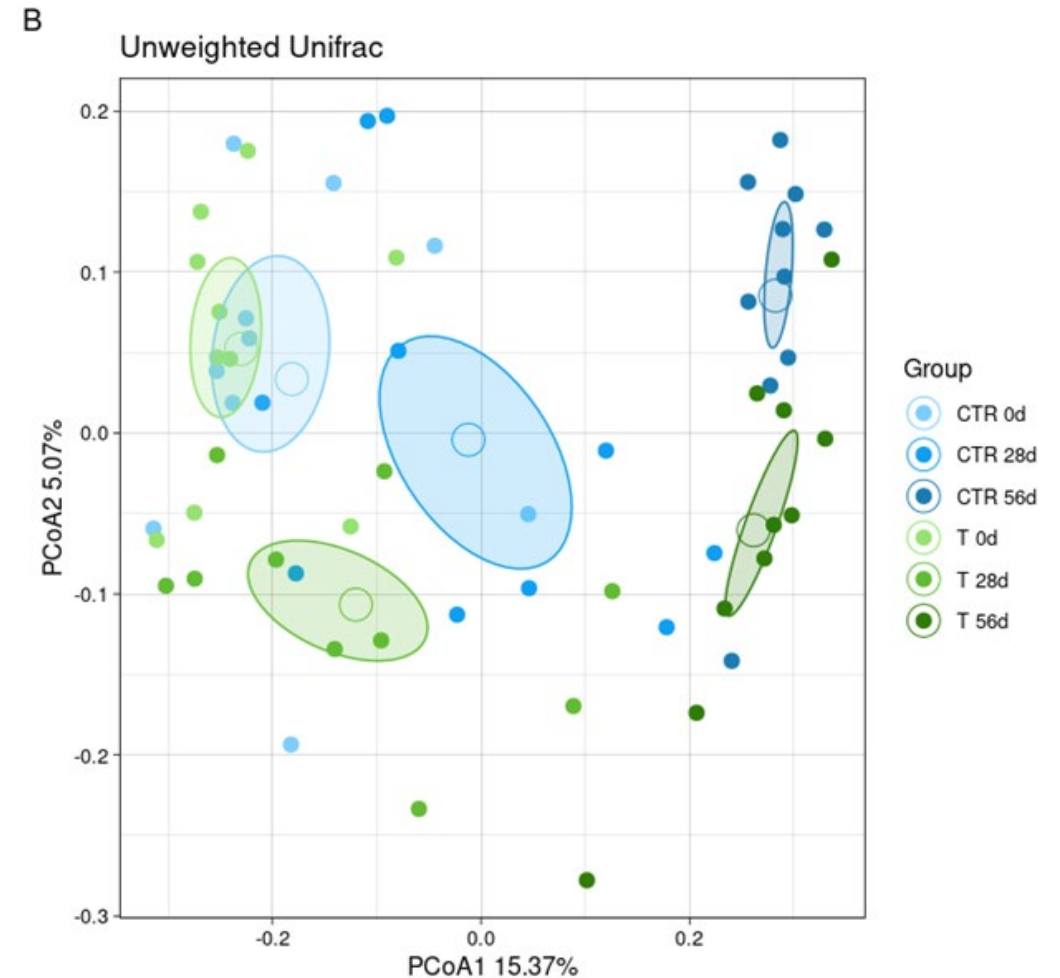
Feng & Wang. 2020. Gut microbes, 12(1), 1801944.



