

EAAP 2014
Copenhagen, Denmark
25 - 29 August 2014

65th annual meeting of the European Federation of Animal Science



***In vitro* incubation of dairy cow diets:**

2. Relations between measures and estimates of methane yield

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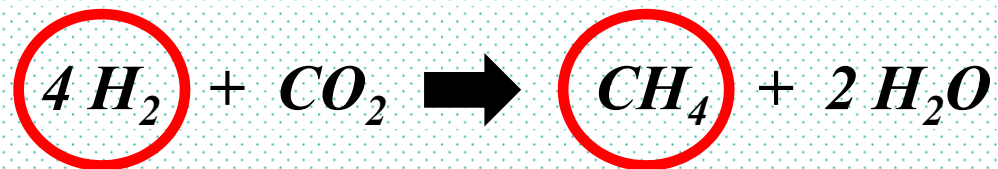
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Background

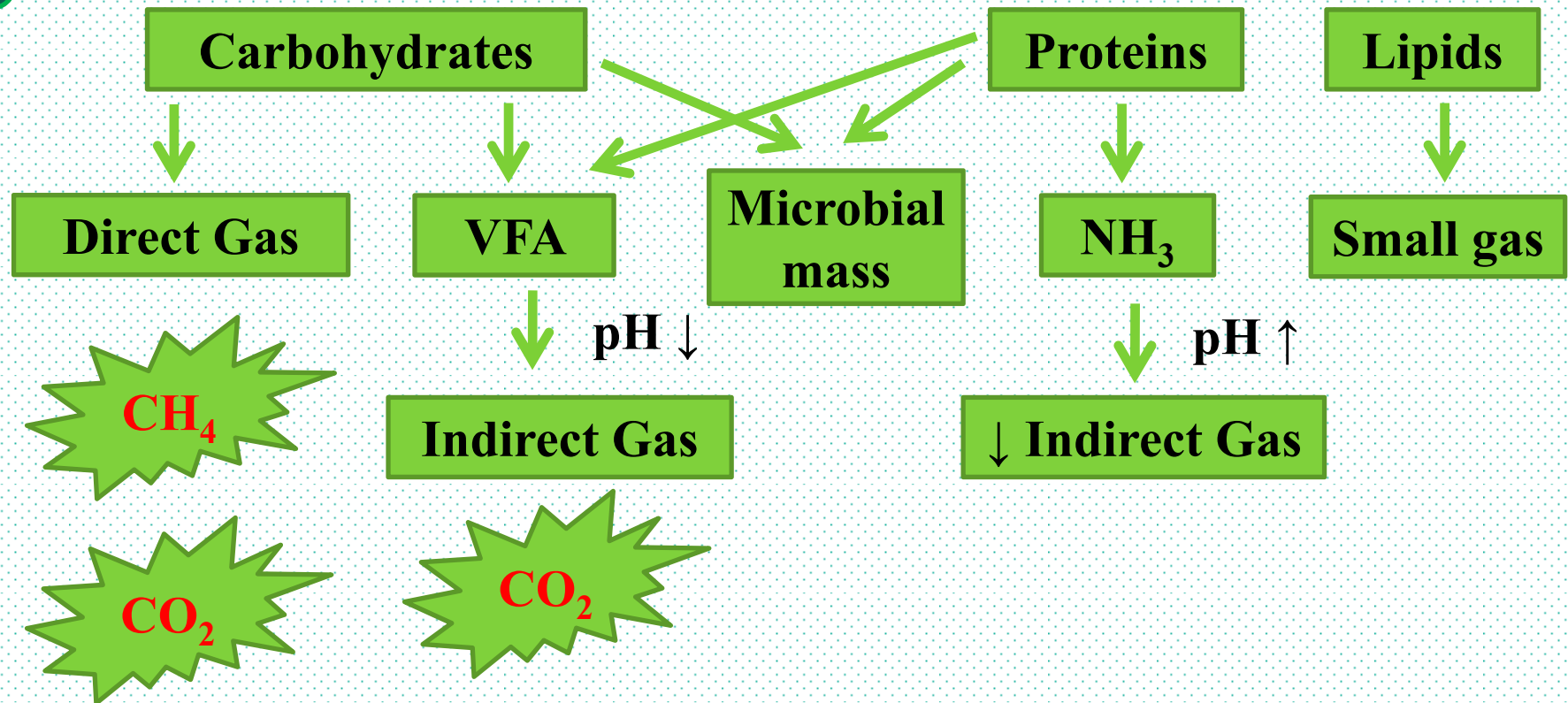
When single feeds were fermented *in vitro* for 48 h, methane (CH_4) and volatile fatty acids (VFA) productions were closely related (Blümmel *et al.*, 1999)

