EAAP European Federation of Animal Science

In vivo evaluation of tannins and essential oils mixtures as additives for dairy cows

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Background

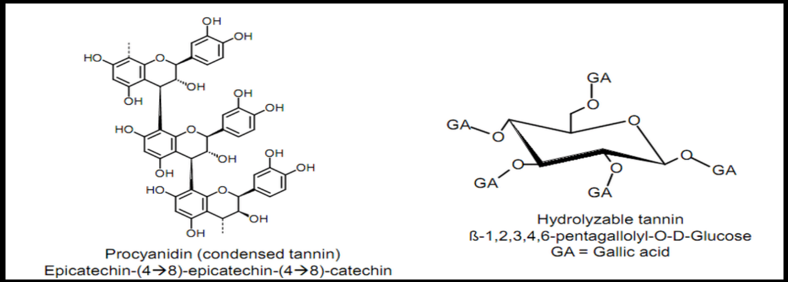
- Feed additives as nutritional strategy to mitigate the environmental impact of ruminants (Lind et al., 2023)
- Some products are already available on the market (e.g., Agolin Ruminant, Switzerland)

agolin®

Background

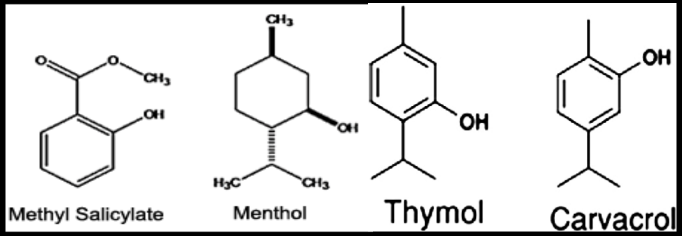
- Selected **mixture additives** reported interesting mitigating properties both on ruminal **CH_4** and **NH_3** in an in batch fermentation (Foggi et al., 2022) and in a RUSITEC system (unpublished)

Tannins (Vasta et al., 2019)



- Effect on biohydrogenation
- Anti-methanogenic and fibrolytic activities
- by-pass proteins +++

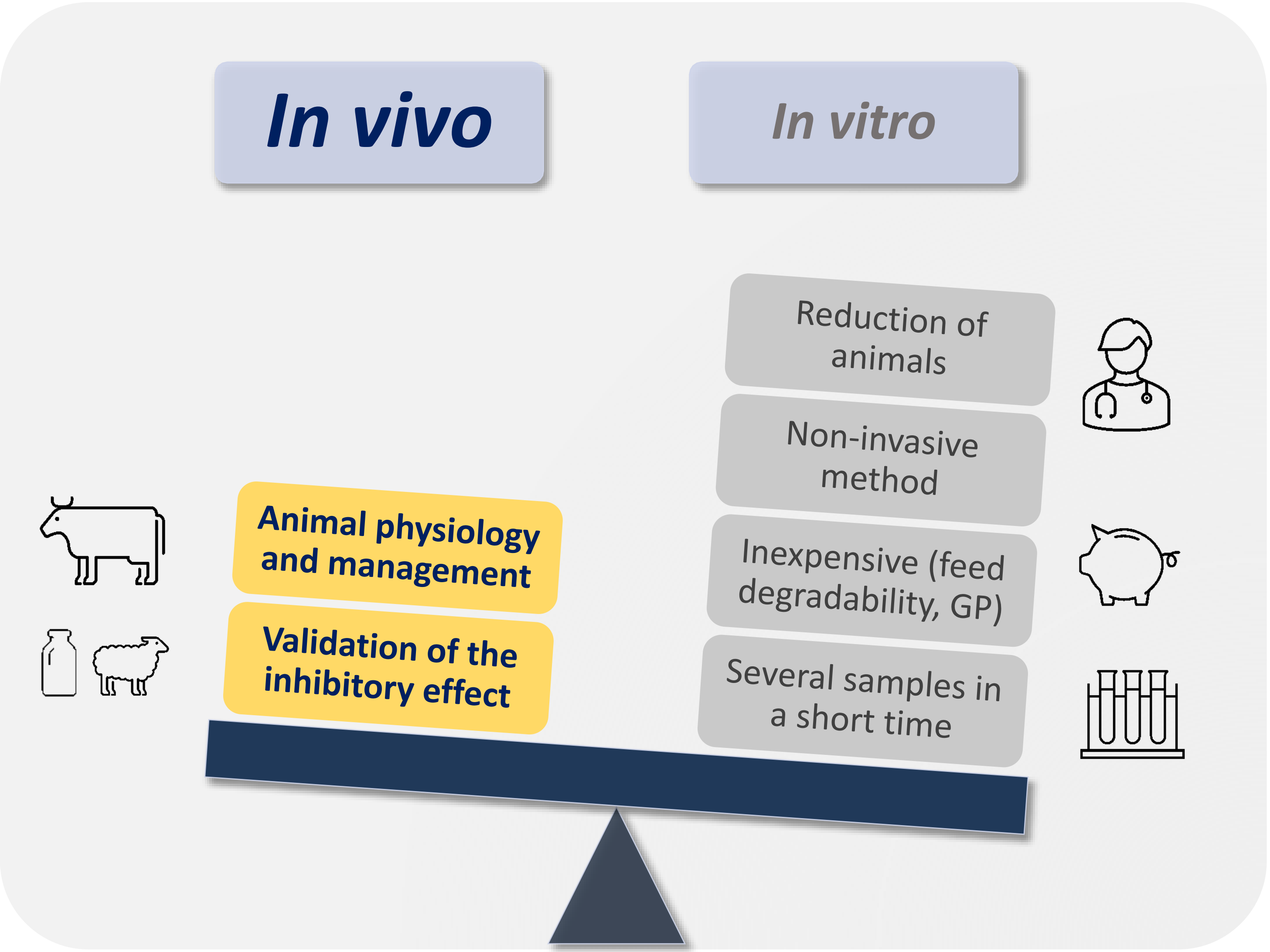
Essential oil compounds (Cobellis et al., 2016)



