The Effect of Dietary Nitrate on Enteric Methane Emissions and Methaemoglobin in Ruminants: *a Meta-Analysis*

Jolien Veneman, John Newbold, Hink Perdok, Jamie Newbold

February 2016

Introduction

NITRATE

- Nitrate acts as alternative hydrogen sink when being reduced to ammonia (Ungerfeld and Kohn, 2006)

$$\mathbf{NO_3^- + H_2 \rightarrow NO_2^- + H_2O}$$

$$\mathbf{NO_2^- + 3 \cdot H_2 + 2 \cdot H^+ \rightarrow NH_4^+ + 2 \cdot H_2O}$$

- Both reactions s have a greater negative ΔG value than methane formation, through the reduction of CO_2, therefore nitrate reduction would be favoured over methanogenesis as a hydrogen sink
- If nitrate is completely reduced to ammonia then 100 g nitrate could ‘compete’ with 25.8 g of methane (stoichiometric potential)
- When nitrite forms, and is not quickly reduced to ammonia, it can be absorbed across the rumen wall to bind to Heamoglobin, forming Metheamoglobin (MetHb). This can lead to reduced performance or in extreme situations death at high levels of MetHb

Introduction

META- ANALYSIS

- Several newly published studies on nitrate supplementation and methane emissions
  - Not included in Lee and Beauchemin (Dec., 2014) → 11 comparisions.

Aim

- Study the effect of nitrate supplementation on methane emissions (g/kg DMI) → Calculate efficacy of nitrate
- The effect of nitrate on MetHb%
- Which moderators influence this effect

Materials and Methods;
Data selection

STUDIES

- 18 papers

Total comparisons between a control and nitrate treatment

  - 27 for Methane yield, 21 for MetHb%

Inclusion criteria;

- Within each experiment, control and nitrate diets contained equal concentrations of crude protein.
- Methane yield was expressed as methane emissions per kg dry matter intake (DMI) and MetHb as % of total haemoglobin.

Materials and methods; *analysis*

METAFOR PACKAGE IN R (VIECHTBOUWER, 2010)

- Mean difference (MD) → (Treatment – Control*) can be used when units (and range) are the same*
- Using Random effects model to test overall effect of nitrate;
  - $\theta_i = \mu + u_i$
- Mixed effects model to test effect of moderators;
  - $\theta_i = \beta_0 + \beta_1 x_{1p} + \ldots\ldots + \beta_p x_{ip} + u_{i,}$
- Each moderator was first tested individually and included if P<0.10 to construct final model
  - Animal type (beef cattle, dairy cattle or sheep; class)
  - Dose (g NO3/kg DM; continuous)
  - Feeding management (ad lib or fixed; class)
  - Treatment duration (weeks; continuous)
  - Treatment type (coated or plain Ca Nitrate; class)

Results

METHB (N=21)

Overall significant increase in MetHb% by 1.2% units (±0.35SE; P<0.001)

Significant effect for **feeding management**

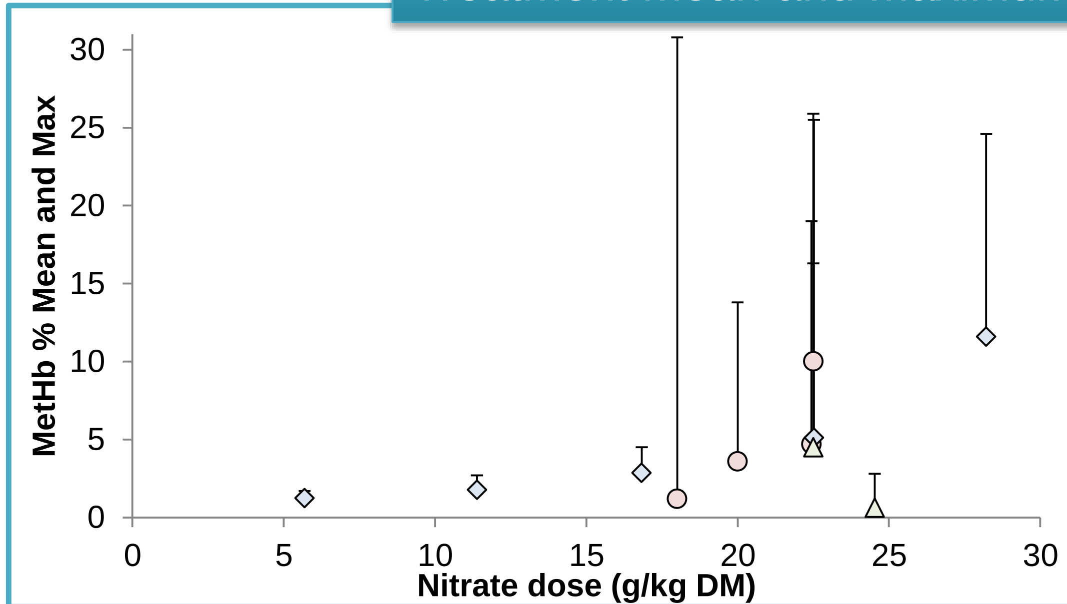
Restricted fed (n=11)

