Impact of menthol supplementation on calcium absorption in ruminants

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PerformaNat feeding science

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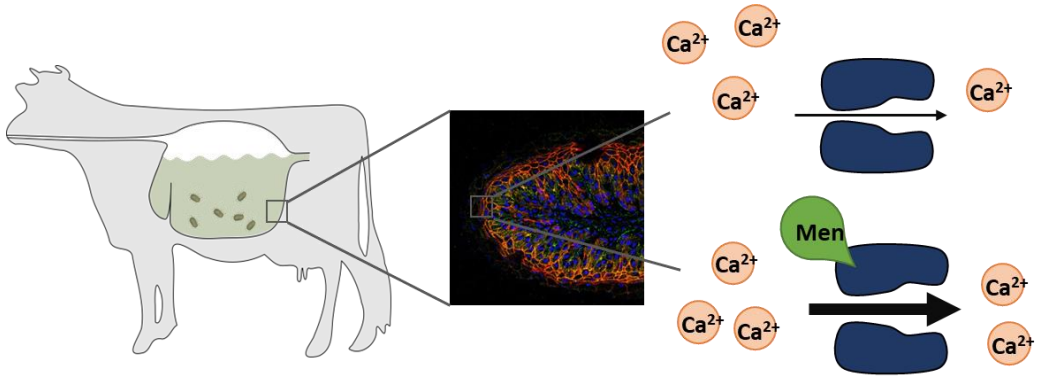
overview

1. Why does calcium metabolism matter in dairy cows?
2. Calcium absorption mechanisms in ruminants
3. Evidence for menthol-sensitive ruminal calcium uptake from *in vitro, ex vivo* and *in vivo* studies:

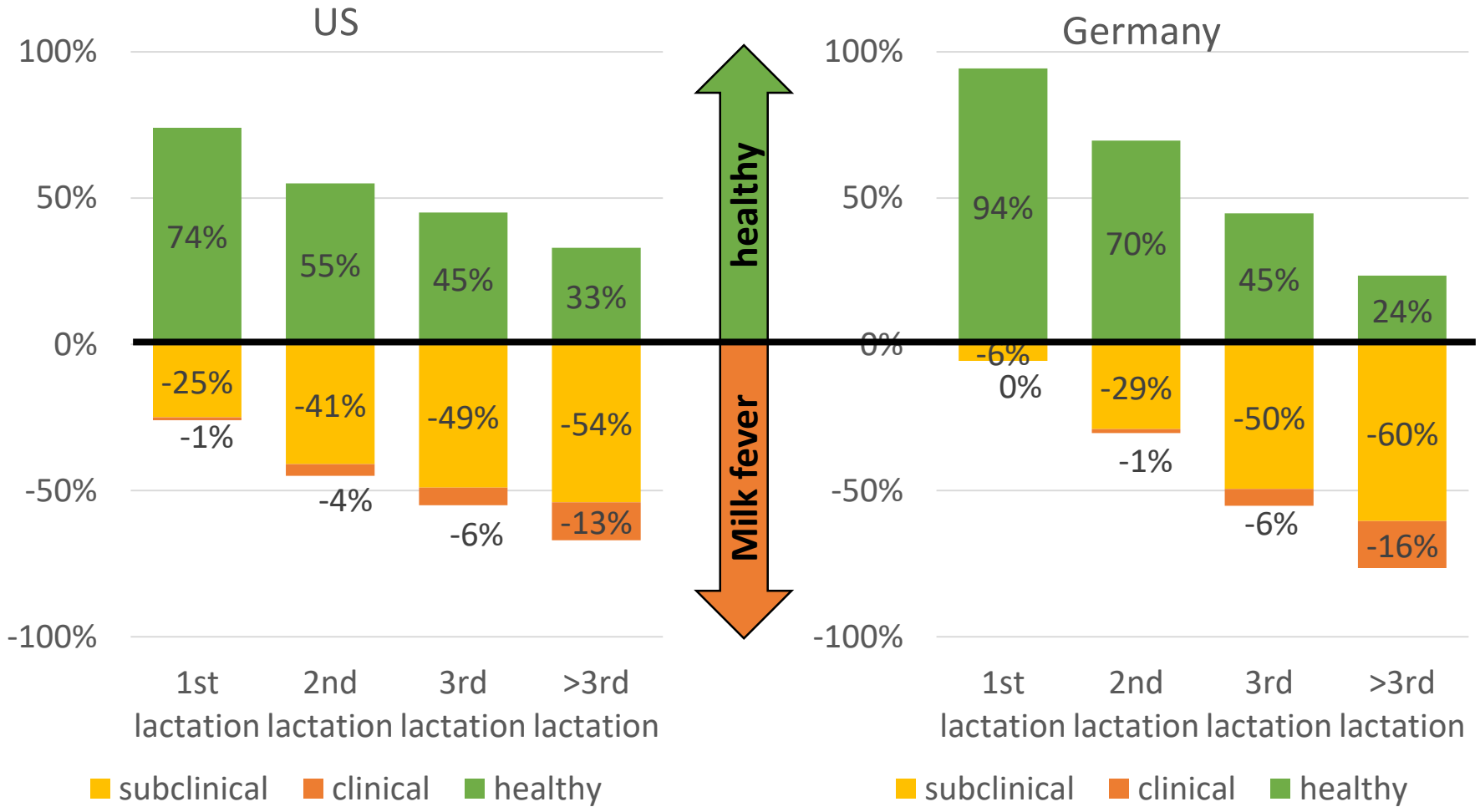
    a) *ex vivo* experiments with isolated ruminal epithelium
    b) *in vitro* experiments with TRPV3 overexpressing HEK cells
    c) *in vivo* feeding study with lactating dairy cows
    d) *in vivo/ex vivo* study with growing sheep

