NUTRITION – CARBOHYDRATE, NITROGEN, LIPID STUDIES

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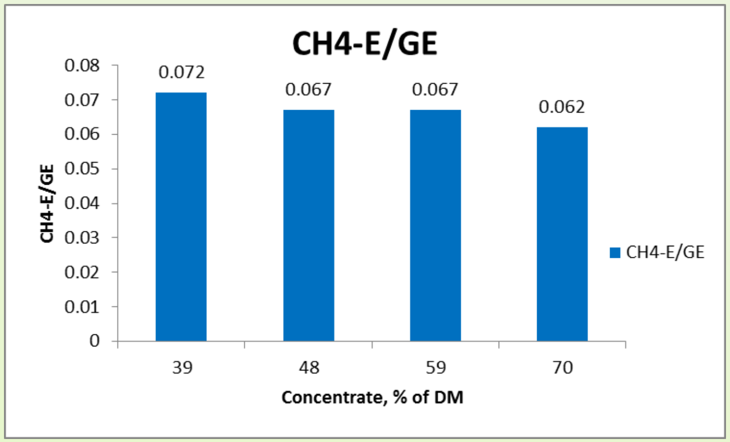
RUMINOMICS

CARBOHYDRATES

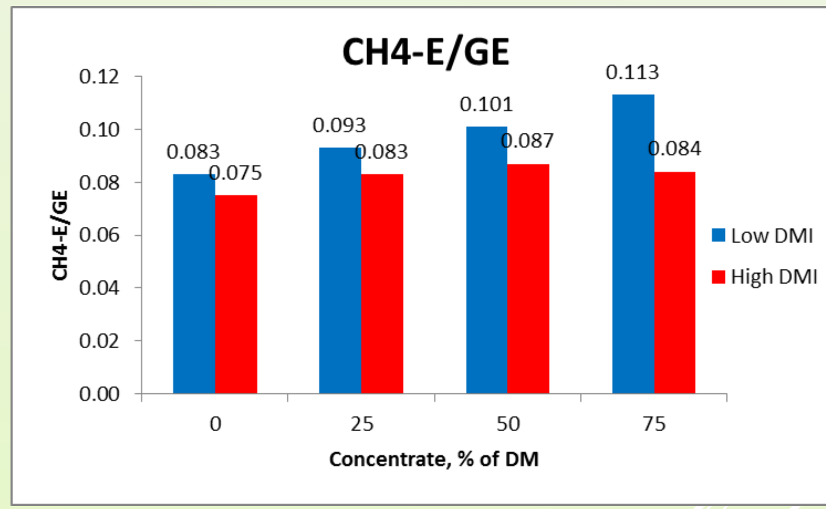
- A common assumption is that high concentrate diets produce less methane than high forage diets
- The effects are clearly evident for feed-lot diets (≥90% concentrates that produce 2 – 3% CH_4 of GE
- For the range of dairy cow diets (0 – 60% concentrates) effects not very large per unit of intake but per unit of products high concentrate diets may produce less CH_4
- Grain can be used directly as human food (a lot of wheat fed to dairy cows) or more efficiently to pigs and poultry with much less CH_4

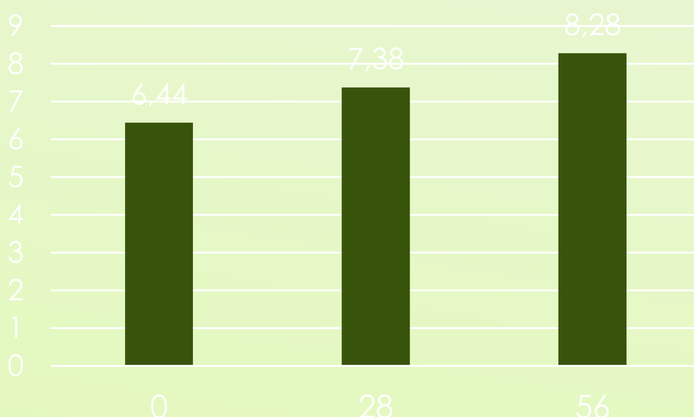
EFFECT OF PROPORTION OF CONCENTRATE ON CH_4 EMISSIONS IN DAIRY COWS, GROWING CATTLE AND SHEEP



Ferris et al., 1999

Moss et al. 1995





Beever et al. 1988

CHARACTERISING THE IMPACT OF DIETARY CARBOHYDRATE ON THE COMPOSITION AND FUNCTION OF THE RUMEN MICROBIOME IN LACTATING COWS.

- Early-cut high D silage was gradually (0, 33, 67, 100%) replaced with low D silage + barley
- Diets formulated to produce same amount of milk
- 4 x 4 production study with intake, production and gas production (CH_4 and CO_2) with 20 cows
- 4 x 4 flow study using omasal (and reticular) sampling method, triple marker system and ^{15}N as microbial marker
    - Ruminal and total digestion of nutrients
    - Rumen fermentation and microbiome

DIET FORMULATION

	Diet[1]			
	L	LE	EL	E
LC Silage	421	329	189	0
EC Silage	0	164	378	641
Forage total	421	494	567	641
Barley	472	404	335	266
Rapeseed expeller	97	93	89	84
NaCl	3.2	3.2	3.1	3.1
$CaCO_3$	6.6	6.2	5.8	5.3

[1]Diet LC silage: EC silage: L = 100:0, LE = 67:33; EL = 33:67, E = 0:100.