- Widespectrum antimicrobial activity
- Penetrate Gram +, protozoa membranes
  Low MW also Gram -

Aim of the trial

This study aimed to evaluate the in vivo effects of the 3 additives (2 newly formulated with tannins and EOC and 1 commercial) studied with RUSITEC



Experimental design

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32x

TMR	*g/kg*
Corn silage	370
Grass silage	300
Grass hay	130
Concentrate (wheat, corn gluten, soybean meal)	190
Wheat bran premix containing additives	10

No additive — K

Producer's recommendations — PC

Same concentration as **RUSITEC** experiment 100-133 mg/L — A

(Thyme and Oregano EO, limonene)

Same concentration as **RUSITEC** experiment 100-133 mg/L — B

(Thymol, Carvacrol and Eugenol)

After 2 weeks, groups were balanced according to milk yield (35±5), DIM (62±27), parity (1,2, ≥3), DMI, body weight, methane emission

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Materials and Methods

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Adaptation to the basal diet | Adaptation to the treatments | **Experimental period**

1 2 3 4 5 6 7 8 9 10 11 12

- **Daily**
  - feed intake
  - milk production
  - CH_4 (GreenFeed, C-Lock Technology Inc., Rapid City, SD) (Denninger et al., 2019)
- **Weekly**
  - milk and feed gross composition
- **Specific weeks**
  - Rumen fluid (before feeding) analysed on site for ruminal pH, NH_3, and acidified for later VFA analysis
  - Faeces (3-day pool) gross composition
  - Milk fatty acids (FA) composition (GC-FID) (Conte et al., 2016)

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Trial conducted in a free-stall barn in Posieux during winter, Agroscope (Switzerland)

Statistical analysis

The following linear mixed model was use for ANOVA (*R software v. 4.2.0, R Core Team*):

$$Y_{ijn} = \mu + T_i + wk_j + T_i x wk_j + parity\_grouped_W + A(T)_k + e_{ijwkn}$$

- T= treatment (K, PC, A, B)
- wk= week (8-12)
- parity grouped= “1”;”2”,”>3”

Calculations

- 4% **FCM** (Fat-corrected milk, kg) = 0.4 x milk (kg/d) + 15 x milkfat (kg/g) (*NRC, 2001*)
- CH_4: **Yield**, g on kg of DMI; **Intensity**, g on kg of FCM
- **Total-tract apparent digestibility**: 100*(1 - ([Nutrient]$_{feces}$ / [Nutrient]$_{feed}$ *[ADL]$_{feed}$/ *[ADL]$_{feces}$) *(Van Soest, 1994)*

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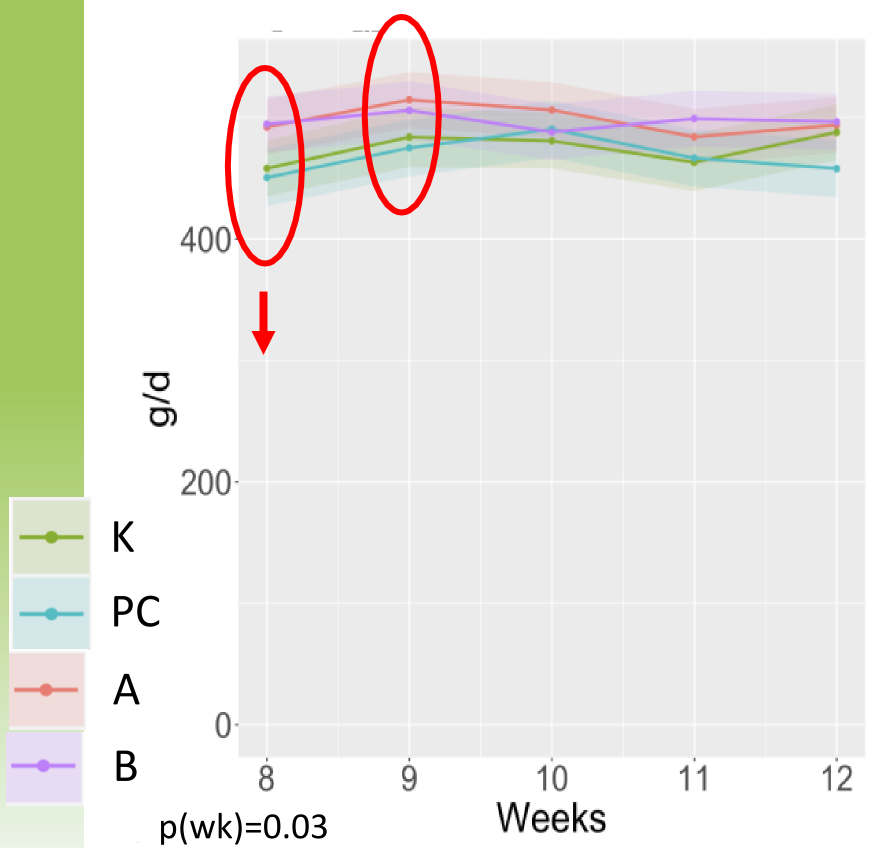
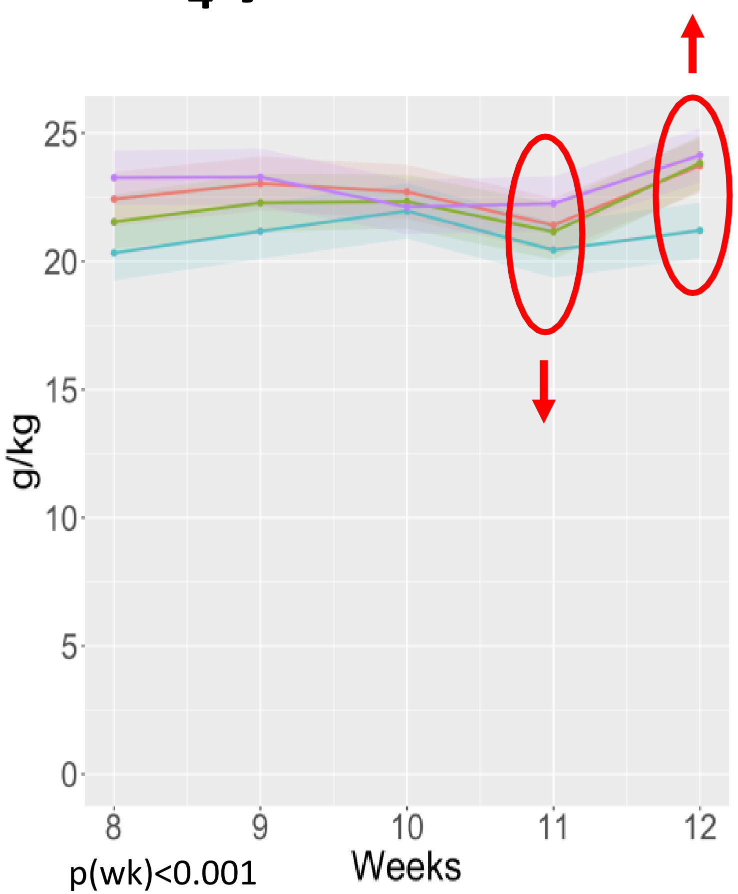
Results – CH_4