4. summary



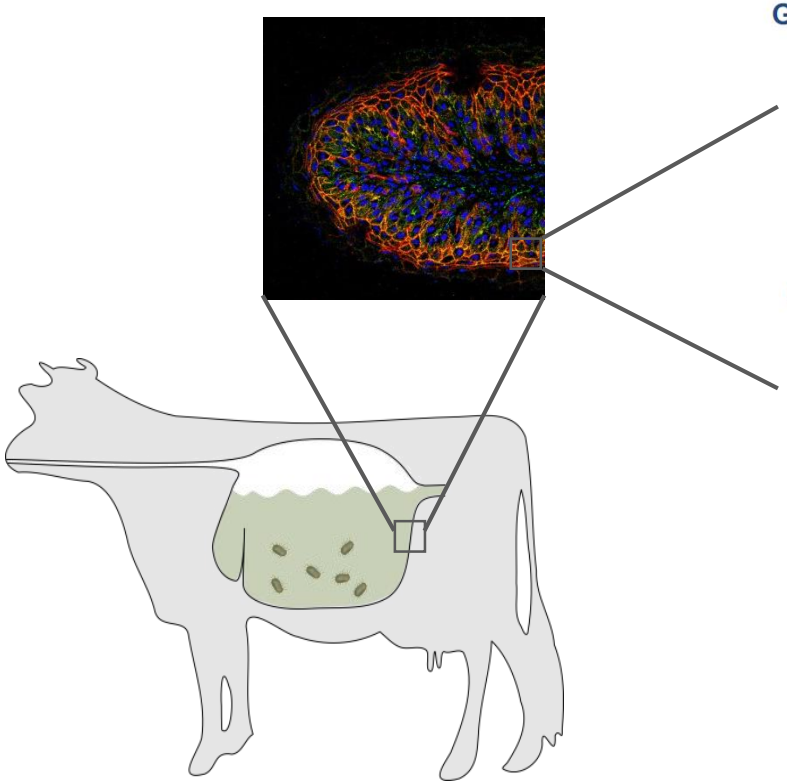
1. occurrence of milk fever

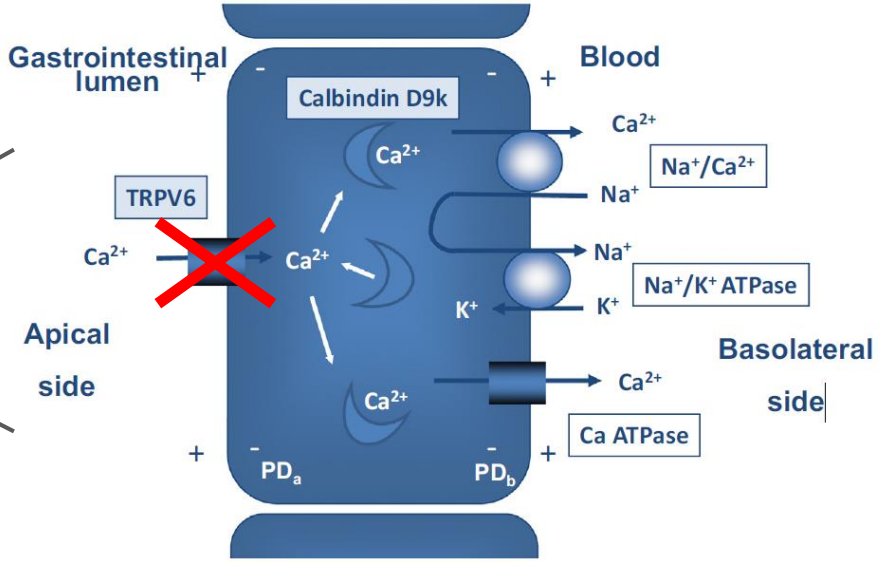
Milk fever risk increases by 9% with every lactation (DeGaris et al., 2008)