Results: fecal microbiota composition



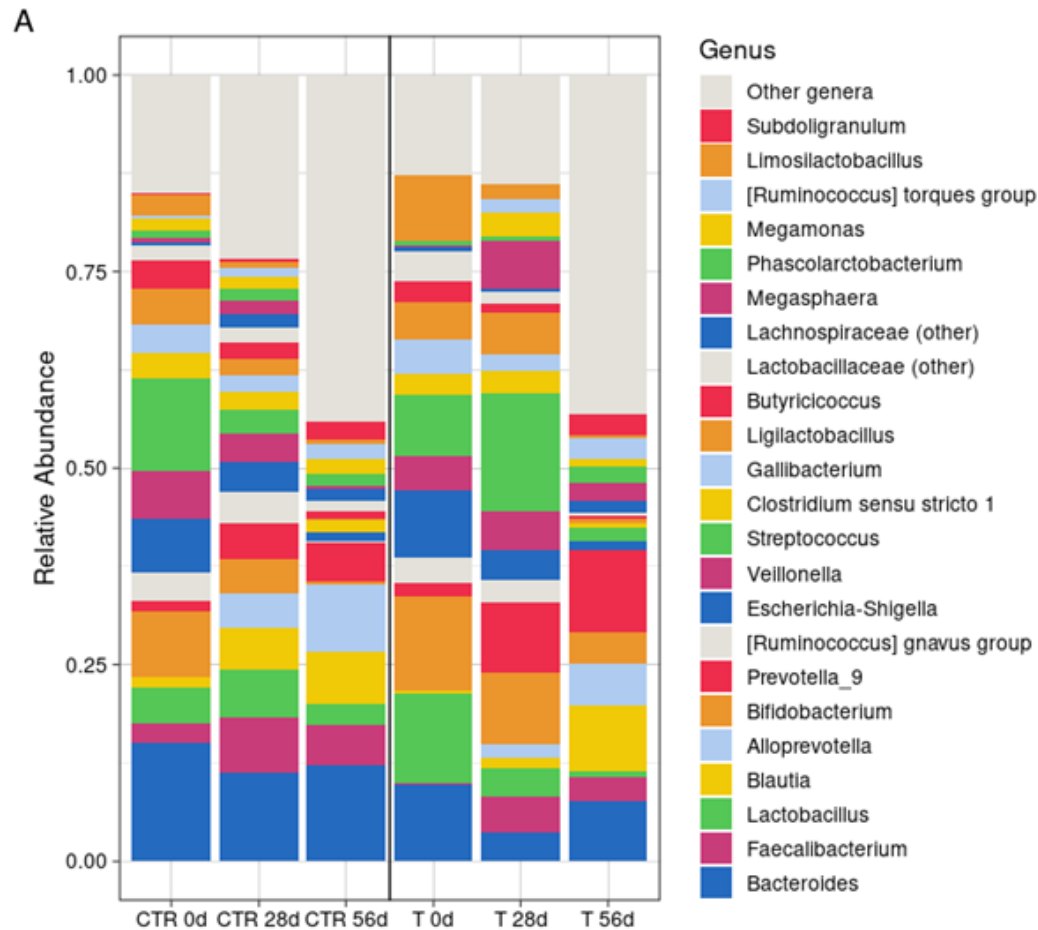
Gradually increase in diversity and richness indexes in both groups.



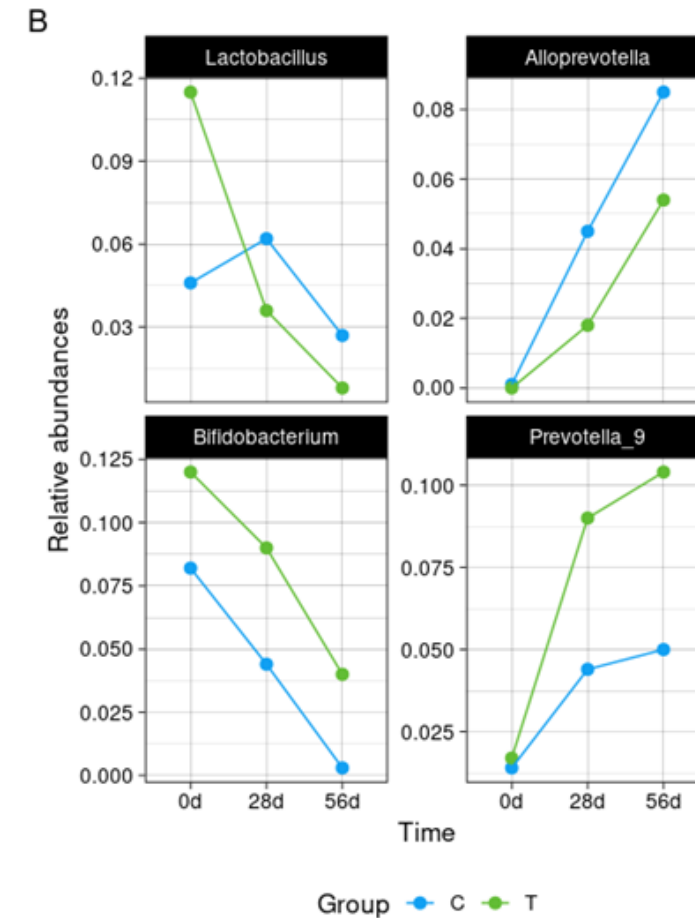
Marked differences when analysing weighted and Unweighted distances at 28 and 56 d ($p < 0.05$)



Results: fecal microbiota composition



Lactobacillus spp. tended to decrease whereas *Bifidobacteria* were maintained higher in T