Molar proportions of main VFA (acetate, propionate, and n-butyrate) influence H_2 formation in the rumen



In vitro CH_4 and total gas production (GP) could be predicted from VFA production

On equal in vitro degradability carbon fixed in gas, VFA and microbial mass can greatly change



In vitro systems are NOT dynamic

When feed sample is incubated for long times

Digestible fraction is completely fermented

Status of energy deficiency occurs

Microbial lysis starts

↓
↑ GAS

↓
NH₃

→

↓ GAS

COMPLEX EFFECTS TOTALLY INDEPENDENT
FROM FEED FERMENTATION

Aim

AIM

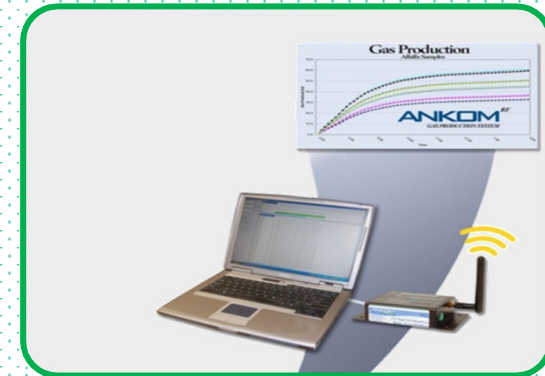
To evaluate relations between values of GP and CH₄

1. **Actually measured *in vitro***
2. **Predicted from *in vitro* molar proportions of VFA**

Diets

- ✓ A **Reference Diet (RD)** → representative of dairy cow diets commonly used in Veneto region (North-East of Italy)
- ✓ Other 7 diets were formulated changing the proportions of CP, NDF and lipids, within the limits of viable:
 - ~ Low protein
 - ~ High protein
 - ~ Low fibre
 - ~ High fibre
 - ~ Low lipid
 - ~ High lipid, fat supplement (calcium soaps of palm)
 - ~ High lipid, extruded oilseeds

Measurement of gas production and CH₄



MATERIAL &
METHODS



**Runs were carried out as described
in the previous presentation**

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Predicted values of GP and CH₄ from VFA

DIRECT GAS, *mmoL/g DM*

$$\text{CO}_2 \text{ ferm} = \text{acet}/2 + \text{prop}/4 + 1.5 \times \text{n-but}$$

$$\text{CH}_4 \text{ ferm} = \text{acet} + 2 \times \text{n-but} - \text{CO}_2 \text{ ferm}$$

INDIRECT GAS, *mmoL/g DM*

$$\text{CO}_2 \text{ buff} = \text{acet} + \text{prop} + \text{n-but} + \text{iso-but}$$

TOTAL GAS PRUDUCTION, *mL/g DM*

$$(\text{CO}_2 \text{ ferm} + \text{CO}_2 \text{ buff} + \text{CH}_4 \text{ ferm} + \text{CO}_2 \text{ iso-but}) \times 0.0821 \times 312$$

0.0821 = gas constant

312 = temperature (expressed in Kelvin)

All equations are derived from Blümmel *et al.* (1999)