Absolute CH_4



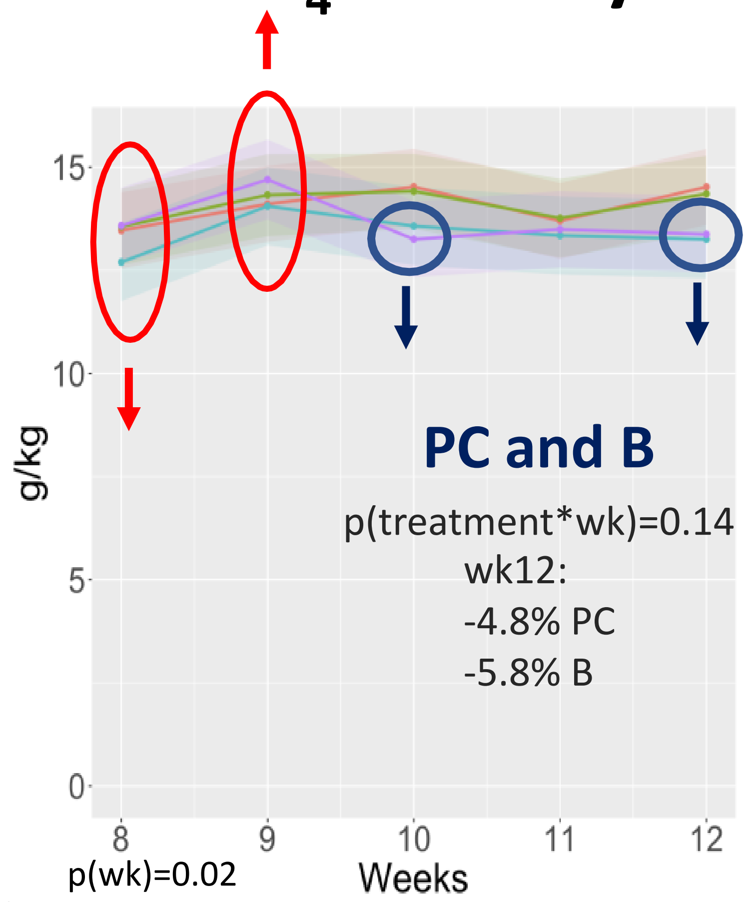
p(wk)=0.03
p(parity)<0.001

CH_4 yield



p(wk)<0.001

CH_4 intensity



p(wk)=0.02

Previously the commercial additive (PC) was reported to significantly lower *in vivo* up to CH_4 **-12.9%**, after 4 adaptation weeks (Belanche et al., 2020)