RUMEN FERMENTATION

	Diet[1]					P-value	
	L	LE	EL	E	SEM	Lin	Quadr.
pH	6.04	6.15	6.05	6.15	0.083	0.13	0.70
Ammonia N, mg/L	32.3	42.0	46.1	46.1	4.83	0.08	0.35
Total VFA, mmol/L	109	103	108	103	4.3	0.27	0.71
Molar proportion, mmol/mol							
Acetate (C_2)	684	688	686	687	6.4	0.82	0.80
Propionate (C_3)	167	170	181	178	5.9	0.14	0.57
Butyrate (C_4)	106	98	94	98	5.6	0.14	0.20
Isovalerate	30.5	30.6	26.8	27.7	0.70	0.01	0.60
Valerate	12.5	12.8	11.4	10.1	1.38	0.03	0.28
VFA ratios							
C_2 / C_3	4.10	4.06	3.81	3.87	0.148	0.19	0.76
$(C_2 + C_4) / C_3$	4.73	4.65	4.34	4.42	0.175	0.15	0.64
CH_4VFA[2]	0.369	0.367	0.359	0.362	0.0046	0.17	0.56

TOTAL DIGESTIBILITY

	Diet[1]				SEM	P-value	
	L	LE	EL	E		Lin	Quadr.
Digestibility, g/kg							
DM	672	668	685	715	8.4	<0.01	0.09
OM	681	677	692	722	8.5	<0.01	0.10
CP	651	638	685	708	12.8	<0.01	0.22
NDF	484	502	584	647	13.4	<0.01	0.14
pdNDF	654	659	737	787	17.1	<0.01	0.23
NDS[2]	806	801	777	785	8.7	0.07	0.47
MFOM[3], g/kg DMI	112	110	116	106	4.8	0.66	0.43
DNDF/DOM[4], g/kg	276	307	372	419	8.1	<0.01	0.36

NDF DIGESTION

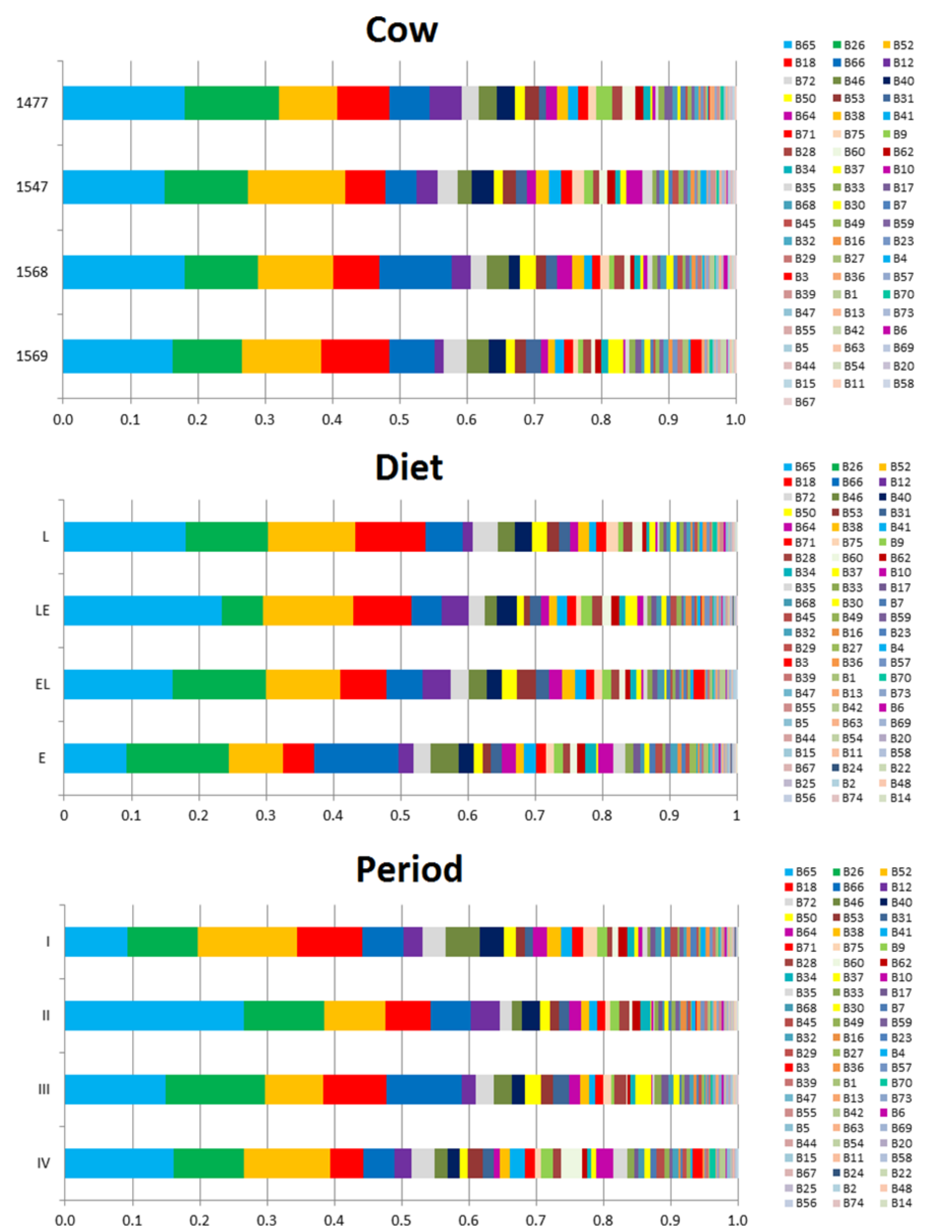
	Diet[1]				SEM	P-value	
	L	LE	EL	E		Lin	Quadr.
NDF, kg/d							
Intake	7.37	7.43	7.84	7.72	0.306	0.12	0.62
Flow into omasum	4.32	4.00	3.66	3.06	0.102	<0.01	0.10
Faecal output	3.78	3.70	3.25	2.73	0.156	<0.01	0.13
Digestibility, g/kg							
NDF rumen	410	458	531	602	14.8	<0.01	0.35
pdNDF rumen	554	601	671	732	18.7	<0.01	0.62
NDF total	484	502	584	647	13.4	<0.01	0.14
pdNDF total	654	659	737	787	17.1	<0.01	0.23
NDF rumen / total	845	911	909	932	26.0	0.04	0.37

[1]Diet LC silage: EC silage: L = 100:0, LE = 67:33; EL = 33:67, E = 0:100.