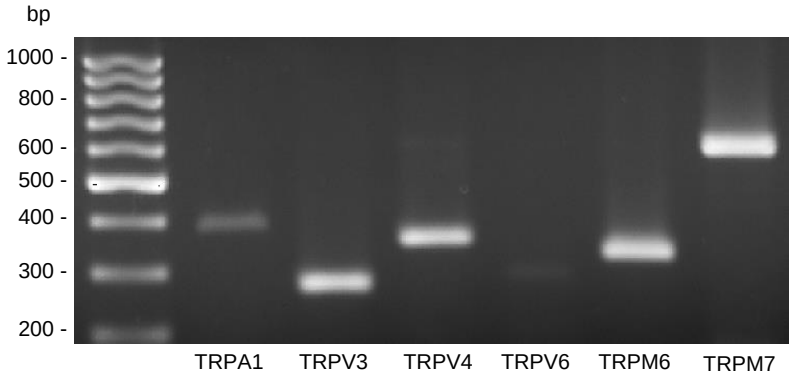


(Venjakob et al., 2017, Reinhard et al. 2011)

2. calcium absorption in dairy cows



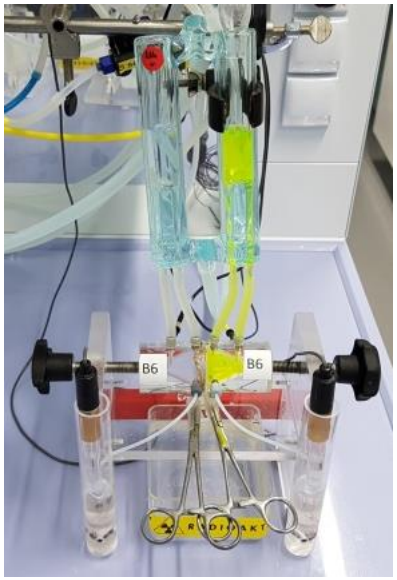


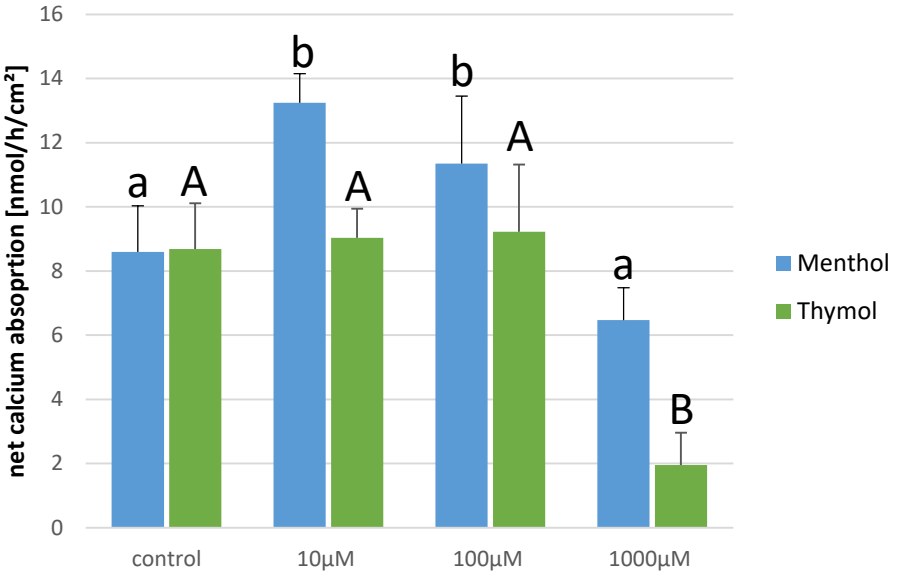


Gel electrophoresis loaded with PCR products from ruminal epithelium, Rosendahl et al. 2016

