Effects of *Clostridium butyricum* supplementation on gut microbiota, intestinal and ruminal integrity, and growth performance in dairy calves

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INTRODUCTION

Clostridium butyricum is a butyrate-producing probiotic that has gained interest as an alternative to antibiotics in dairy calves. It promotes intestinal microbial stability, enhances gut barrier integrity, and modulates local immune responses, thereby supporting gastrointestinal health.

OBJECTIVE

This study evaluated the effects of *C. butyricum* supplementation on intestinal microbiota, gastrointestinal morphology, and health outcomes in Holstein calves during the pre-weaning period.

METHODOLOGY

STUDY DESIGN

A total of 150 Holstein calves were randomly assigned at birth to two groups.

n = 150 at birth

CONTROL (C)	TREATMENT (Cb)
75 calves (72 ♀ / 3 ♂)	75 calves (72 ♀ / 3 ♂)
Pasteurised milk + starter concentrate	Pasteurised milk + starter concentrate + 4 g *C. butyricum* in milk

Randomized allocation within blocks of 24 female and 1 male calf, across 6 repetitions.

MONITORING THROUGHOUT THE STUDY

- **Passive immunity** (serum IgG at 24 h, Brix)
- **Growth parameters** Calves were measured (weight/height) on day 1, days 28 ± 7, 56 ± 7, 90 ± 7, and 120 ± 7.
- **Faecal enteropathogens** (Rotavirus, Coronavirus, *E. coli* K99, *Cryptosporidium* spp., *Salmonella* spp.)
- **Clinical health** (diarrhoea, respiratory disease, morbidity, mortality)

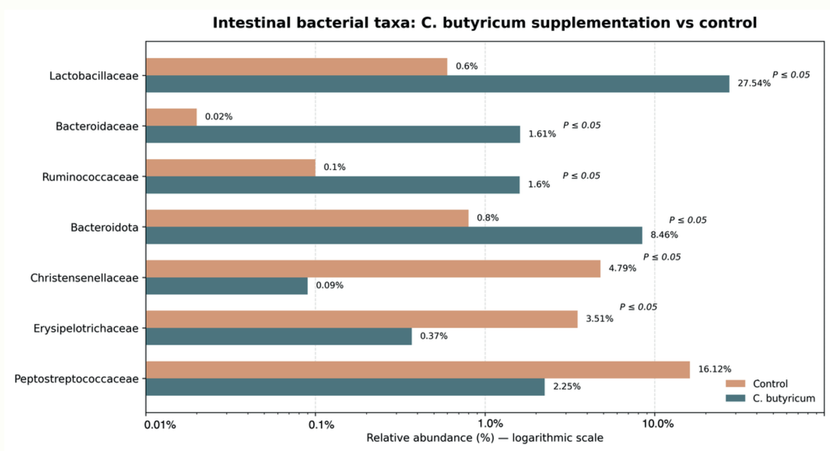
SAMPLING AT 28 DAYS

Intestinal and ruminal samples were collected from male calves (n = 3 per group).

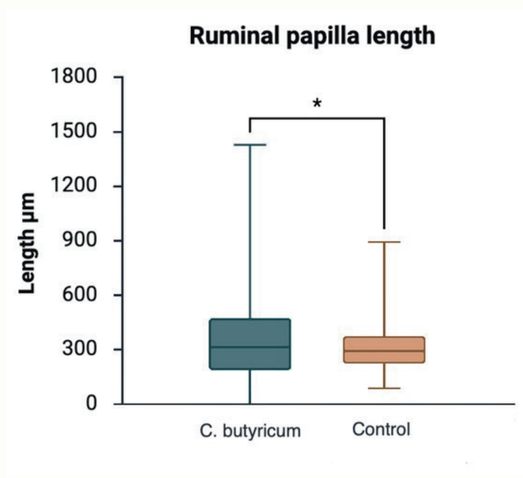
INTESTINAL SAMPLES	RUMINAL SAMPLES
Tissue and content (colon and ileum)	Tissue and content (rumen)
Microbiome analysis Histopathological analysis	Microbiome analysis Histopathological analysis

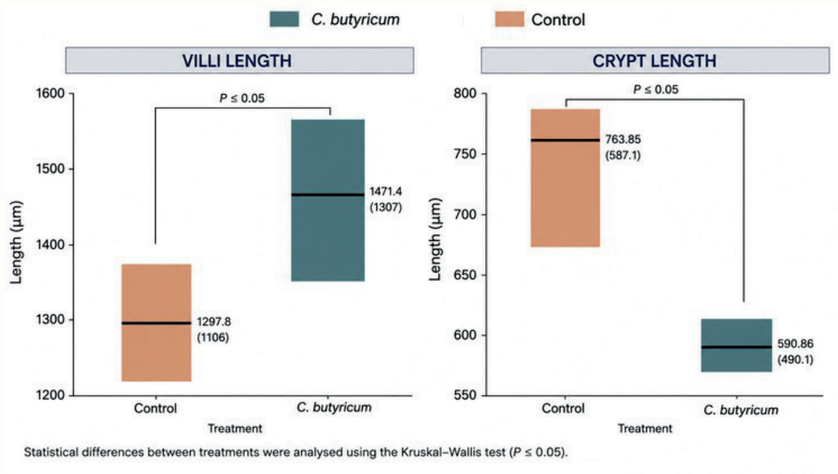
Microbiome and histopathological analyses