N DIGESTION

	Diet[1]				SEM	P-value	
	L	LE	EL	E		Lin	Quadr.
Bacteria (B)	0.0578	0.0543	0.0545	0.0483	0.00231	0.03	0.57
Large particles (LP)	0.0287	0.0292	0.0290	0.0251	0.00174	0.06	0.09
Small paricles (SP)	0.0352	0.0354	0.0341	0.0309	0.00265	0.24	0.51
Fluid	0.0540	0.0516	0.0523	0.0459	0.00219	0.05	0.39
Ratio							
LP : B	0.497	0.537	0.531	0.520	0.0255	0.50	0.26
SP : B	0.609	0.651	0.625	0.642	0.0428	0.72	0.78
Nitrogen, g/d							
Intake	498	495	516	514	24.2	0.39	0.98
Omasal flow							
Total N	503	522	420	433	32.6	0.05	0.93
Microbial N	326	365	282	302	26.8	0.21	0.71
Feed N[2]	177	156	138	131	17.7	0.09	0.71
Faeces	172	178	162	148	10.4	0.08	0.35
MN, g/kg OMTDR	28.6	32.2	23.7	26.2	1.91	0.12	0.76
Ruminal N degr., g/kg	642	682	727	745	28.2	0.03	0.69
N digestibility, g/kg	651	638	685	708	12.8	<0.01	0.22

[1]Diet LC silage: EC silage: L = 100:0, LE = 67:33; EL = 33:67, E = 0:100



Cow
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1569
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
B65 B26 B52 B18 B66 B12 B72 B46 B40 B50 B53 B31 B64 B38 B41 B71 B75 B9 B28 B60 B62 B34 B37 B10 B35 B33 B17 B68 B30 B7 B45 B49 B59 B32 B16 B23 B29 B27 B4 B3 B36 B57 B39 B1 B70 B47 B13 B73 B55 B42 B6 B5 B63 B69 B44 B54 B20 B15 B11 B58 B67
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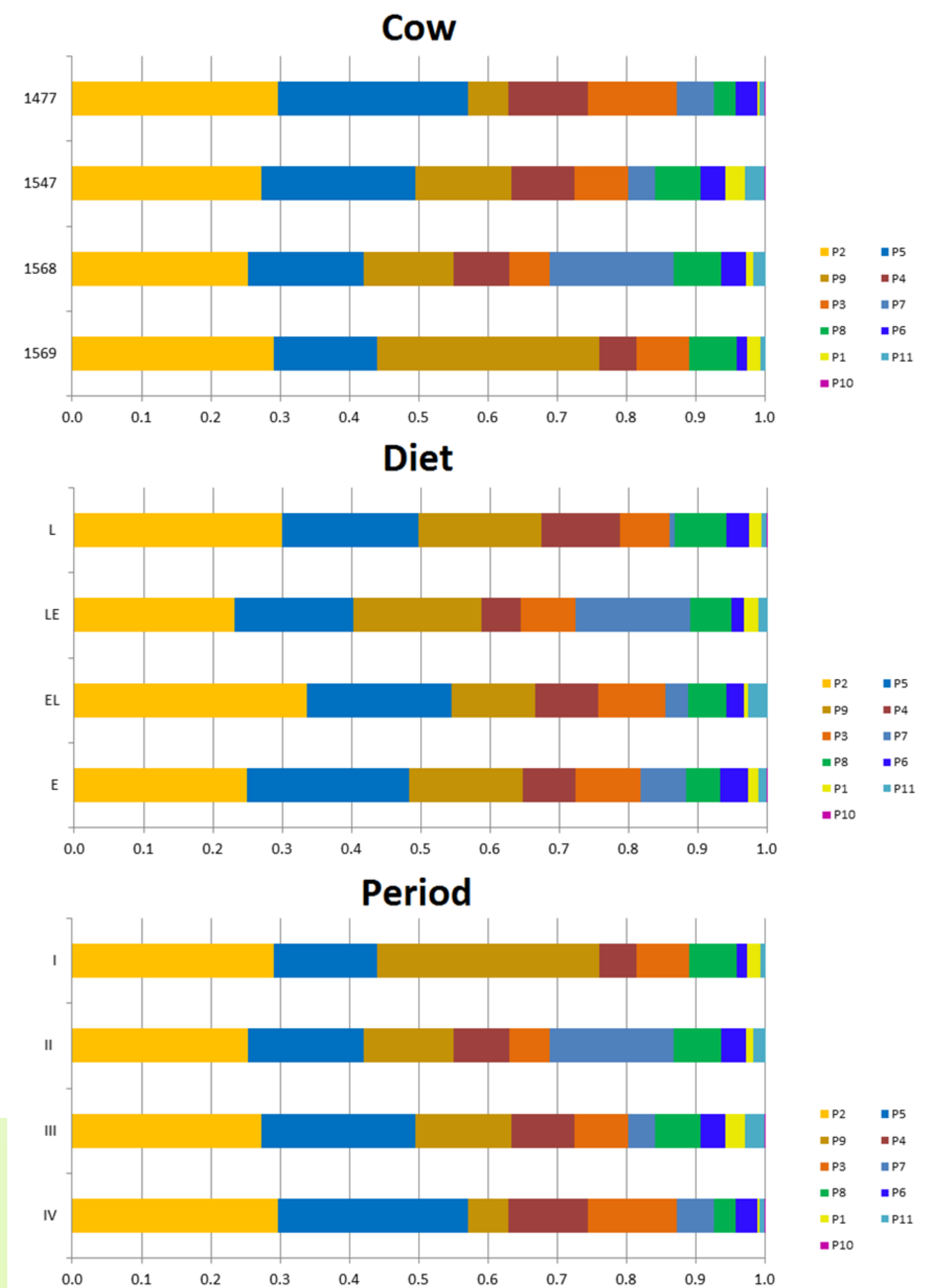