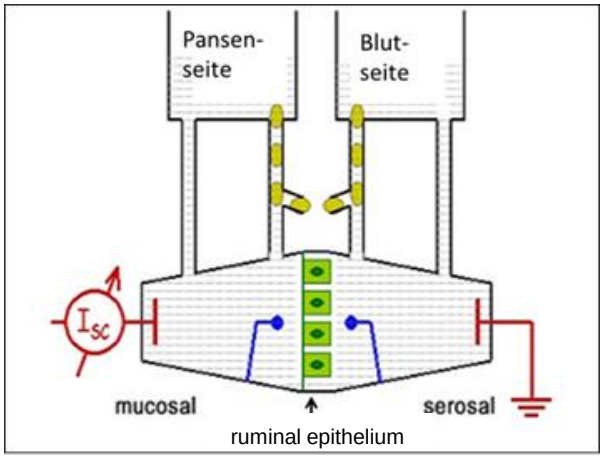
Source: Martin-Tereso & Martens 2014, Rosendahl et al. 2016

3a) *ex vivo* experiments





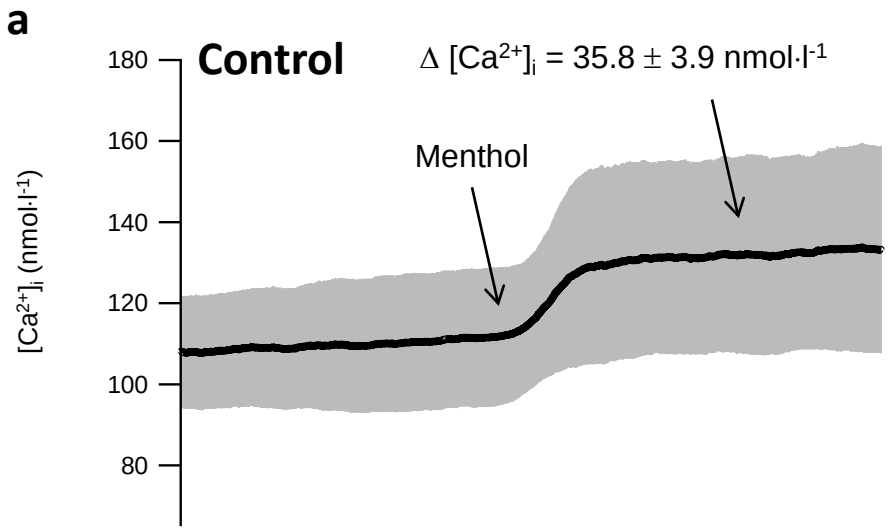
One way repeated ANOVA, SigmaPlot, $P<0.05$; N=4, n=9