- MetHb +2.4% (±0.47SE; P<0.001)
- Max values increased at dose >18 g NO3/kg DM

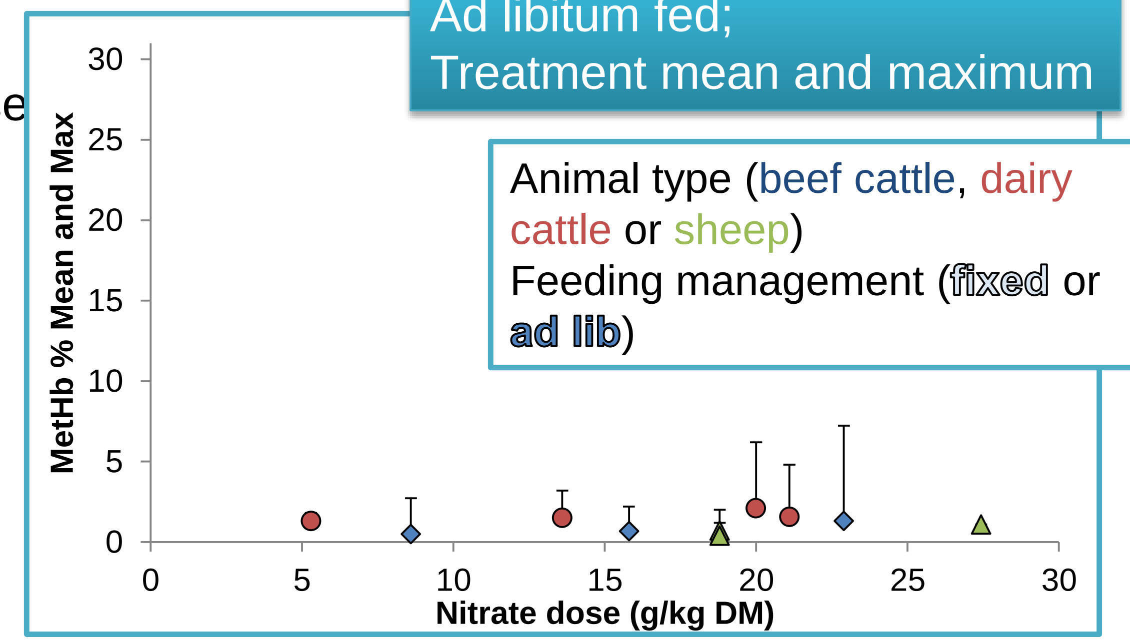
Ad libitum fed (n=10)

- MetHb +0.4% (±0.31SE; P=0.17)
- Max MetHb level did not exceeded 7.2%

Restricted/fixed fed;
Treatment mean and maximum



Ad libitum fed;
Treatment mean and maximum



Results

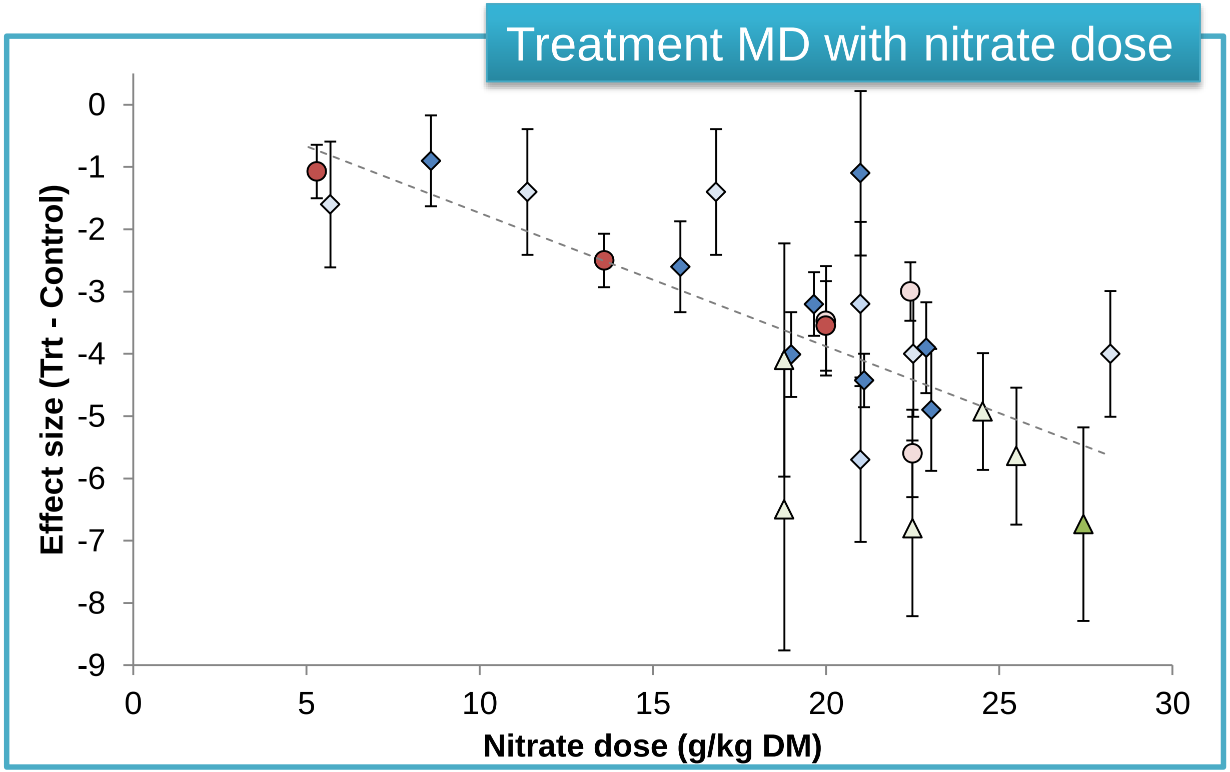
METHANE (G/KG DMI; N=27)

Overall significant reduction in methane yield (-3.6 g/kg DMI; ±0.33SE; P<0.001)

Significant **effect of dose**

- nitrate decreases methane by **0.21g** (±0.035SE; P<0.001) per g nitrate

Animal type (beef cattle, dairy cattle or sheep)
Feeding management (fixed or ad lib)