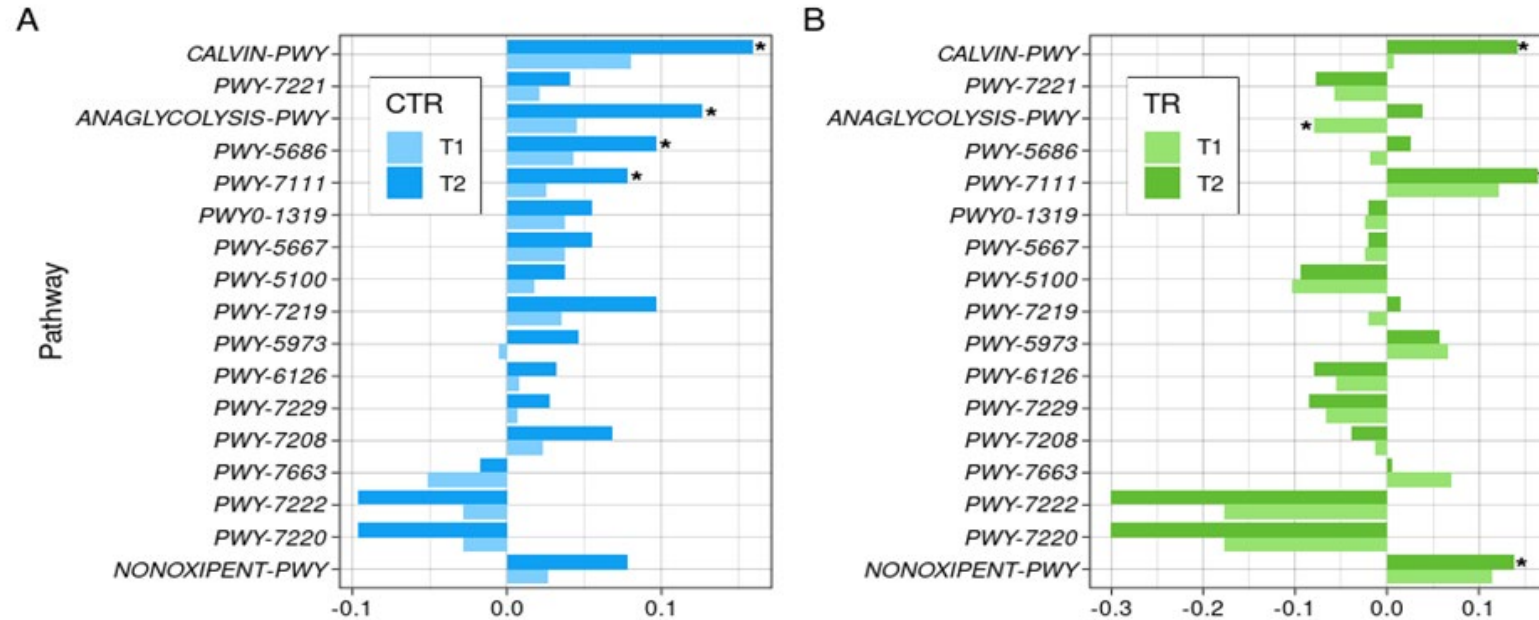


Blautia and Prevotellaceae (*Prevotella* + *Alloprevotella*) increased in T group



Results: functional metabolic prediction

* $p < 0.05$



NONOXIPENT-PWY pathway increased in treated group at 56 d



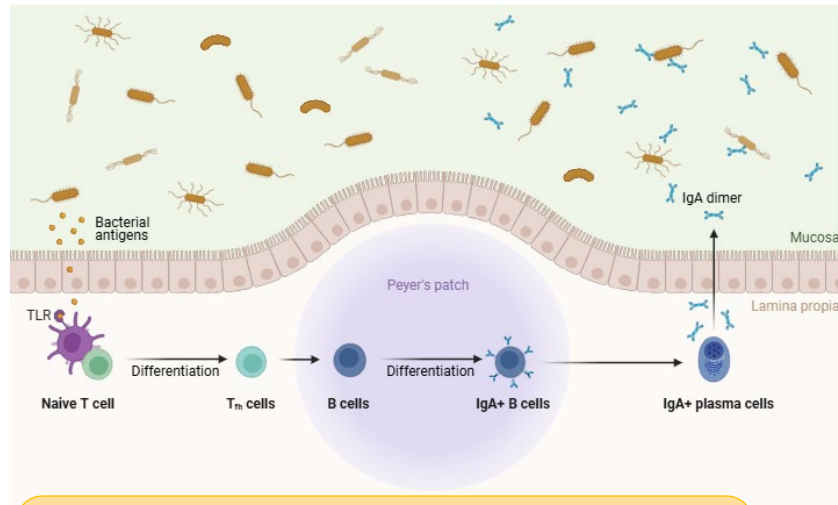
Related to glycolysis and regulatory processes of minor pathways (nucleotide biosynthesis)



Conclusions

Bacillus coagulans DSM32016

plasma IgG and serum T-AOC



Microbiota modulation and functional pathways conditioning

Performance and fecal score

***Bacillus coagulans* DSM 32016** represent a suitable solution in supporting gut health of calves



Thanks for the attention!



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