Cow
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0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
P2 P5 P9 P4 P3 P7 P8 P6 P1 P11 P10

CHEMICAL COMPOSITION OF DIETS IN DAIRY COW TRIAL (N = 16)

	Diet[1]			
	L	LE	EL	E
In DM[2], g/kg				
OM	948	945	941	937
CP	157	164	171	180
EE	31.3	32.8	34.3	36.0
NDF	363	380	399	414
iNDF	87.7	84.7	80.2	72.7
pdNDF	275	296	319	341
NFC	397	368	337	307
Starch	251	224	195	170
Feed values[3]				
MP	95.8	96.3	96.9	98.2
PBV	14.5	20.3	26.7	33.9
ME (MJ/kg DM)	11.6	11.6	11.7	11.8

[1]Diet LC silage: EC silage: L = 100:0, LE = 67:33; EL = 33:67, E = 0:100.

INTAKE AND MILK PRODUCTION

	Diet[1]					P-value	
	L	LE	EL	E	SEM	Lin	Quadr.
Intake[2], kg/d							
DM	22.8	20.9	20.4	19.5	0.57	<0.01	0.17
CP	3.75	3.58	3.64	3.64	0.1	0.36	0.20
NDF	8.28	7.99	8.14	8.07	0.26	0.43	0.42
Starch	5.71	4.65	3.98	3.31	0.11	<0.01	0.03
ME, MJ/d	265	244	238	230	6.49	<0.01	0.11
MP	2.18	2.01	1.98	1.91	0.05	<0.01	0.12
PBV	0.33	0.43	0.55	0.67	0.02	<0.01	0.52
Production, kg/d							
Milk	28.5	28.1	27.9	27.7	1.23	0.16	0.80
ECM[3]	30.0	29.7	29.7	29.7	1.17	0.55	0.72
Composition, g/kg							
Fat	43.0	43.4	43.9	44.7	0.91	0.05	0.74
Protein	37.0	36.7	36.2	36.3	0.63	0.05	0.43
Lactose	46.4	46.8	46.9	46.8	0.60	0.15	0.17
Milk urea, mmol/L	3.46	3.59	3.97	4.25	0.19	<0.01	0.34
Yield, g/d							
Fat	1216	1209	1221	1229	50.2	0.60	0.73
Protein	1047	1022	1001	996	37.2	0.01	0.45
Lactose	1318	1312	1305	1292	55.9	0.32	0.84
Efficiency							
ECM/DMI, kg/kg	1.32	1.43	1.46	1.52	0.05	<0.01	0.40
Milk N/N intake, g/kg	293	299	288	285	9.6	0.11	0.37
BW[4], kg	631	633	632	629	17.9	0.74	0.51

CH_4 AND CO_2 DATA

	Diet[1]					P-value	
	L	LE	EL	E	SEM	Lin	Quadr.
CH_4, g/d	440	441	446	444	14.9	0.69	0.92
CO_2, g/d	13271	13012	13152	12949	297	0.24	0.85
CH_4/CO_2, g/kg	33.2	33.7	33.8	34.2	0.67	0.15	0.93
CH_4, g/kg DMI	19.4	21.1	21.9	22.7	0.67	<0.01	0.26
CH_4, g/kg ECM	14.8	15.0	15.2	15.2	0.63	0.44	0.89
CO_2, g/kg DMI	583	626	649	663	17.4	<0.01	0.10
CO_2/ECM, g/kg	448	444	450	444	17.5	0.86	0.95
N excess[2], g/kg ECM	13.8	13.2	13.7	14.0	0.68	0.53	0.21

[1]Diet LC silage: EC silage: L = 100:0, LE = 67:33; EL = 33:67, E = 0:100.

[2] Calculated as [N intake (g/d) – Milk N yield (g/d)] / ECM yield (kg/d).

CONCLUSIONS FROM CHO STUDY

- No differences in production – consistent with diet formulation
- The amount of concentrate can be decreased without decreases production or increases in CH_4 by improving forage quality
- High quality silage improved apparent feed efficiency
- CH_4/DMI increased with the proportion of EC silage
  - Higher digestibility
  - Lower DMI
  - Lower efficiency of microbial cell synthesis
  - Differences in fermentable substrate (pdNDF vs. starch)
- No consistent differences in microbial population

THE EFFECTS OF DIETARY PROTEIN CONTENT AND COMPOSITION ON THE COMPOSITION AND FUNCTION OF THE RUMEN MICROBIOME IN LACTATING COWS.