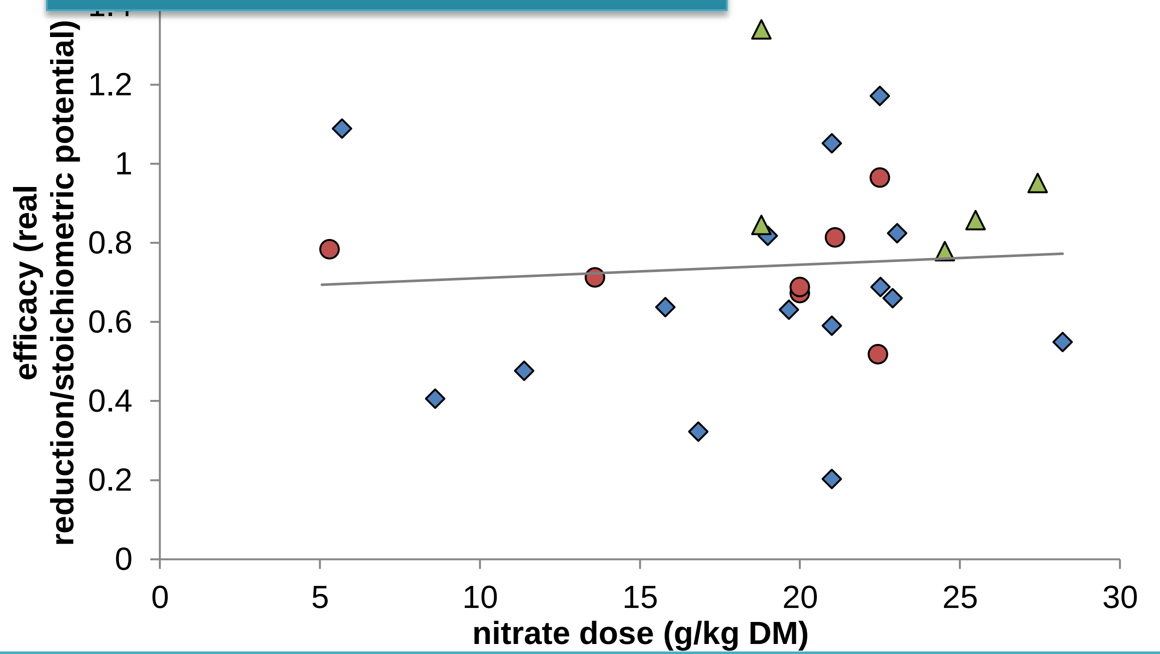
Results

METHANE (G/KG DMI; N=27)

→ 20.8 gram per 100 gram instead of 25.8 gram = 80.6% efficacy

No apparent decrease in methane decreasing efficacy with increasing nitrate dose

Efficacy per comparison with increasing dose of nitrate



Animal type (beef cattle, dairy cattle or sheep)
Feeding management (fixed or ad lib)

Results

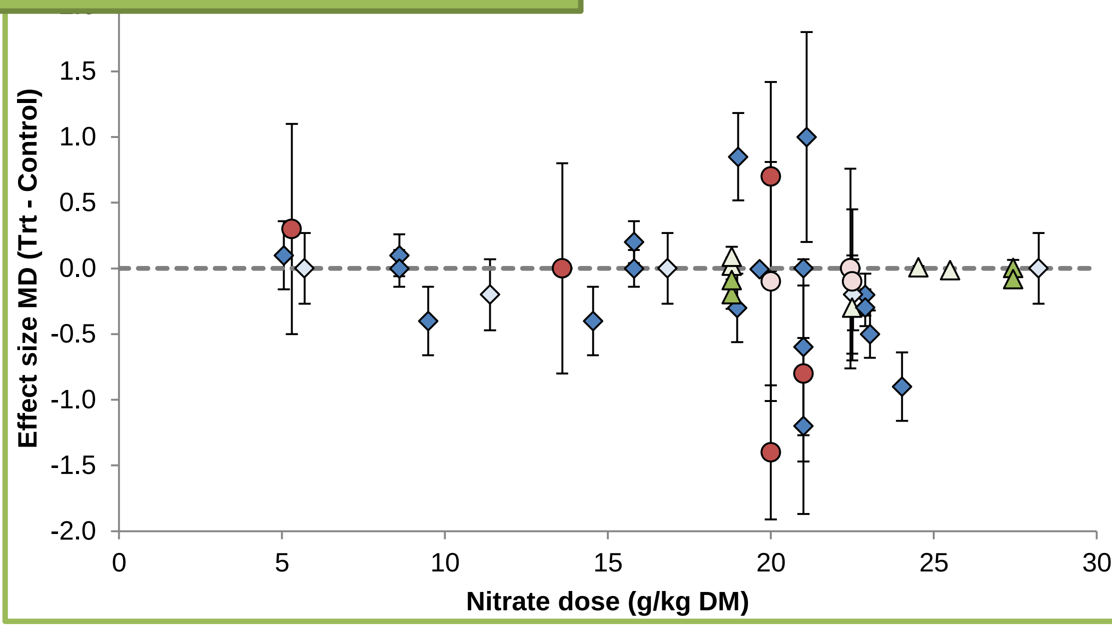
DMI (N=39)

Small overall negative effect -0.12kg (SE±0.06:P<0.003)

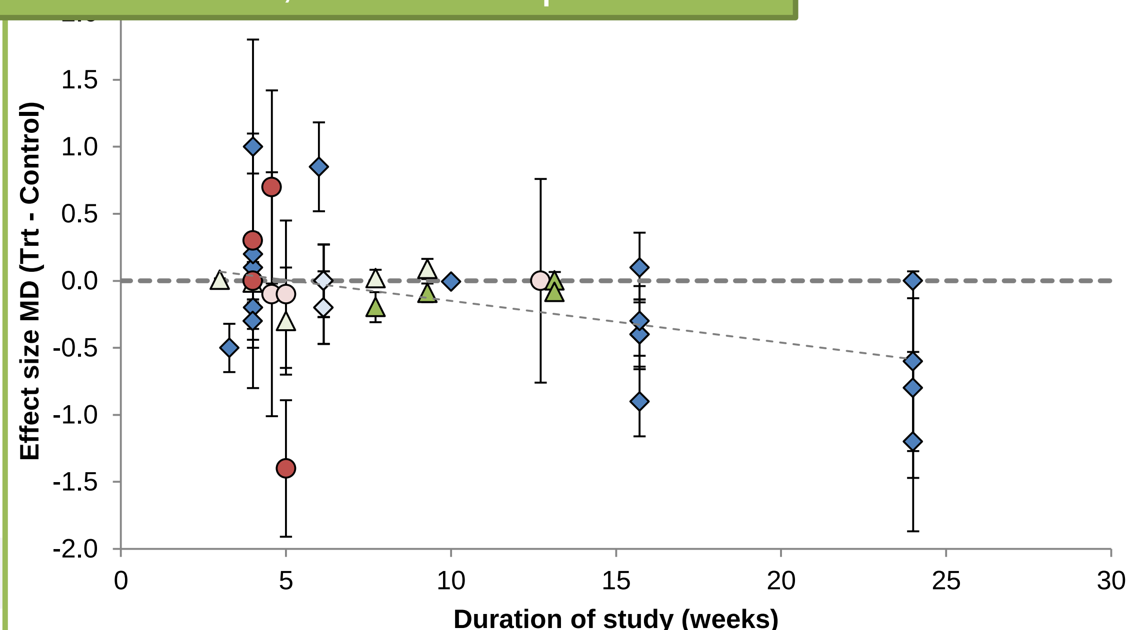
- No significant effect of dose, animal type, coating or feeding management.
- Significant effect of exp. Duration with decrease in DMI with longer experiments