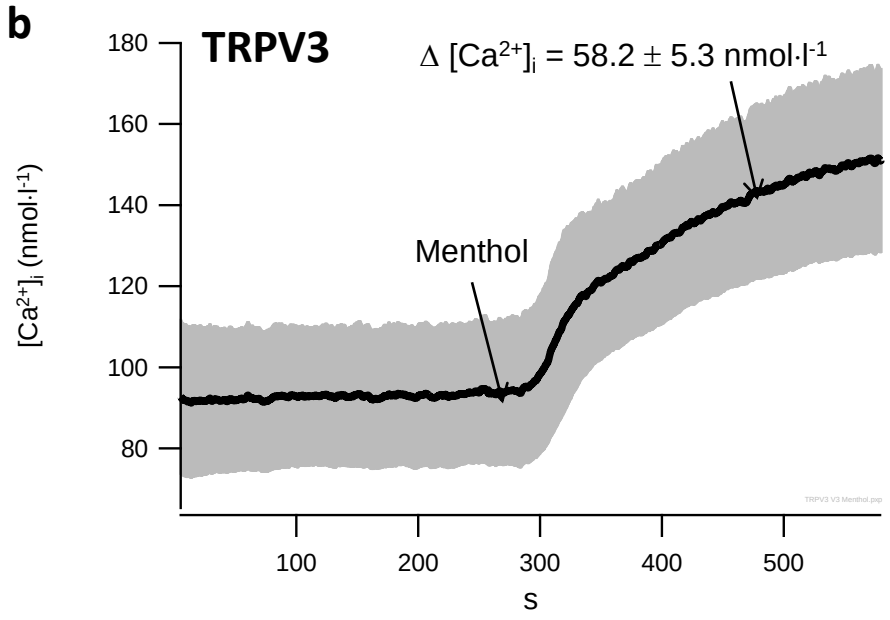


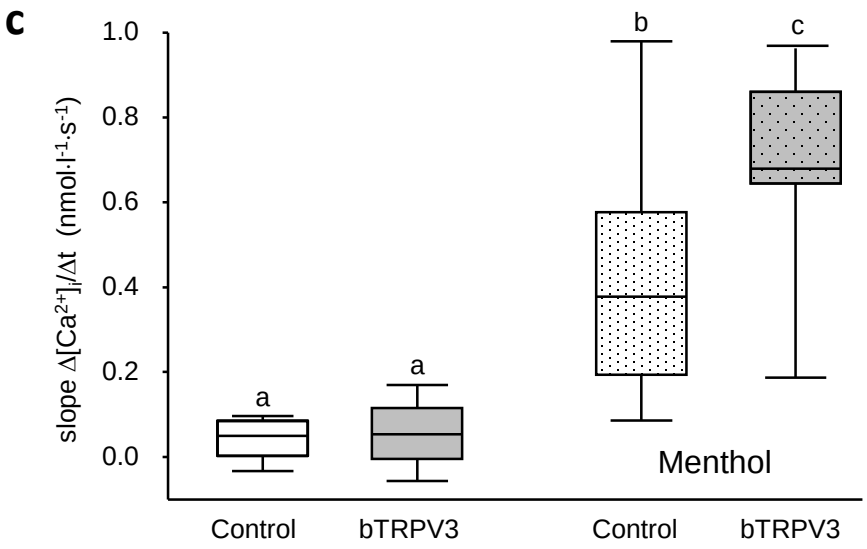
- Low dose menthol increased calcium absorption
- No effect of low dose Thymol, high dose: net absorption decreased

Rosendahl et al. 2016

3b) *in vitro* experiments







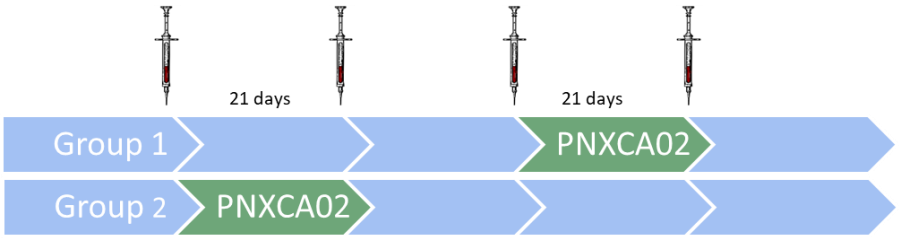
One way ANOVA, SigmaPlot, *P*=0.003; n=10

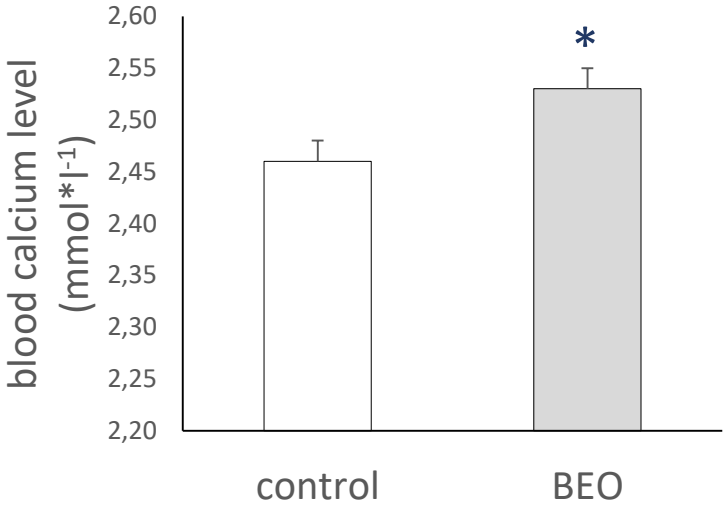
TRPV3 overexpression in HEK cells increased calcium uptake with 1mM Menthol

Schrapers et al. 2018

3c) *in vivo* experiment

- 72 mid-lactating cows (2x2 cross over design) were fed with a menthol based feed additive (BEO)
- blood samples were taken before and after 21 days of supplementation