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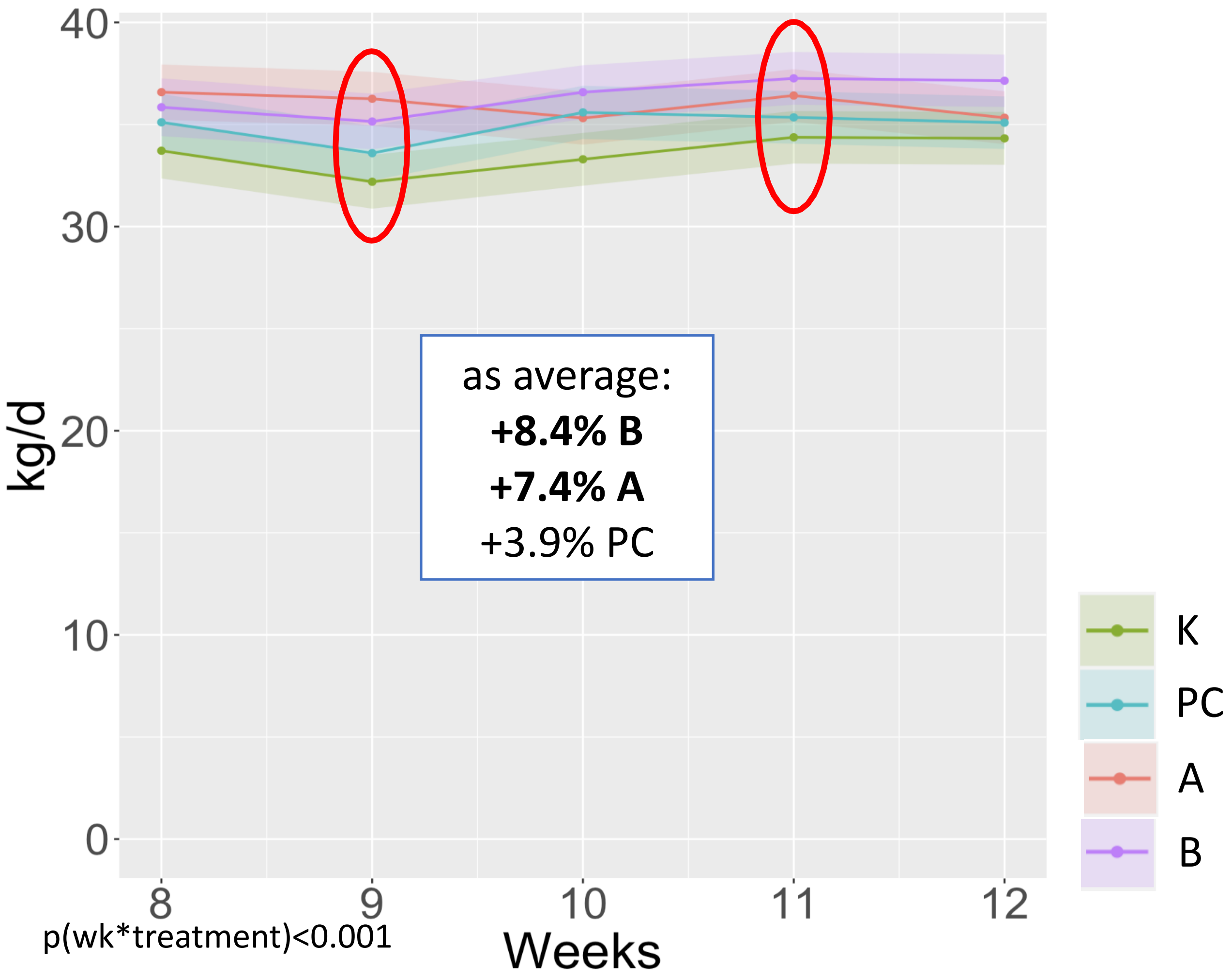
Results – Animal performances

Milk production (FCM)



p(wk*treatment)<0.001

- Beginning of week 8: **DIM 118±27 days,**
- **DMI** did not differ across groups

- In a meta-analysis, Agolin caused an increase of milk yield (+4%; Belanche et al., 2020)
- Similar concentration of oregano extract did not affect the milk yield (Kolling et al., 2018)

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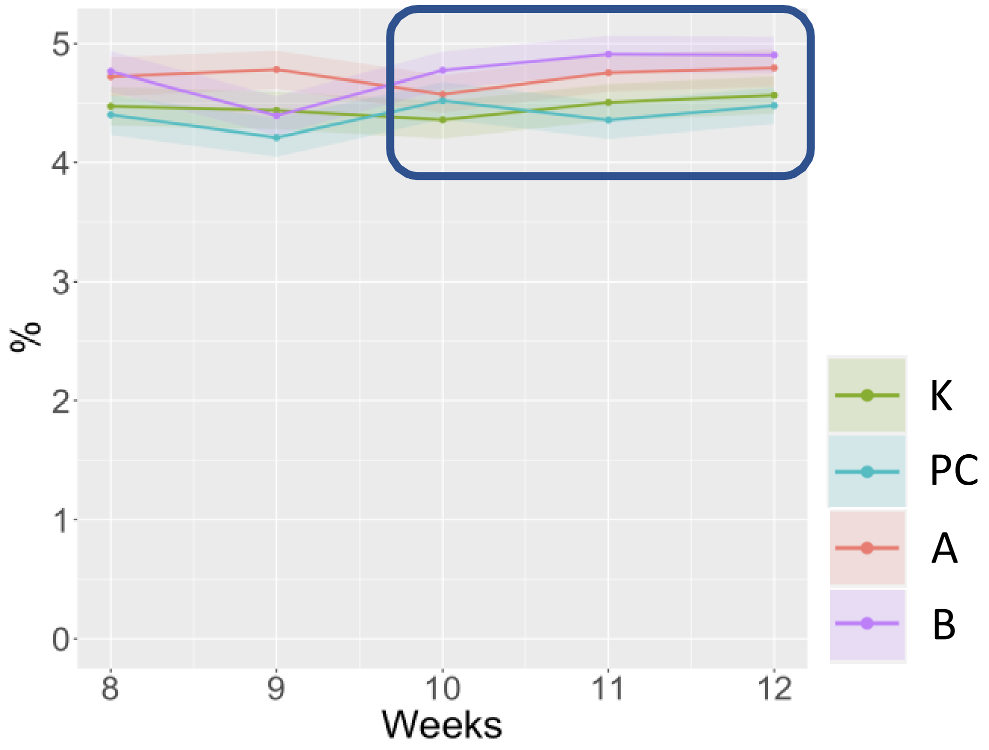
Results – Milk quality

Milk fat



p(wk*treatment)<0.001

Milk fat yield
+6.0% A (+120 g/d)
+11.8% B (+140 g/d)

Milk FA composition

- **C4:0** (p=0.10) and **C16:0** (p=0.04)

B > A > PC > K

de novo FA

- **C18:1c9** (p<0.001)