Animal type (beef cattle, dairy cattle or sheep)
Feeding management (fixed or ad lib)

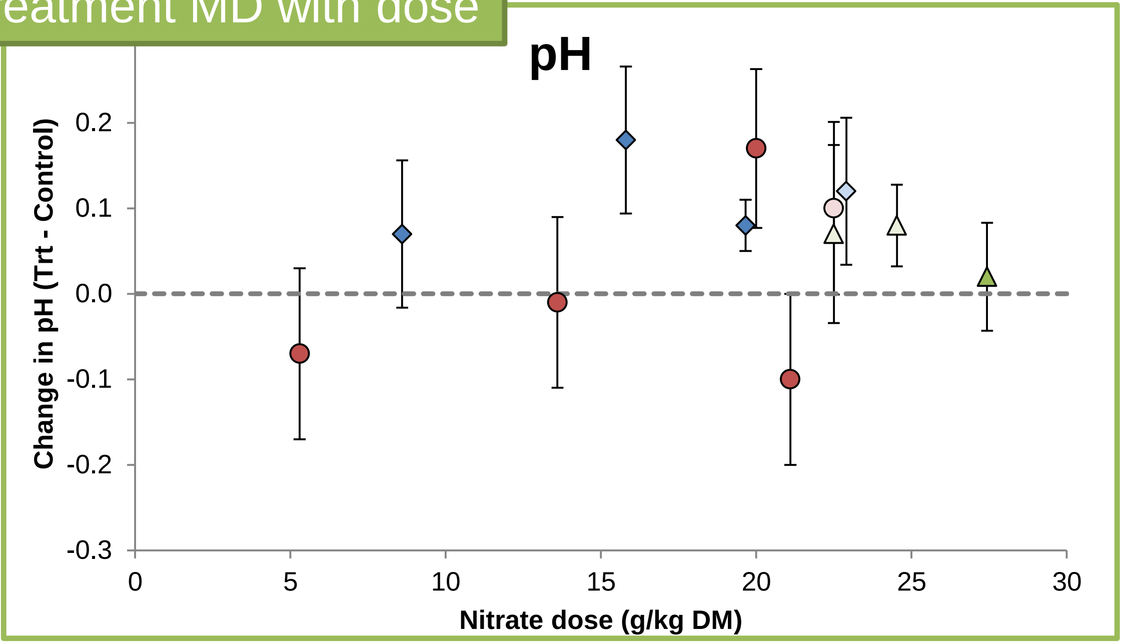
Treatment Mean Difference



Treatment MD, effect of exp. duration

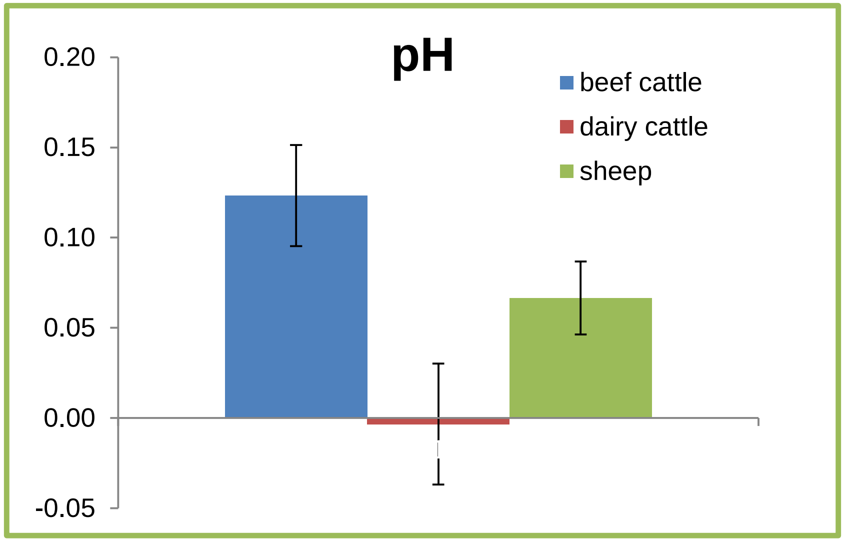


Results



PH (N=11)

- Overall increase in pH with 0.06 points (SE±0.003; P=0.003)
- Significant effect of animal (P=0.0148) with sheep and beef cattle showing a significant increase, but not in dairy cattle.