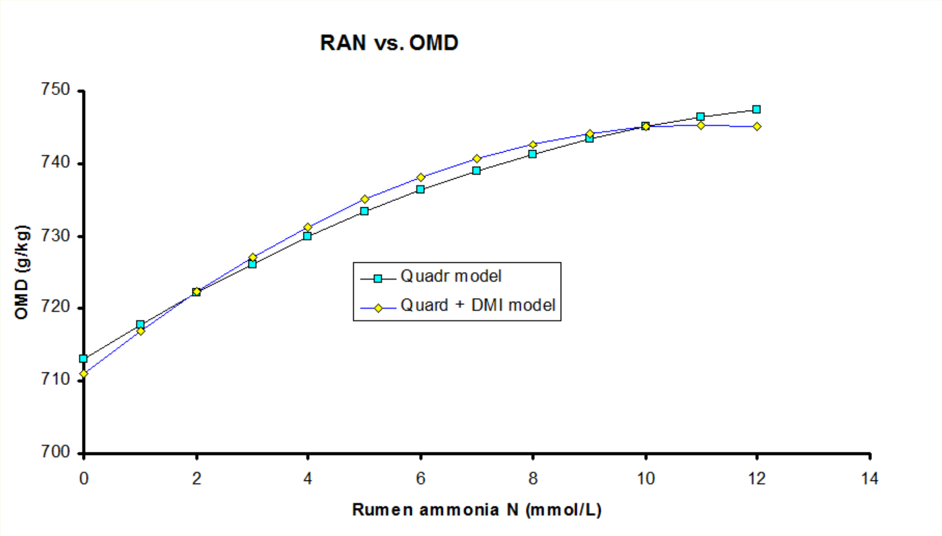
- Meta-analysis of between-animal variation in rumen ammonia N and milk urea concentrations, and associations to the efficiency of N utilization.
- 4 x 4 flow study using omasal (and reticular) sampling method, triple marker system and ^{15}N as microbial marker
- Barley replaced with heat-treated rapeseed meal
- Methane and CO_2 measurements with the same protein treatments in production study with 28 cows.

CONCLUSIONS OF META-ANALYSIS

- Between cow CV in rumen ammonia N (RAN) and milk urea N (MUN) 0.11 – 0.14 when the effects of diet and period removed
- MUN and RAN negatively related to milk N efficiency (MNE)
- The effects per unit of MUN much smaller compared to variation resulting from diet manipulation
- MUN + RAN explained variation in MNE better than MUN or RAN alone
- RAN and MUN positively related to diet digestibility

RESULTS / CONCLUSIONS

- RAN (flow dataset) and MUN (production dataset) positively related digestibility
- No effect of RAN on efficiency of MPS or flow of microbial N
- The effect can be related to rumen protozoa; negative($P < 0.01$) relationship between RAN and Prop/But ratio in rumen VFA
- Are protozoa more useful than harmful in dairy cows fed at high DMI, when intraruminal recycling of N less important than in animals fed at M



DESIGN & DIETS

- Latin Square design with 4 cows in 4 periods

Composition of experimental diets

	Diet			
	B	RL	RM	RH
Ingredient proportions, g/kg DM				
Silage	544	549	561	552
Barley	441	357	271	198
Expro RSM	0	79	154	235
NaCl	3	3	3	3
Minerals	11	11	11	11
Chemical composition, g/kg DM				
DM, g/kg	411	427	440	465
OM	929	924	920	915
CP	133	149	165	181
Fat	21	26	30	36
Starch	247	204	160	125

N DIGESTION

	Diet[1]				SEM	*P* -value[2]
	C	CL	CM	CH		Linear
Intake, g/d						
N	319	367	431	481	10	<0.01
Omasal flow, g/d						
NAN	370	402	422	438	17.6	0.03
NANMN[3]	83	128	160	193	4.5	<0.01
Microbial NAN	**286**	**274**	**262**	**244**	**16.2**	**0.10**
Rumen digestibility, g/k	740	647	630	594	17.2	<0.01
True NAN						
Total tract digestibility,	649	673	714	730	12.5	<0.01
Apperent N						
Microbial 15N enrichme	0.058	0.056	0.046	0.049	0.012	<0.01
Microbial efficiency,						
g NAN/kg OMTDR[6]	**29.3**	**29.5**	**26.5**	**24.1**	**1.81**	**0.06**