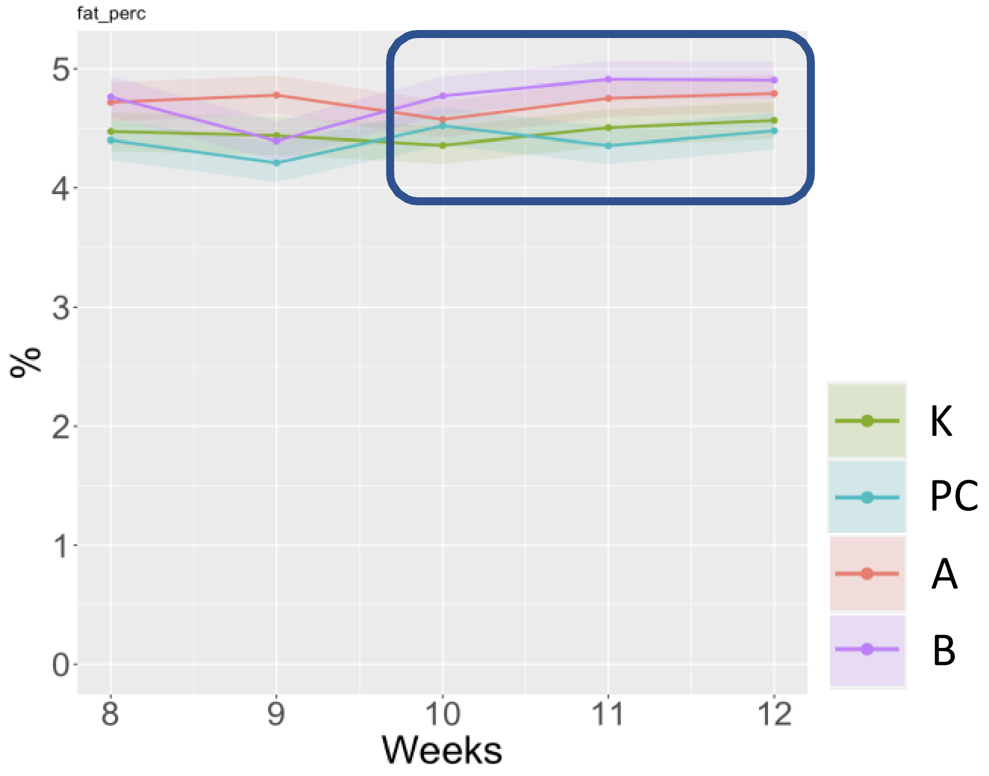
K > PC > A > B

(Turini et al., 2020)