Animal type (beef cattle, dairy cattle or sheep)
Feeding management (fixed or ad lib)

Results

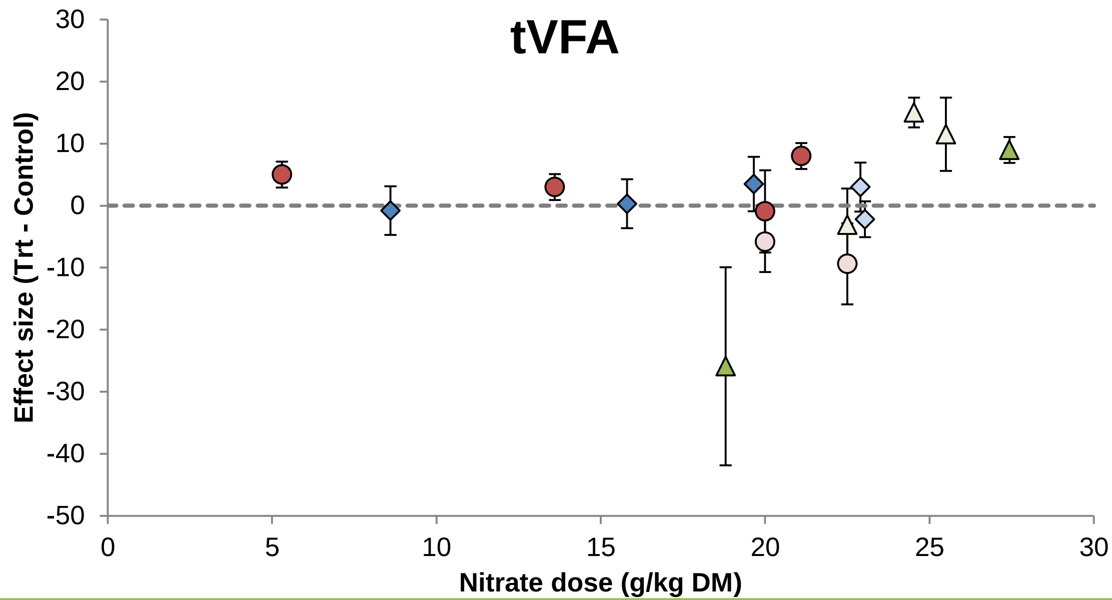
TOTAL VFA

(n=11)

- Overall no effect of nitrate on VFA
- Tendency (P=0.093) for effect of animal type with only sheep showing a significant increase in tVFA

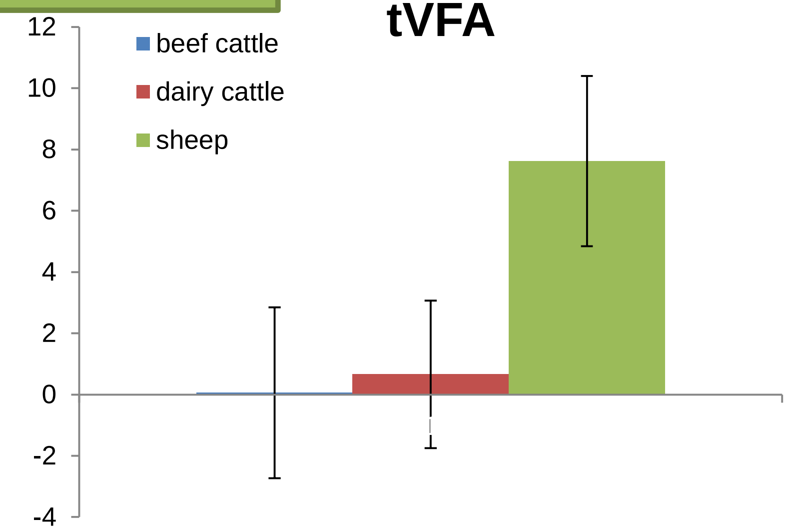
Animal type (beef cattle, dairy cattle or sheep)
Feeding management (fixed or ad lib)