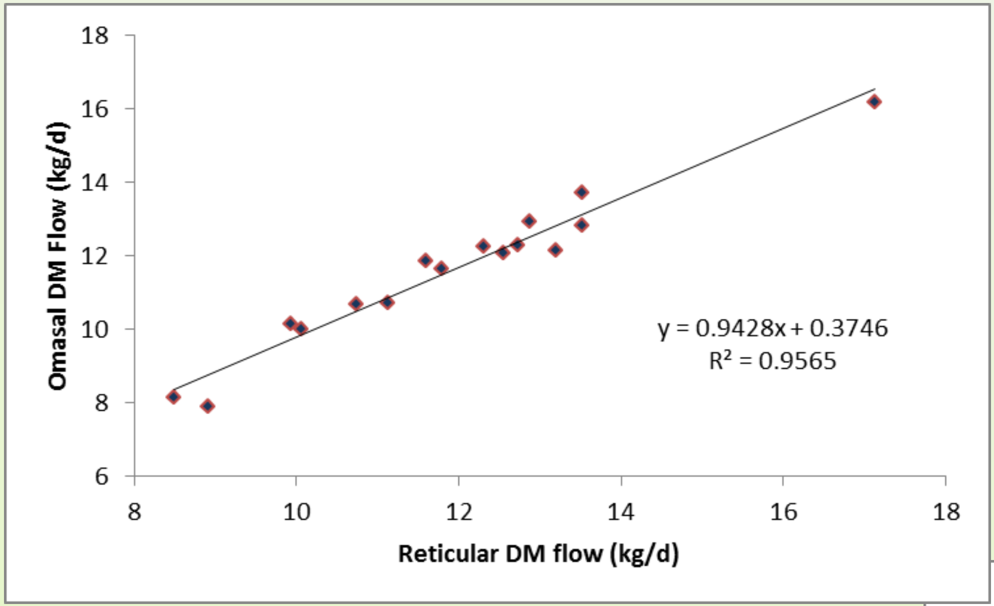
METHANE EMISSIONS

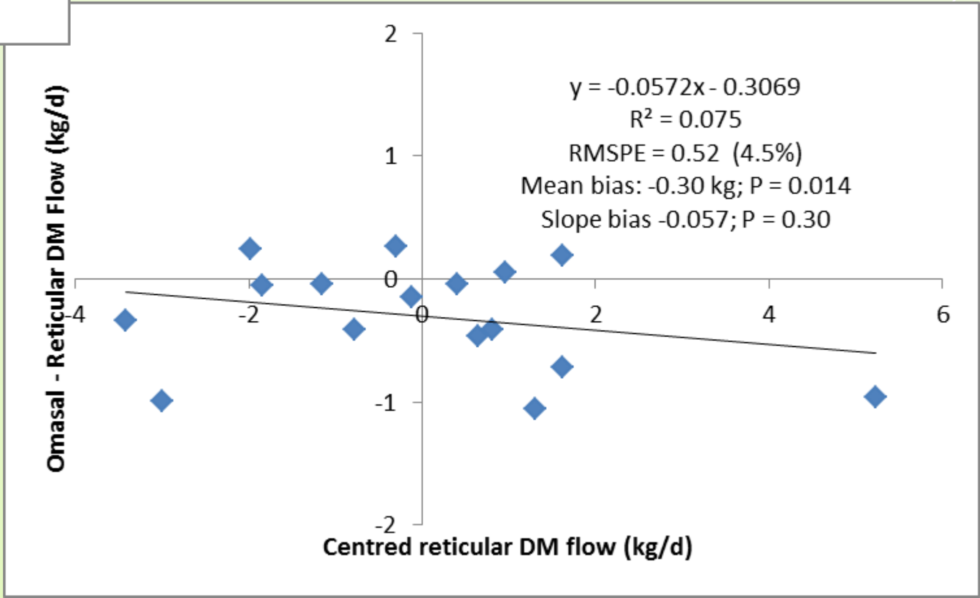
	Control	Rapeseed meal			
		Low	Medium	High	SEM
CH_4					
g/d	463	458	447	456	17.0
g/kg DMI	24.1	22.6	22.6	22.5	0.63
g/kg ECM	17.5	16.9	16.1	15.8	0.71
CH_4 / CO_2, g/kg	41	40.7	39	38.8	0.82

OMASAL VS. RETICULAR SAMPLING

- Ruminal / reticular / omasal sampling compared
- Digesta sampled from reticulum was sieved trough 10 mm screen
  - Resulted in similar particulate matter distribution to omasal digesta and faeces
- Good relationship in DM flow between reticular and omasal sampling ($R^2 = 0.96$); rumen not as good
- N flow could be overestimated with reticular sampling
  - Selective retention of protozoa
  - Accumulation of feed protein particles in reticulum?

RETICULAR VS. OMASAL DM FLOW





RUMEN MICROBIOME DATA

Table 7. Effect on the relative abundance (%) for prevalent bacterial species in the rumen, between diets with increasing supplementation of heat-moisture treated canola meal.

		Diet[1]				SEM	*P*-value[2]	
		C	CL	CM	CH		Linear	Quadratic
B1	Acholeplasma laidlawii	1.18	0.00	0.10	0.00	0.606	0.25	0.41
B2	Acinetobacter radioresistens	3.35	0.00	0.00	0.00	1.675	0.23	0.36
B3	Acinetobacter sp. TS25	0.40	0.00	0.00	0.00	0.200	0.23	0.36
B4	Anaerosinus glycerini	0.28	0.18	0.00	0.00	0.186	0.27	0.80
B5	Anaerosporobacter mobilis	1.18	0.83	0.73	0.55	0.690	0.55	0.90
B6	Anaerovibrio lipolyticus	0.00	0.13	0.00	0.00	0.063	0.67	0.36
B7	Anaerovibrio sp. RM50	0.00	0.00	0.00	0.13	0.063	0.23	0.36
B8	Bacillus beijingensis	0.00	0.00	0.00	0.28	0.138	0.23	0.36
B9	Bacillus sp. 01082	0.28	0.00	0.00	0.00	0.138	0.23	0.36
B10	Bacillus sp. ABPL 106	0.00	1.85	5.60	5.55	1.749	0.04	0.61
B11	Bacillus sp. EET103Mm 30.10	1.65	5.38	1.90	2.40	1.587	0.87	0.35
B12	Bacillus sp. MHS022	0.00	0.00	0.40	0.00	0.200	0.67	0.36
B13	Bacillus sp. P-149	0.30	0.00	0.00	0.25	0.160	0.84	0.14
B14	Bacillus sp. SGE126(2010)	1.28	0.83	0.10	0.00	0.438	0.06	0.70
B15	Bifidobacterium pseudolongum	0.28	0.00	0.00	0.00	0.138	0.23	0.36
B16	Butyrivibrio fibrisolvens	0.00	0.90	0.65	0.73	0.364	0.28	0.30
B17	Butyrivibrio hungatei	0.00	0.00	0.13	0.00	0.063	0.67	0.36
B18	Campylobacter hyointestinalis subsp. hyointestinalis	0.00	0.50	0.00	0.00	0.250	0.67	0.36
B19	Candidatus Clostridium timonensis	0.28	0.00	0.00	0.13	0.169	0.57	0.28
B20	Clostridium aminophilum	0.00	0.13	0.13	0.00	0.072	1.00	0.13
B21	Clostridium celerecrescens	0.58	0.18	0.00	0.00	0.271	0.17	0.49
B22	Clostridium sp.	0.00	0.55	0.25	0.00	0.261	0.81	0.18
B23	Clostridium sp. 619	0.00	0.55	0.00	0.00	0.275	0.67	0.36
B24	Clostridium sp. Clone-17	0.00	0.00	0.25	0.00	0.125	0.67	0.36
B25	Clostridium sp. IrT-JG1-67	0.00	0.18	0.00	0.00	0.088	0.67	0.36
B26	Clostridium sp. K8	0.00	0.18	0.00	0.00	0.088	0.67	0.36
B27	Clostridium sp. YIT 12069	0.10	0.13	0.13	0.00	0.060	0.30	0.25
B28	Comamonas sp. P3-1	0.00	1.55	5.73	6.78	2.617	0.08	0.93
B29	Desemzia incerta	1.08	0.00	0.00	0.00	0.538	0.23	0.36