RESULTS

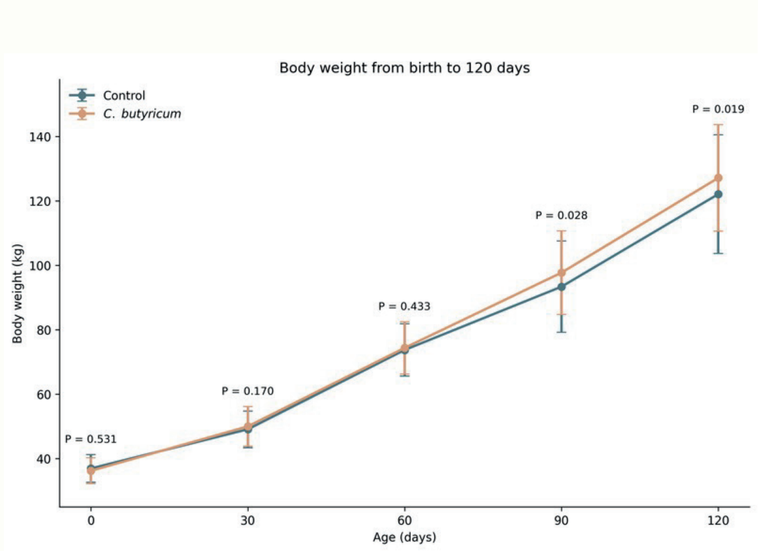


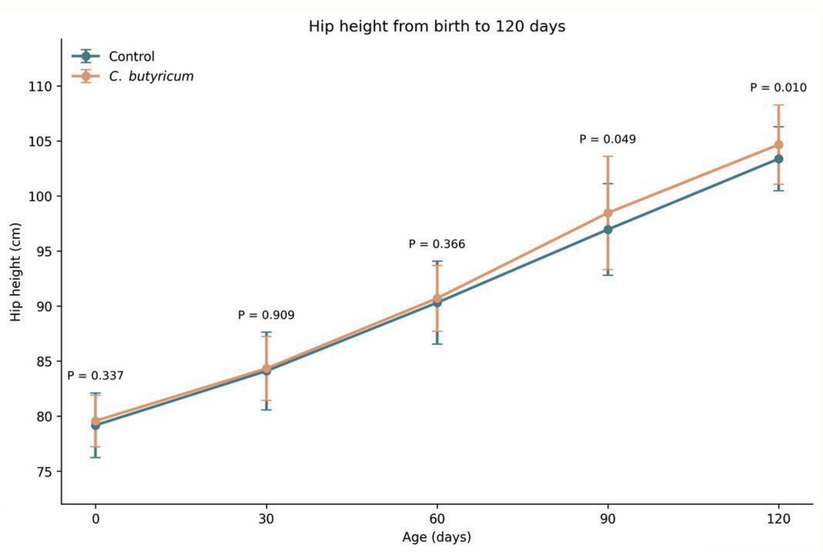
Taxon abundances were compared using the Kruskal–Wallis test (SPSS v29.0.2.0). Statistical significance was set at P ≤ 0.05.





Statistical differences between treatments were analysed using the Kruskal–Wallis test ($P \leq 0.05$).





COST PER ANTIMICROBIAL (EUR)

Antimicrobial	*C. butyricum*	Control	Difference (EUR) (Control – *C. butyricum*)
Paromomycin sulphate	€365.12	€445.91	€80.79
Sulfas	€46.34	€52.97	€6.63
Enrofloxacin	€74.34	€85.53	€11.19
Marbofloxacin	€73.46	€195.91	€122.45
Penicillin	€24.18	€48.37	€24.19
Gentamicin	–	€10.81	€10.81
Butylscopolamine	€7.16	€16.49	€9.33
TOTAL	€590.60	€755.99	€165.39

CONCLUSION

✓ Improved gut microbial balance and gastrointestinal integrity.
✓ Reduced inflammation and neonatal disease.
✓ Increased growth performance and structural development.
✓ Supplementation with *Clostridium butyricum* reduced antimicrobial treatment costs by approximately €186 per study group, representing a 21.9% reduction compared with the Control group.
✓ *Clostridium butyricum* is a promising non-antibiotic strategy to improve dairy calf health and productivity.

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