Treatment MD



Remove ‘only’ before sheep

Treatment MD

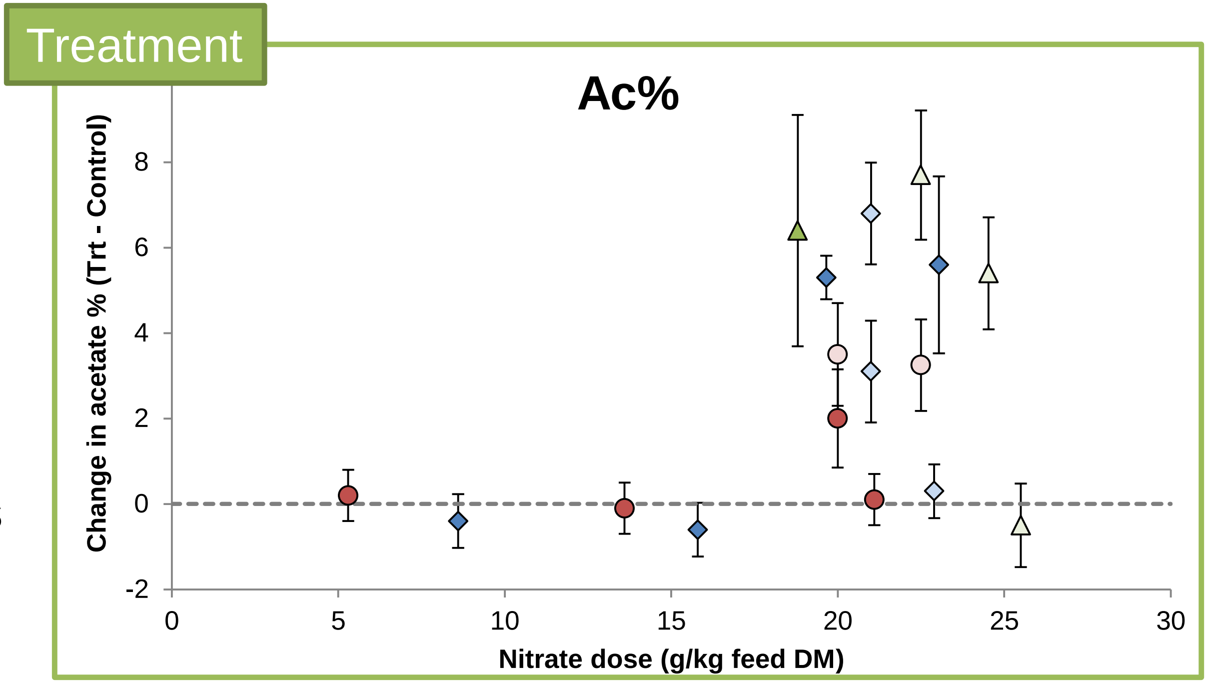


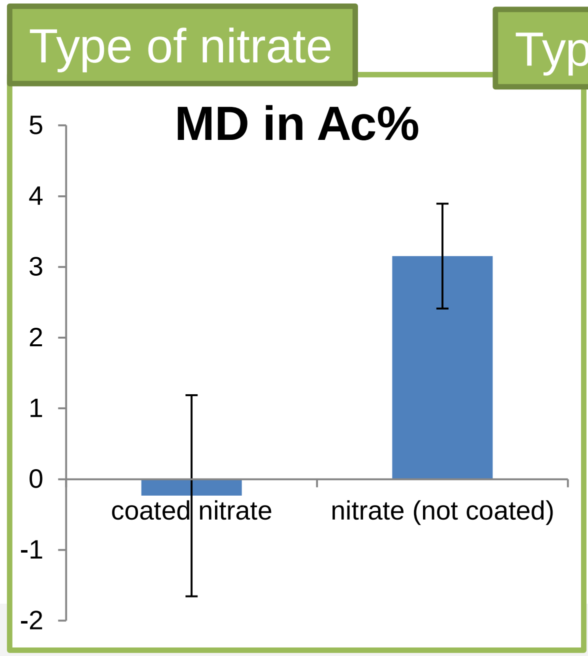
Results

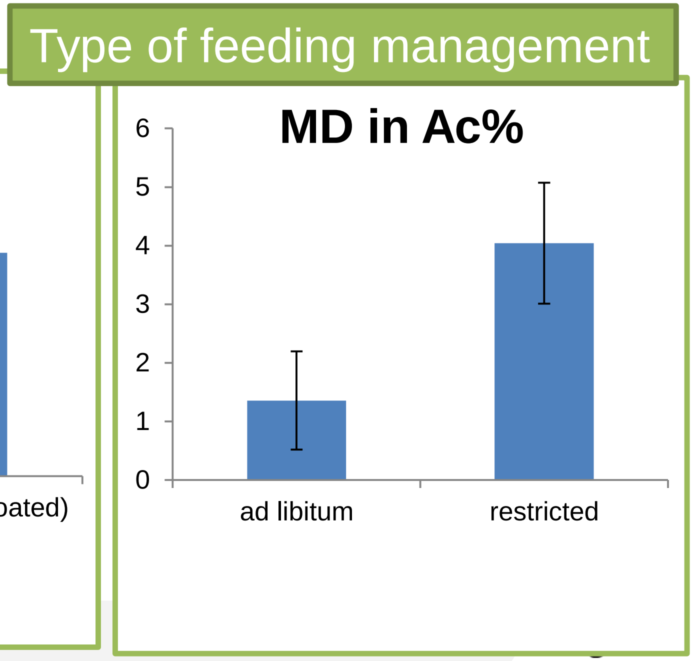
ACETATE %

(n=15)

- Overall nitrate increases Acetate by 2.46% (P<0.001)
- Effect of nitrate or coated nitrate, with coated nitrate not increasing Ac%
- Effect of feeding management, with restricted feeding increasing Ac% more







Animal type (beef cattle, dairy cattle or sheep)
Feeding management (fixed or ad lib)

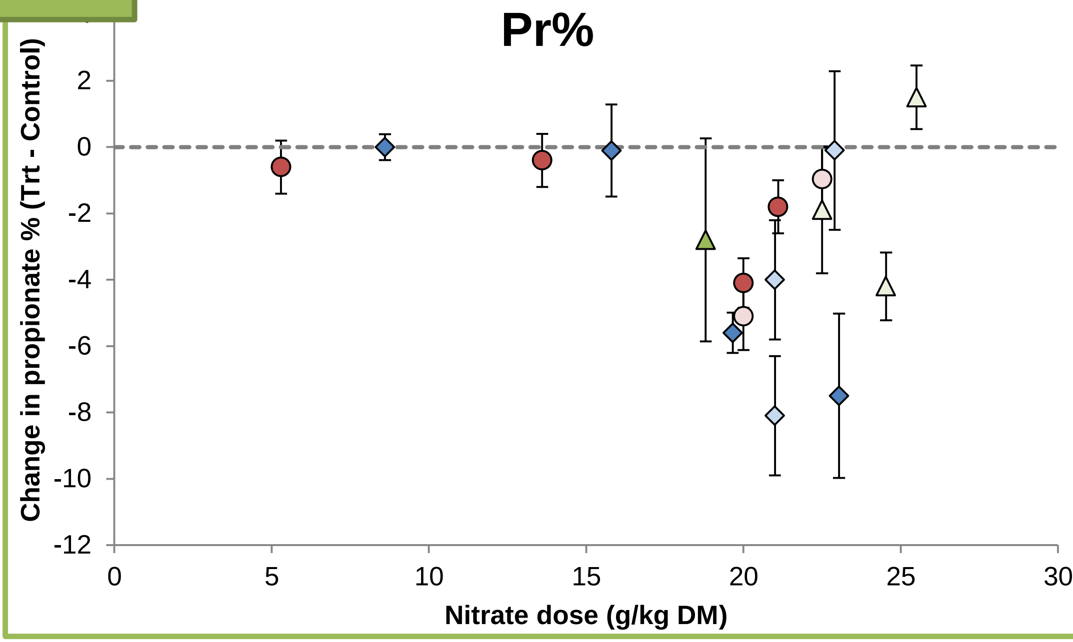
Results

PROPIONATE %

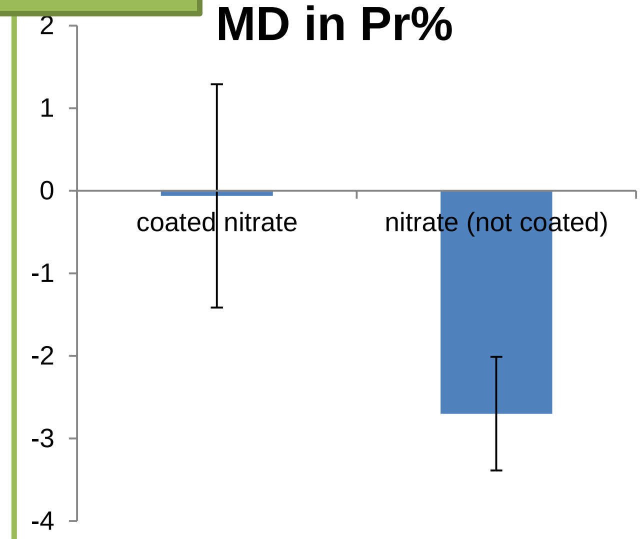
(n=15)