No consistent diet effects – even less $P < 0.05$ differences than could be expected by random…

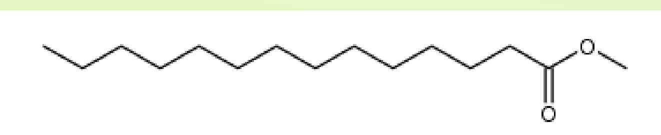
CONCLUSIONS – PROTEIN EFFECTS

- Optimal protein supplementation can slightly decrease CH_4/DMI
  - Protein fermentation produce less CH_4 than CHO fermentation
  - Increased DMI → less CH_4 per kg DMI
- CH_4 per unit of product decrease more due to increased production
- BUT N emissions per unit of product increase; depending how much of manure N is converted N_2O overall GHG may not change + still increased N emissions
- Reduced ruminal protein degradability can decrease the efficiency of microbial N synthesis → overall benefits of reducing ruminal protein degradability less than expected

Experimental objectives

To examine the influence of supplementing grass silage based diets with myristic acid, rapeseed, safflower or linseed oils on animal performance, ruminal CH_4 emissions, nutrient digestion, milk fatty acid composition and rumen microbiome.

Myristic acid (14:0)
methyl ester



Rapeseed
18:1

Safflower
18:2

Linseed
18:3

Table 1. Effect of dietary lipid supplements on DM intake and **milk production**

	Treatment					SEM	*P*-value
	Control	Myristic acid	Rapeseed oil	Safflower oil	Linseed oil		
DM Intake (kg/d)	21.8^{a}	**15.0^{c}**	19.1^{b}	20.4^{b}	20.1^{b}	0.96	**< 0.01**
Yield							
Milk (kg/d)	28.7^{a}	**22.1^{b}**	29.0^{a}	29.6^{a}	28.6^{a}	2.97	**< 0.01**
Fat (g/d)	1131^{ab}	**1014^{b}**	1213^{a}	1162^{a}	1213^{a}	107.8	**< 0.05**
Protein (g/d)	977^{a}	**682^{b}**	949^{a}	976^{a}	950^{a}	87.3	**< 0.01**
Lactose (g/d)	1290^{a}	**929^{b}**	1291^{a}	1331^{a}	1291^{a}	137.1	**< 0.01**
Concentration (g/kg)							
Fat	39.2^{c}	**46.7^{a}**	41.8^{bc}	39.3^{c}	**42.8^{b}**	1.29	**< 0.01**
Protein	34.3^{a}	**31.1^{b}**	33.1^{a}	33.1^{a}	33.4^{a}	0.64	**< 0.01**
Lactose	45.1^{a}	**41.7^{b}**	44.2^{a}	44.8^{a}	45.0^{a}	0.68	**< 0.01**

$^{a-c}$ Within a row means without a common superscripts differ ($P < 0.05$).

NO EFFECT ON RUMEN FERMENTATION CHARACTERISTICS OR APPARENT DIGESTIBILITY OF NUTRIENTS

Table 2. Effect of dietary lipid supplements on **rumen methane production**

	Treatment						
	Control	Myristic acid	Rapeseed oil	Safflower oil	Linseed oil	SEM	*P*-value
Methane							
g/d	614[a]	403[c]	475[b]	488[b]	484[b]	34.8	< 0.01
g/kg OM intake	29.5[a]	28.3[c]	26.3[b]	25.2[b]	25.4[b]	1.82	0.09
g/kg milk yield	22.5[a]	19.5[ab]	17.6[b]	17.2[b]	18.0[b]	2.62	< 0.05
% of GE intake	8.09[a]	7.38[b]	6.82[b]	6.56[b]	6.61[b]	0.475	< 0.05

Table 3. Effect of dietary lipid supplements on **milk fatty acid composition** of lactating cows