dietary FA

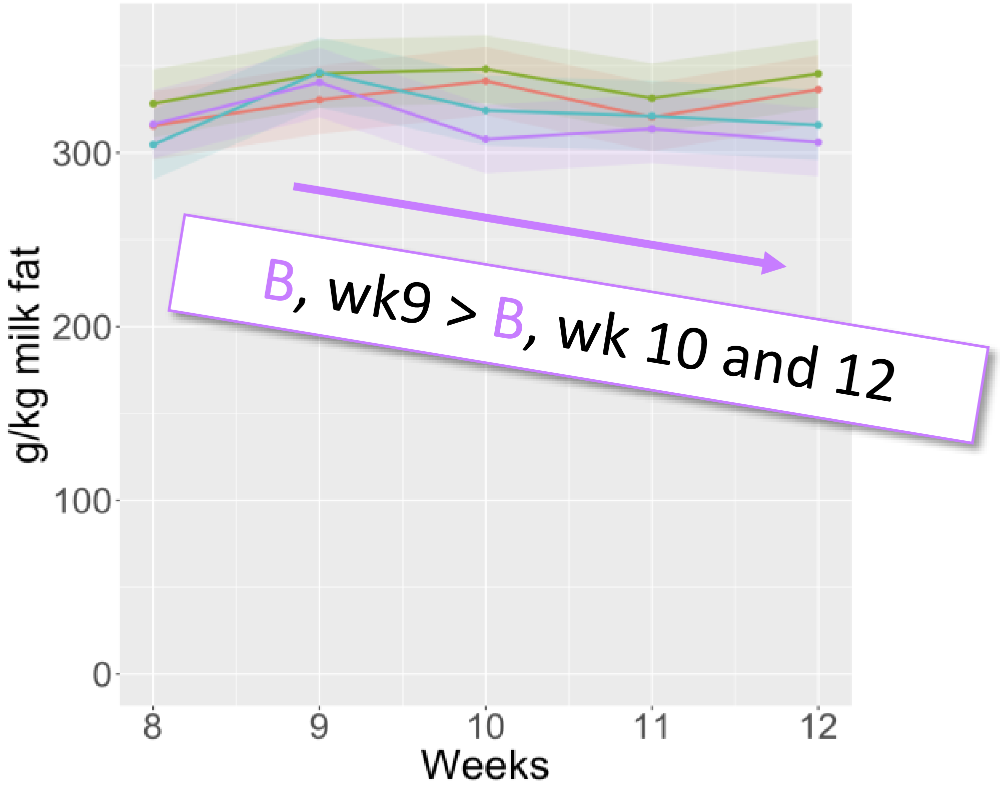
Results – Milk quality

Milk fat



p(wk*treatment)<0.001

CH_4 per unit of milkfat



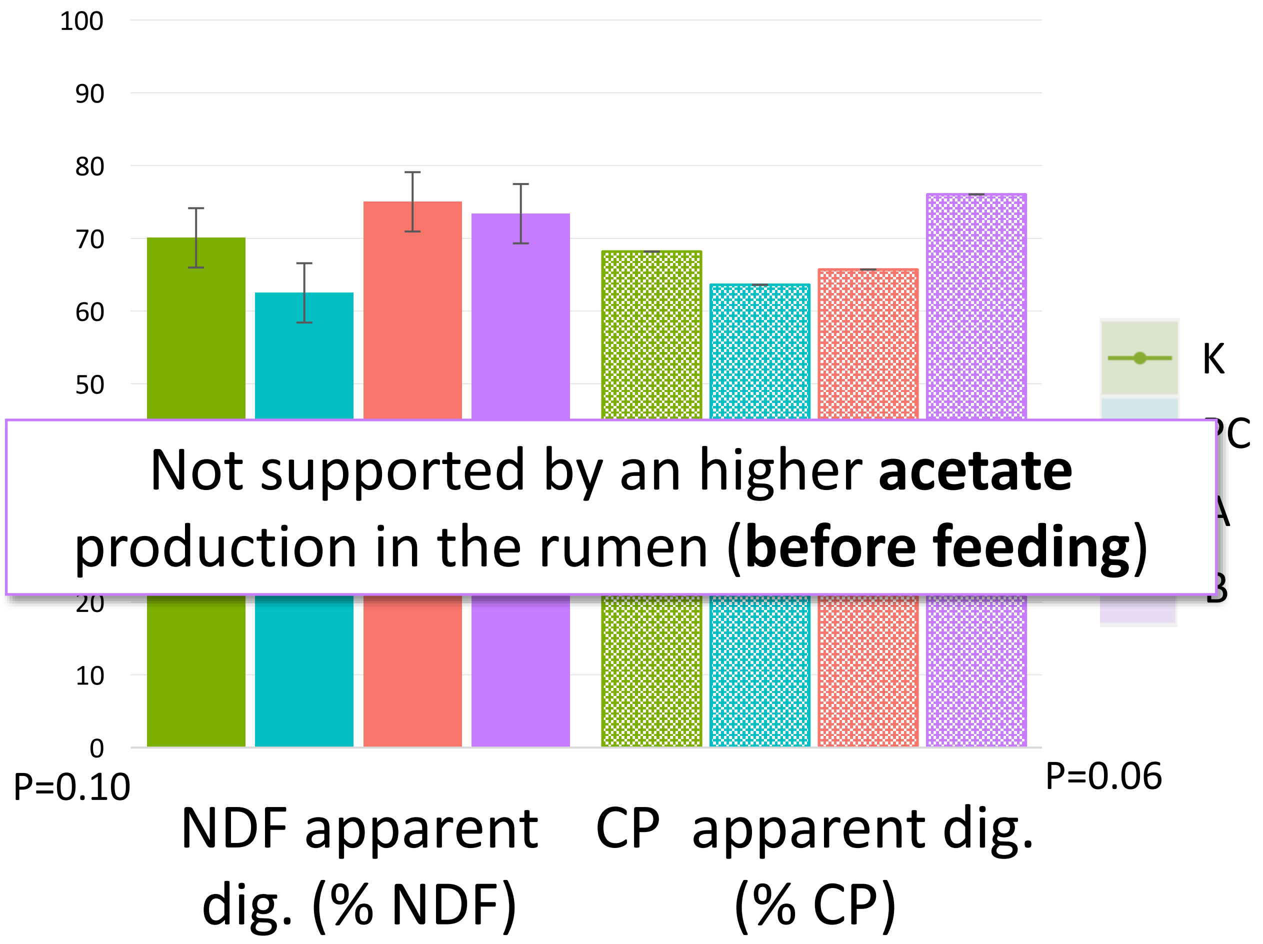
p(wk*treatment)=0.02

Results – Total-tract apparent digestibility

Milk FA composition

- **C13iso** and **C15iso** B > K (p<0.001)
- **C17iso** B and A > K (p<0.01)

Higher content of iso-FA in rumen liquor as biomarker of **cellulolytic bacteria** (Buccioni et al., 2012; Vlaeminck et al., 2004)

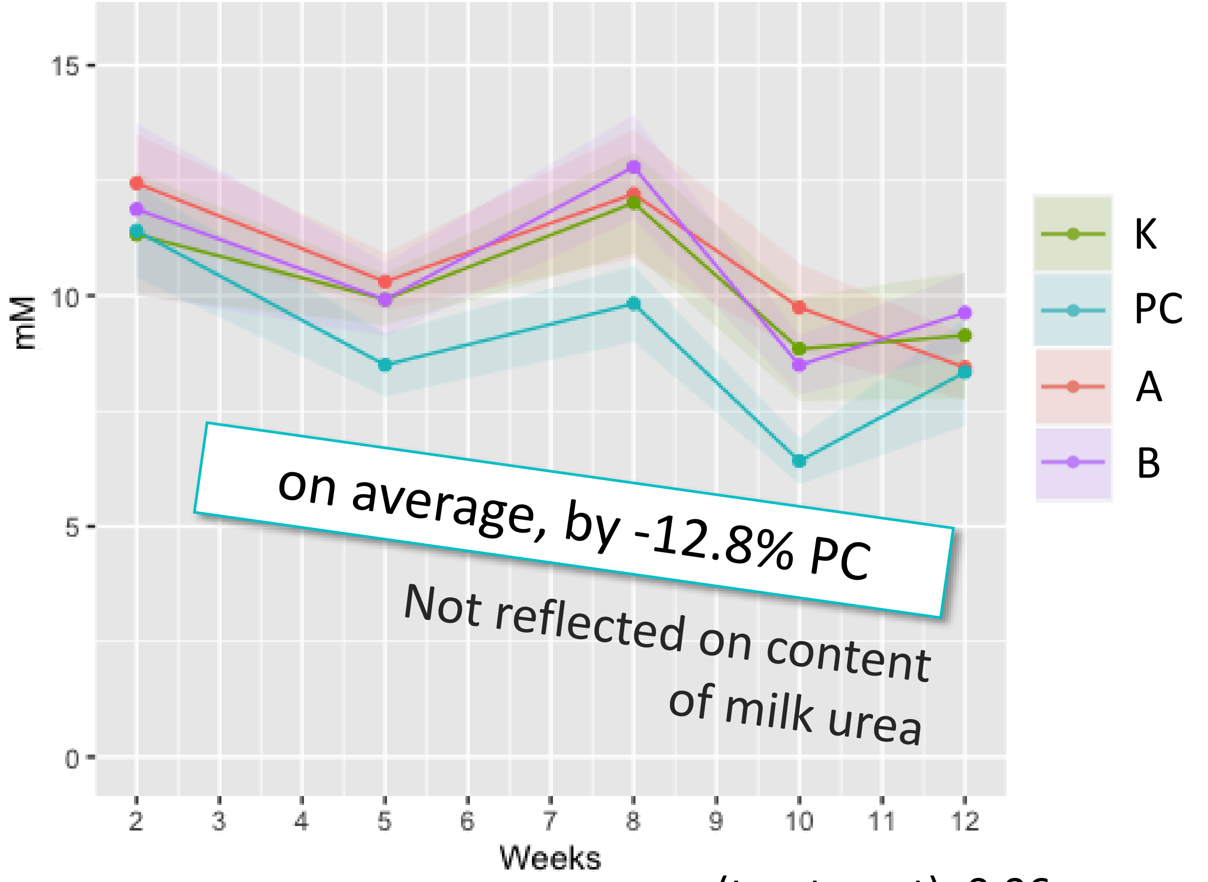


Not supported by an higher **acetate** production in the rumen (**before feeding**)