- Overall nitrate decreases propionate wby 2.17% s (P<0.001)
- Effect of type of nitrate, with coated nitrate not decreasing Pr%

Treatment



Type of nitrate



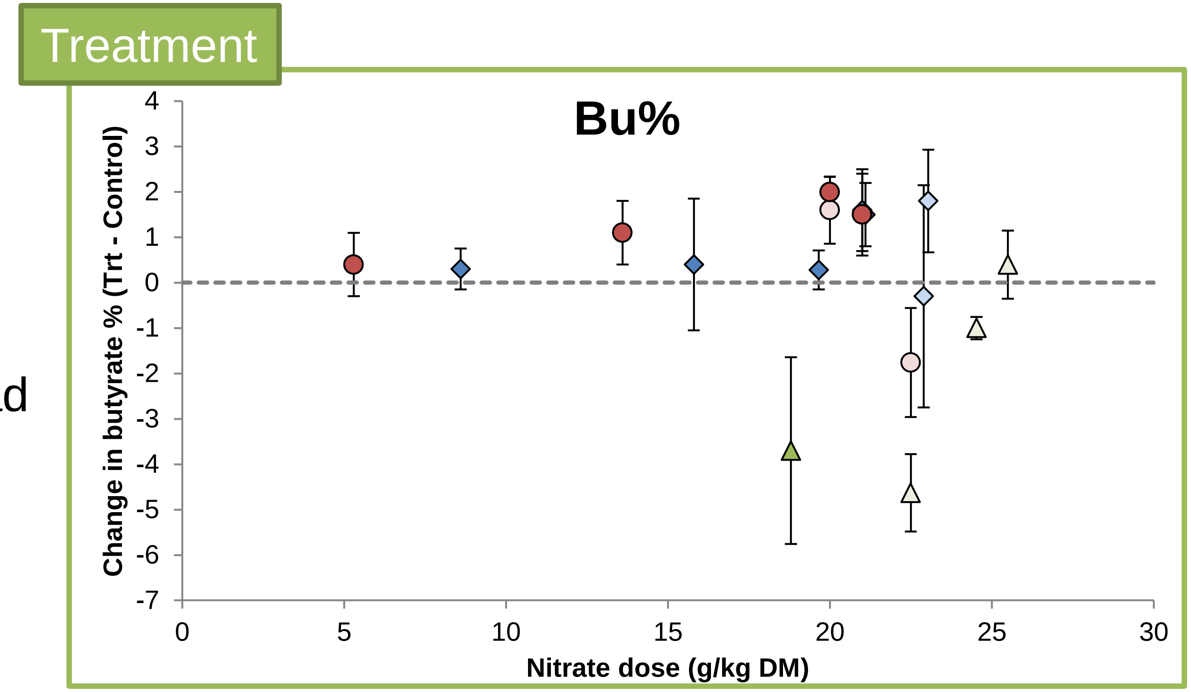
Animal type (beef cattle, dairy cattle or sheep)
Feeding management (fixed or ad lib)

Results

N-BUTYRATE %

(n=15)

- Overall no effect of nitrate on Butyrate % (P=0.94)
- No other moderators had an effect (e.g. dose, duration)



Animal type (beef cattle, dairy cattle or sheep)
Feeding management (fixed or ad lib)

Results

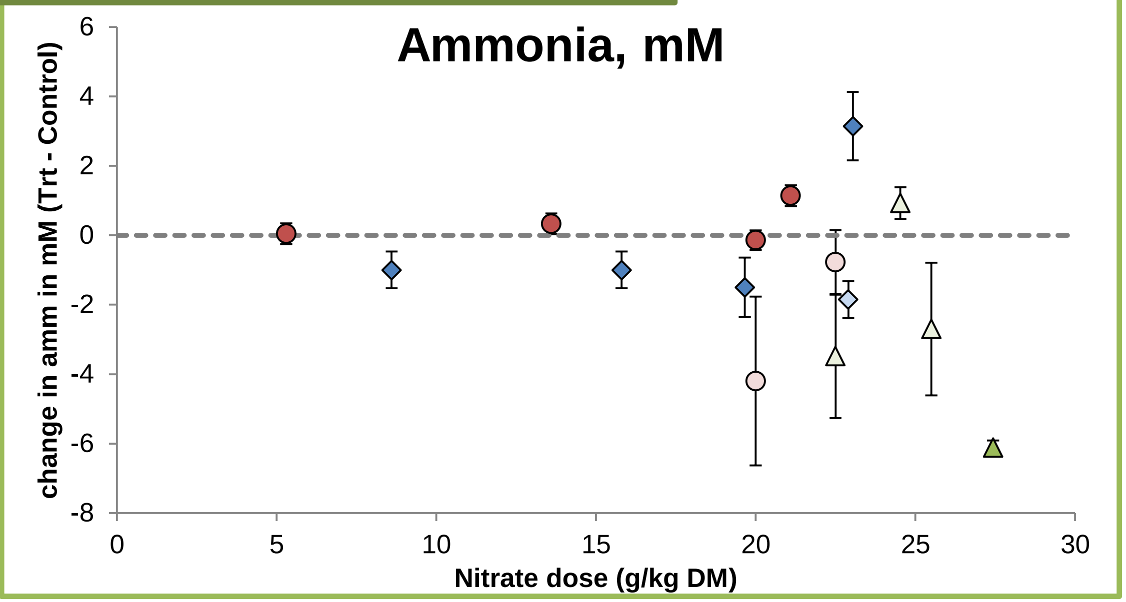
AMMONIA

(n=14)