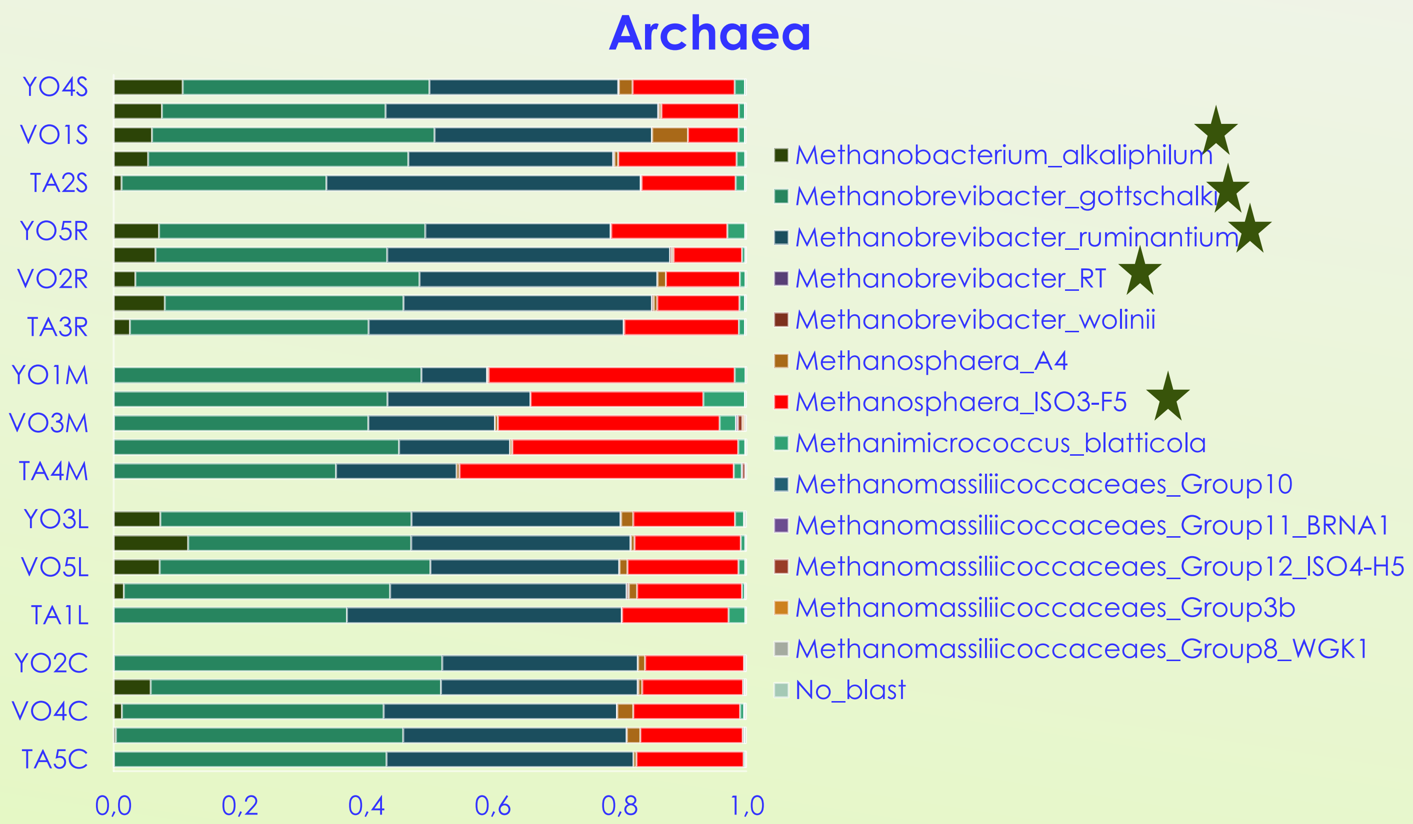
Fatty acid (g/100 g FA)	Treatment					SEM	*P*-value
	Control	Myristic acid	Rapeseed oil	Safflower oil	Linseed oil		
C4-C14	27.7^{b}	**44.2^{a}**	**18.3^{c}**	**18.1^{c}**	**18.8^{c}**	1.01	< 0.01
14:0	12.9^{b}	**31.7^{a}**	**8.51^{c}**	**8.17^{c}**	**8.28^{c}**	0.837	< 0.01
trans-11 18:1	1.39^{cd}	0.97^{d}	1.77^{c}	**3.66^{a}**	**2.43^{b}**	0.269	< 0.01
Σ 18:1	20.5^{b}	20.0^{b}	**36.3^{a}**	**36.5^{a}**	**35.0^{a}**	1.02	< 0.01
Σ 18:2	1.99^{cd}	1.87^{d}	2.19^{c}	**2.62^{b}**	**3.65^{a}**	0.139	< 0.01
Σ CLA	0.738^{cd}	0.611^{d}	0.892^{c}	**1.66^{a}**	**1.23^{b}**	0.109	< 0.01
Σ *trans* FA	5.00^{c}	5.04^{c}	**8.48^{b}**	**11.4^{a}**	**11.8^{a}**	0.46	< 0.01
Σ SFA	70.4^{a}	69.0^{a}	**55.7^{b}**	**54.5^{b}**	**55.1^{b}**	1.32	< 0.01
Σ MUFA	25.6^{b}	27.7^{b}	**40.3^{a}**	**40.4^{a}**	**38.7^{a}**	1.11	< 0.01
Σ PUFA	3.72^{c}	3.08^{d}	3.77^{c}	**4.92^{b}**	**5.92^{a}**	0.259	< 0.01

Fig 7. Distribution of rumen bacteria (OTUs were assigned using the Greengenes 12_10 database)

Bacteria

B10	p_Bacteroidetes	
B12	o_Bacteroidales_1	
B13	o_Bacteroidales_2	B
B14	o_Bacteroidales_f_BS11	
B15	f_Bacteroidaceae	
B16	f_Porphyromonadaceae	
B18	o_Bacteroidales_f_S24-7	F
B28	f_Fibrobacteraceae	
B34	f_Lactobacillaceae	
B62	p_Proteobacteria	P
B65	c_Alphaproteobacteria_o_RF32	
B70	f_Alcaligenaceae	
B79	c_Gammaproteobacteria	
B81	f_Succinivibrionaceae	
B89	p_Spirochaetes_o_PL-11B10	
B99	p_TM7_f_F16	
B104	f_Anaeroplasmataceae	

Fig 8. Distribution of rumen archaea
(OTUs were assigned using RIM-DB database (Seedorf et al., 2014))



GENERAL CONCLUSIONS

- Potential to reduce CH_4 emissions by nutrition rather limited
- Increased proportion of concentrates
  - Increased costs, health problems
  - Grain can be used as human food or more efficiently by simple-stomached animals with much smaller CH_4 emissions
  - Overall effects on GHG?
- Economically optimal fat supplementation
  - Above optimum increased feed costs, reduced intake and fibre digestibility, reduced milk protein content
- Increased protein supplementation may decrease CH_4 per unit of product, but increase N emissions
  - Increased feed costs
  - Is it ethical to feed high quality protein with 10% marginal efficiency to dairy cows