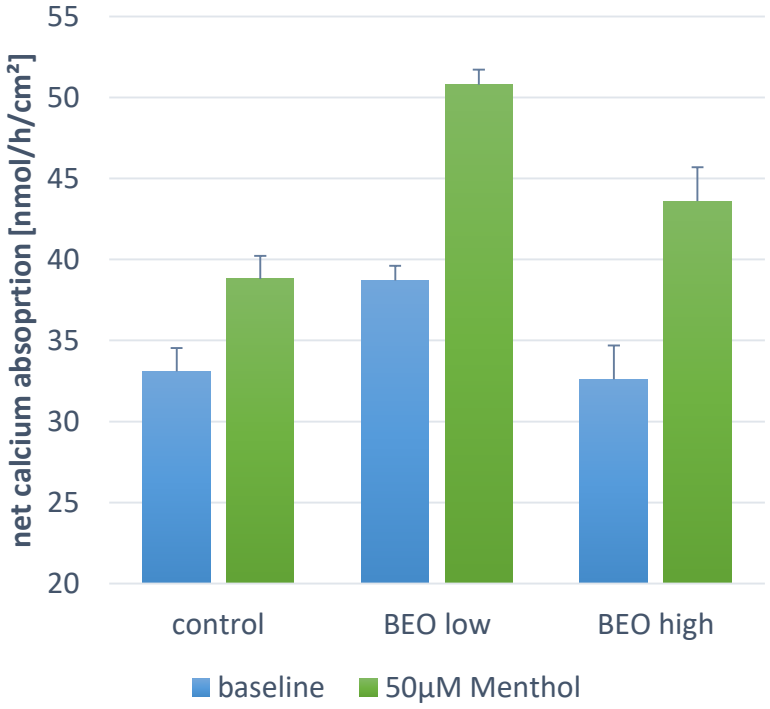
Univariate ANOVA with Covariate, SPSS, $P<0.001$

Blood Calcium levels significantly increased in the BEO group

Braun et al. 2018

3d) *in vivo/ex vivo* experiments

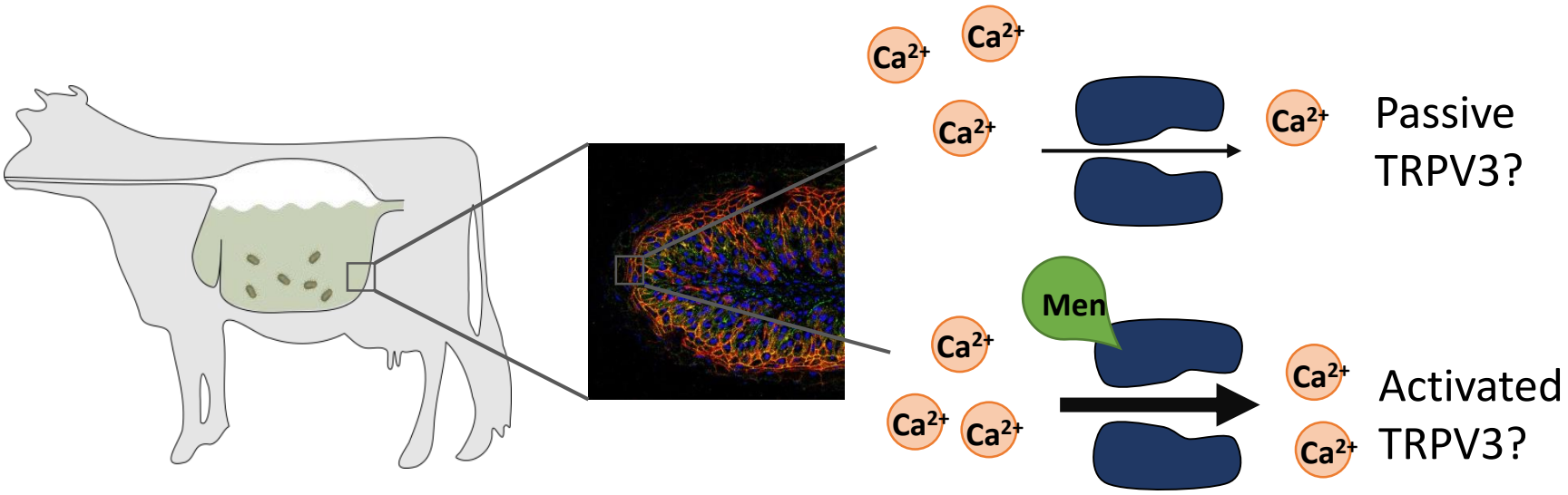
- 24 growing sheep, 3 feeding groups (control, BEO low/high)
- sheep were sacrificed after 28 days of BEO supplementation
- evaluation of ruminal calcium transport rates *ex vivo*