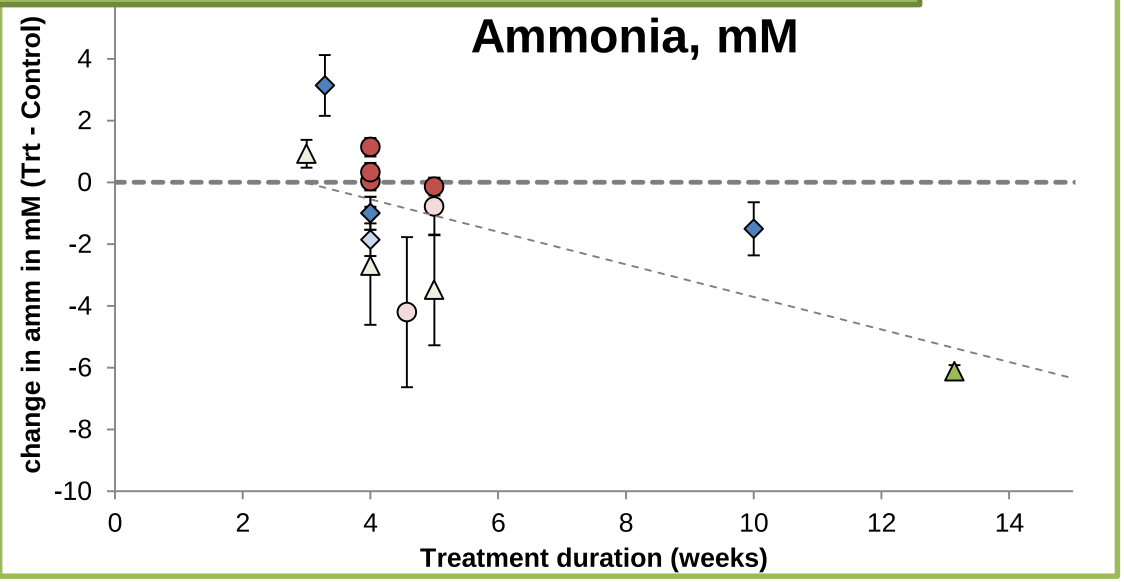
- Overall nitrate showed a tendency to decrease ammonia by 1.1 mmol/L (P=0.08)
- Significant effect of experiment duration (P<0.001) with ammonia decreasing with increasing duration

Animal type (beef cattle, dairy cattle or sheep)
Feeding management (fixed or ad lib)

Treatment effect with nitrate dose



Treatment effect with duration of experiment



Conclusion

META-ANALYSIS

MetHb%

- When nitrate-fed ruminants are limit-fed (e.g. to 95% of *ad libitum* intake) mean MetHb is increased significantly by 2.4% compared to the control.
- MetHb% is not different from the control when cattle or sheep are fed *ad libitum*
- Risk of increased MetHb% is higher in limit fed animals

Methane Yield

- Nitrate reduced methane yield in a linear dose response manner (80.6% efficacy)

Acknowledgements

- ANIMAL CHANGE
- IBERS Aberystwyth University
- Cargill Animal Nutrition

Data (appendix)

INCLUDED IN THE META-ANALYSIS

REF-ID	YEAR	COMPA-RISON	COUNTRY	ANIMAL	FIXED or AD LIB	TREATMENT	DURATION IN WEEKS	MAX* DOSE	n	CH4	DMI	RUMEN	METHB
deRaphélisSoissan 01	2014	1	Australia	Sheep	Fixed	Nitrate	10	20	7	x	x	x	x
El-Zaiat 01	2014	1	Brazil	Sheep	Ad lib	Coated nitrate	13.1	27	6	x	x	x	x
Guyader 01	2015	1	France	Dairy	Fixed	Nitrate	5	23	4	x	x	x	x
Guyader 02	2015	1	France	Dairy	Fixed	Nitrate	5	23	4	x	x	x	x
Hulshof 01	2012	1	Brazil	Beef	Ad lib	Nitrate	3.3	22	14	x	x	x	
Lee 01	2015	3	Canada	Beef	Ad lib	Coated nitrate	4	23	8	x	x		
Lee 02	2015	3	Canada	Beef	Ad lib	Coated nitrate	4	23	8		x	x	x
Li 01	2012	1	Australia	Sheep	Both	Nitrate	7.7	19	5	x	x	x	x
Li 02	2013	1	Australia	Sheep	Both	Nitrate	9.3	19	5	x	x		x
Newbold 01	2014	5	USA	Beef	Fixed	Nitrate	6.1	28	6	x	x		x
Newbold 01	2014	5	Brazil	Beef	Ad lib	Nitrate	15.7	24	5		x		
Nolan 01	2010	1	Australia	Sheep	Fixed	Nitrate	3	25	4	x	x	x	x
Troy 01	2015	4	UK	Beef	Ad lib	Nitrate	24	22	6	x	x	x	
vanZijderveld 01	2010	1	Netherlands	Sheep	Fixed	Nitrate	4	26	5	x	x	x	
vanZijderveld 04	2011	1	Netherlands	Dairy	Fixed	Nitrate	12.7	21	10	x	x		x
Velazco 01	2013	1	Australia	Beef	Ad lib	Nitrate	6	19	11	x	x		
Veneman 01	2015	1	New Zealand	Dairy	Both	Nitrate	4.6	20	6	x	x	x	x
Veneman 01	2015	1	UK	Dairy	Ad lib	Nitrate	5	20	6	x	x	x	x
Olijhoek 01	2014	3	Denmark	Dairy	Ad lib	Nitrate	4	21	4	x	x	x	x

* formulated dose of nitrate in g/kg feed DM