Results – Rumen liquor

- VFA molar proportions did not differ
- Total VFA, on average: 69.0 mM (before feeding)
- pH (7.04±0.04)
- Carrazco et al., 2020 reported a lower ammonia gas emission with Agolin supplementation

Ruminal N-NH_3



p(treatment)=0.06

Discussions and conclusions

- Lack of mitigating effect on CH_4 (at least for the commercial additive) might be caused by the **method of additive administration**
  - In many studies, Agolin Ruminant was administered **top dressed** (e.g., Carrazco et al., 2020), which might have favoured the attainment of the minimal compound active dosage in the rumen (Macheboeuf et al., 2008)
  - Kolling et al. (2018) obtained a significant reduction of CH_4 yield with a similar concentration of oregano extract but **top-dressed**
- Despite this, **A e B increased FCM and milk fat yield** (B additive more than A additive), probably because of an improvement NDF apparent digestibility

what's next?

- Studies to understand the mechanisms of action of such mixtures at the rumen level will be conducted
- Further studies to determine **the effective concentration** *in vivo*, considering the **method of administration** and the estimation of the optimal dosage from ***in vitro* experiments**, are needed

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THANK YOU FOR YOUR ATTENTION

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Supplementary_1 (feed)

	Daily DM intake (kg)	g/100 g DM
Corn Silage	8.18	37.13
Grass Silage	6.72	30.52
Grass Hay	2.90	13.18
Greenfeed	0.89	4.03
Soybean meal 48%RP 88	0.53	2.40
Premix Ferrero3p with or withou additives	0.20	0.79
Corn Gluten 60% RP 90	0.72	3.27
Wheat	1.91	8.69
Total	22.03	100.00

Gross composition		
	g/100 g DM	g/100 g FM
Dry matter	44.8	
Crude Protein	14.9	6.7
Ether Extract	3.1	1.4
NDF	37.7	16.9
ADF	23.2	10.4
ADL	3.0	1.3
Starch	21.7	9.7
Ashes	7.7	3.4

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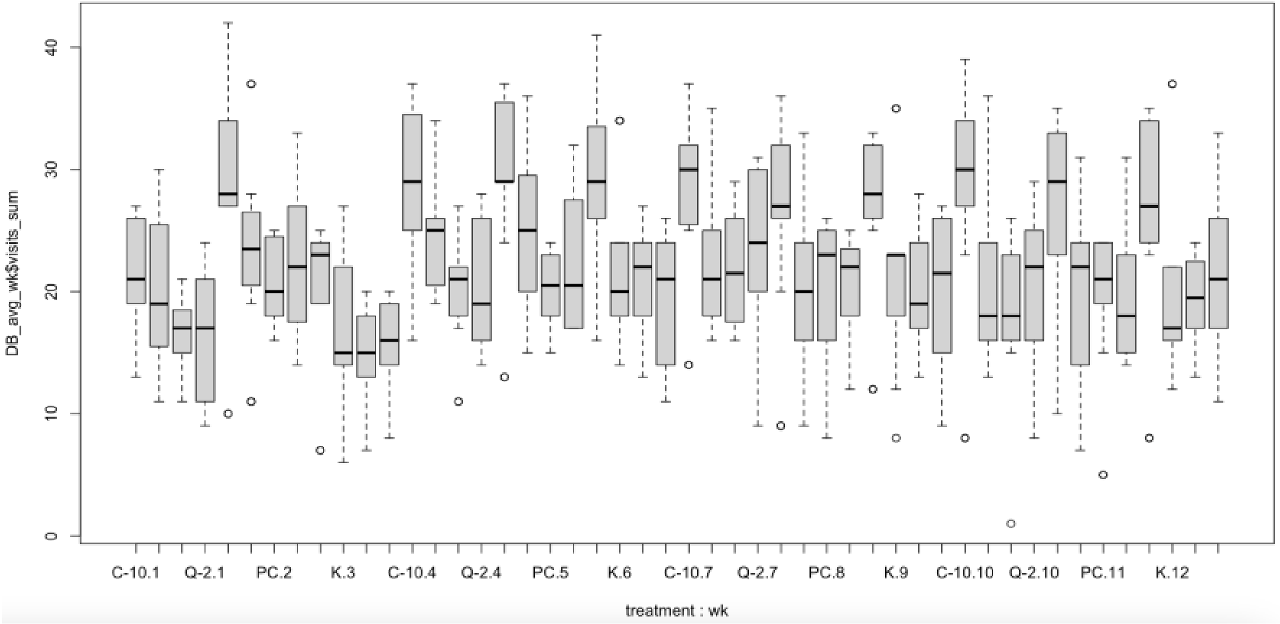
Supplementary_2 (Greenfeed)

6 visits max per day

about 900 g of bait feed per day

8 portions of 32 g of bait feed (pelleted dried whole corn plant) at 20-s intervals.