- Calcium transport was significantly increased in the BEO low group
- Calcium transport further increased by adding menthol

General linear model, Mixed proc, SAS, quadratic response, p= 0.032 (baseline) and p=0.011 (menthol)

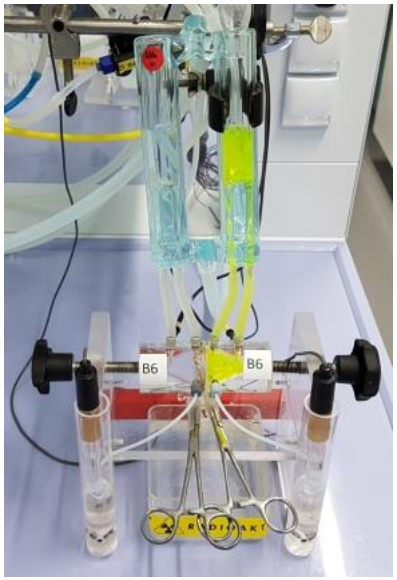
4. summary

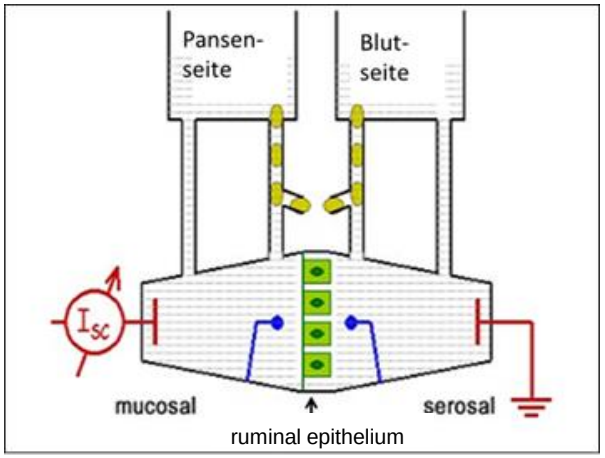


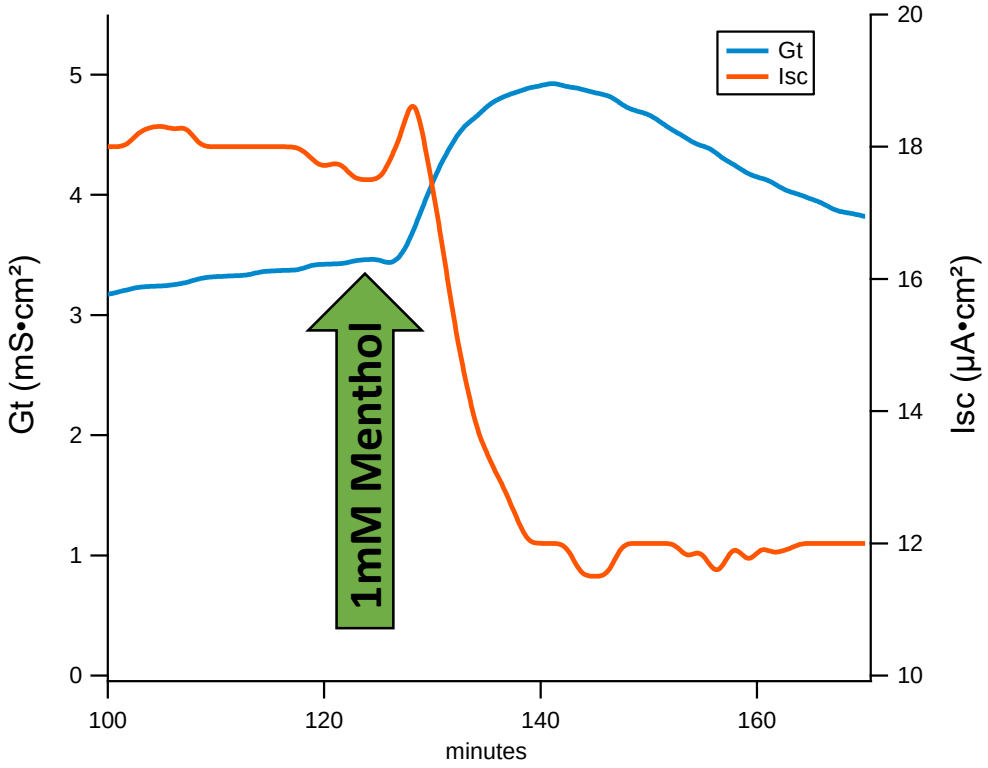
- Menthol increased calcium absorption in
  - Isolated ovine ruminal tissue
  - HEK cells over/expressing TRPV3
  - Serum blood samples of dairy cows fed menthol-based BEO for 20 days
  - ruminal tissue isolated from growing sheeps fed menthol-based BEO for 28 days