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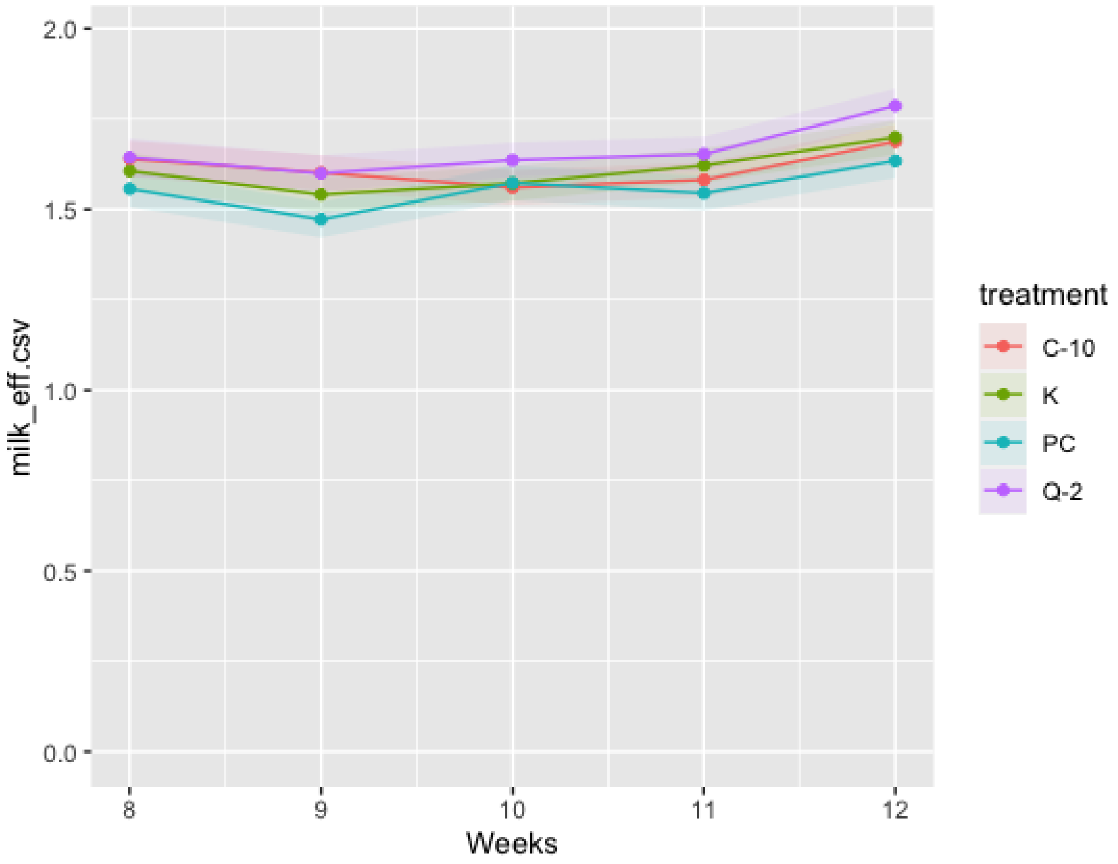
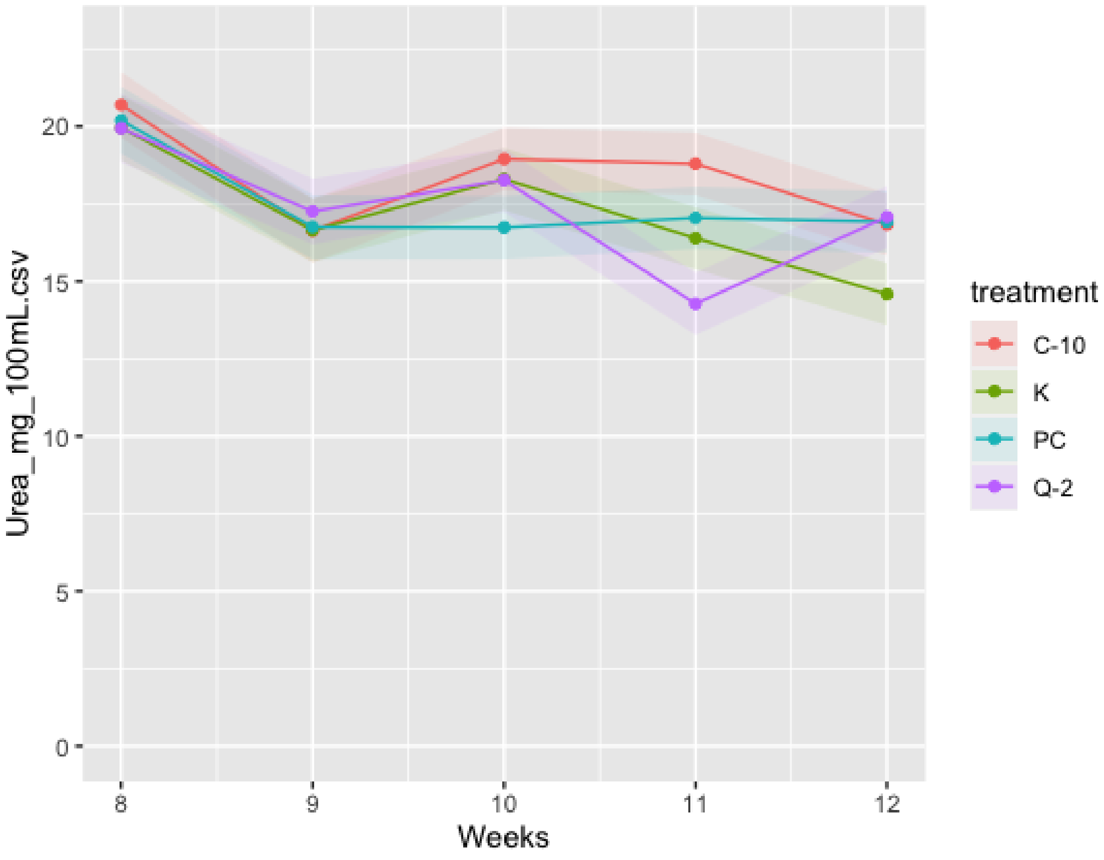
Supplementary_3 (milk)

milk efficiency



Urea in milk



p(wk*treatment)<0.001

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Supplementary_4 (rumen fluid)