Thank you for your attention!

ex vivo experiments



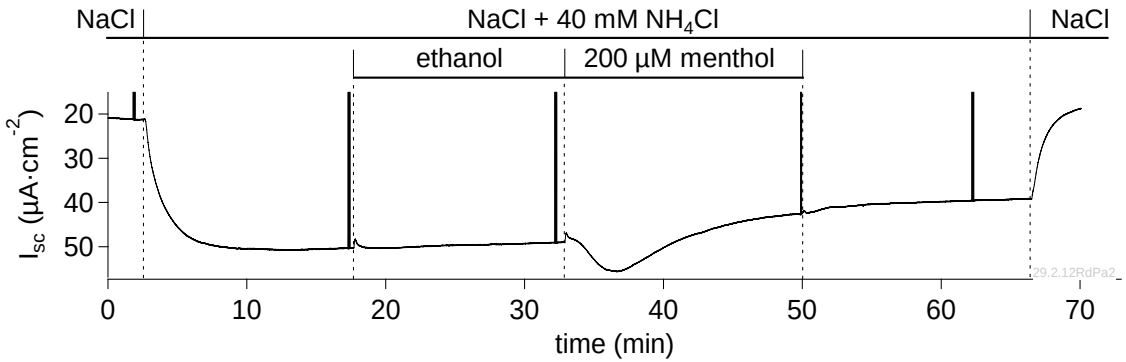




- Menthol increased tissue conductance
- Menthol induced non-selective cation transport (patch clamp data: Na^+ absorption, K^+ secretion)

Rosendahl et al. 2016

Identification of a ruminal calcium channel



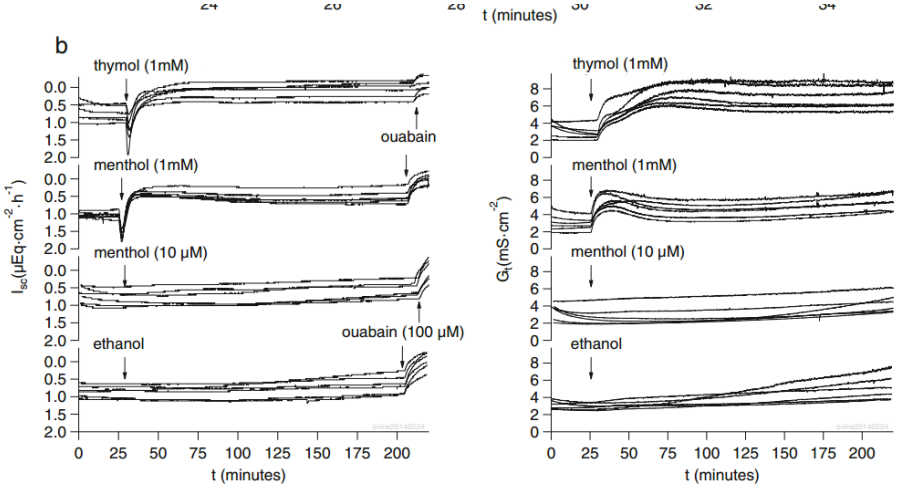


Fig. 7 Original recordings from pieces of ruminal epithelium from one sheep, measured in 24 conventional Ussing chambers in parallel (NaCl on both sides; no cation gradient). **a** Detail from one epithelium: after addition of menthol to the mucosal bath solution, a transient rise in I_{sc} (in µEq·cm^{-2}·h^{-1}) by ΔI_{scPeak} was observed that was coupled to an almost identical change in the conductance by ΔG_{tPeak} (in mS·cm^{-2}). **b** Overlay of the raw data from all 24 chambers with 6 epithelia in each treatment group (time relative to start of measurement in each individual chamber; no current offset). Note the difference in the G_t response of the epithelia to menthol and thymol, which may reflect significant differences in the pharmacological interaction of these agonists with the tissue