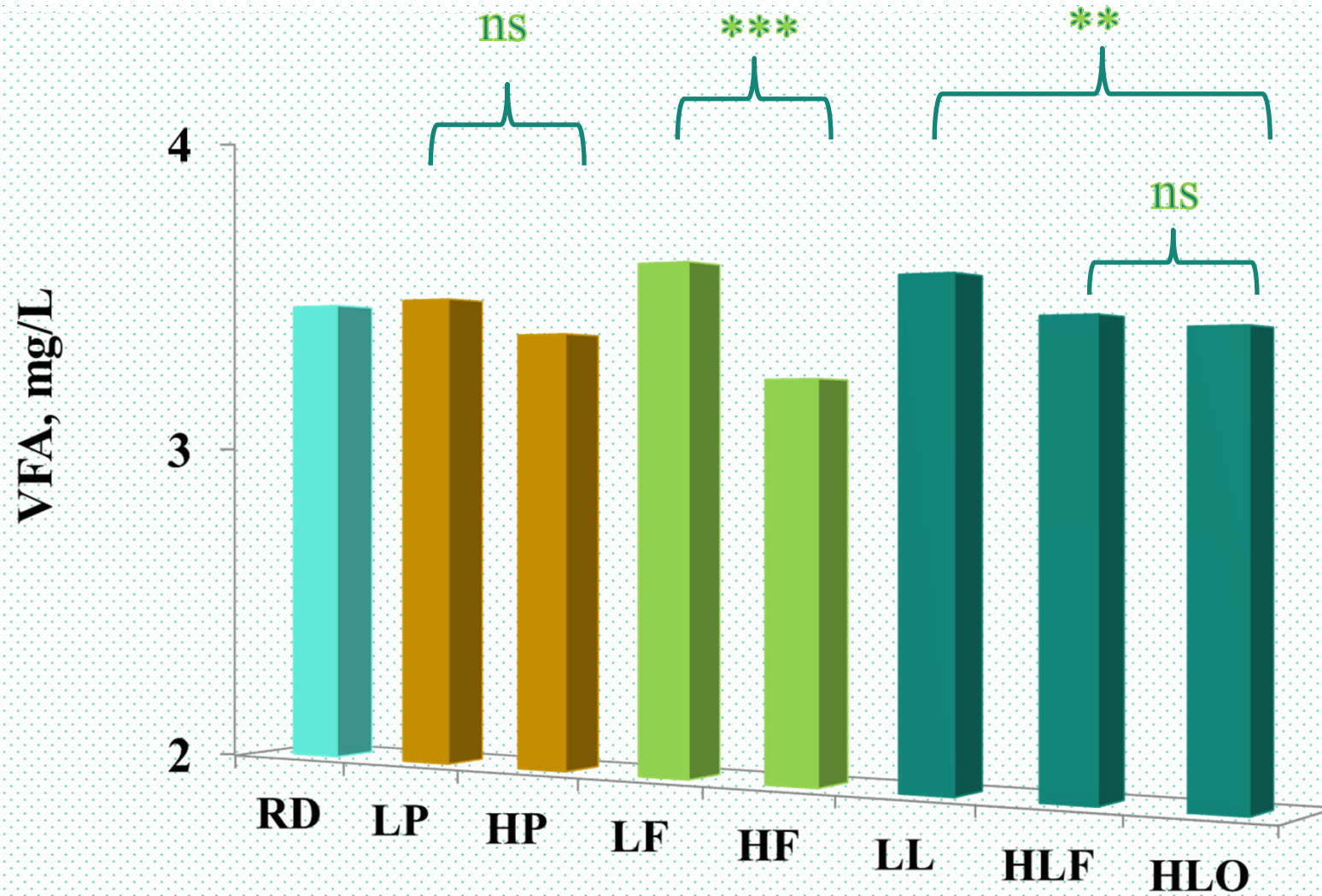
Statistical analysis

All data were submitted to ANOVA (SAS, 2007) using a model that considered as sources of variation:

- 1) *Diet*
- 2) *Incubation time*
- 3) *Run within incubation time*

Interaction between diet and incubation time was never significant ($P > 0.05$), thus it was removed from the model

VFA production of eight diets



VFA production of eight diets

	Acet (%)	Prop (%)	n-but (%)
Diet			
Reference diet	56.3	23.4	14.6
Low protein	56.0	24.3	14.9
High protein	57.1	22.8	13.9
Low fibre	55.9	23.6	14.9
High fibre	58.5	23.1	12.8
Low lipid	56.5	23.8	14.3
High lipid, fat	56.6	23.7	14.1
High lipid, oilseeds	56.6	23.4	14.3
SEM	0.15	0.19	0.15
P values			
Diet	<0.001	<0.001	<0.001

RESULTS &
DISCUSSION

Predicted and measured gas production of eight diets

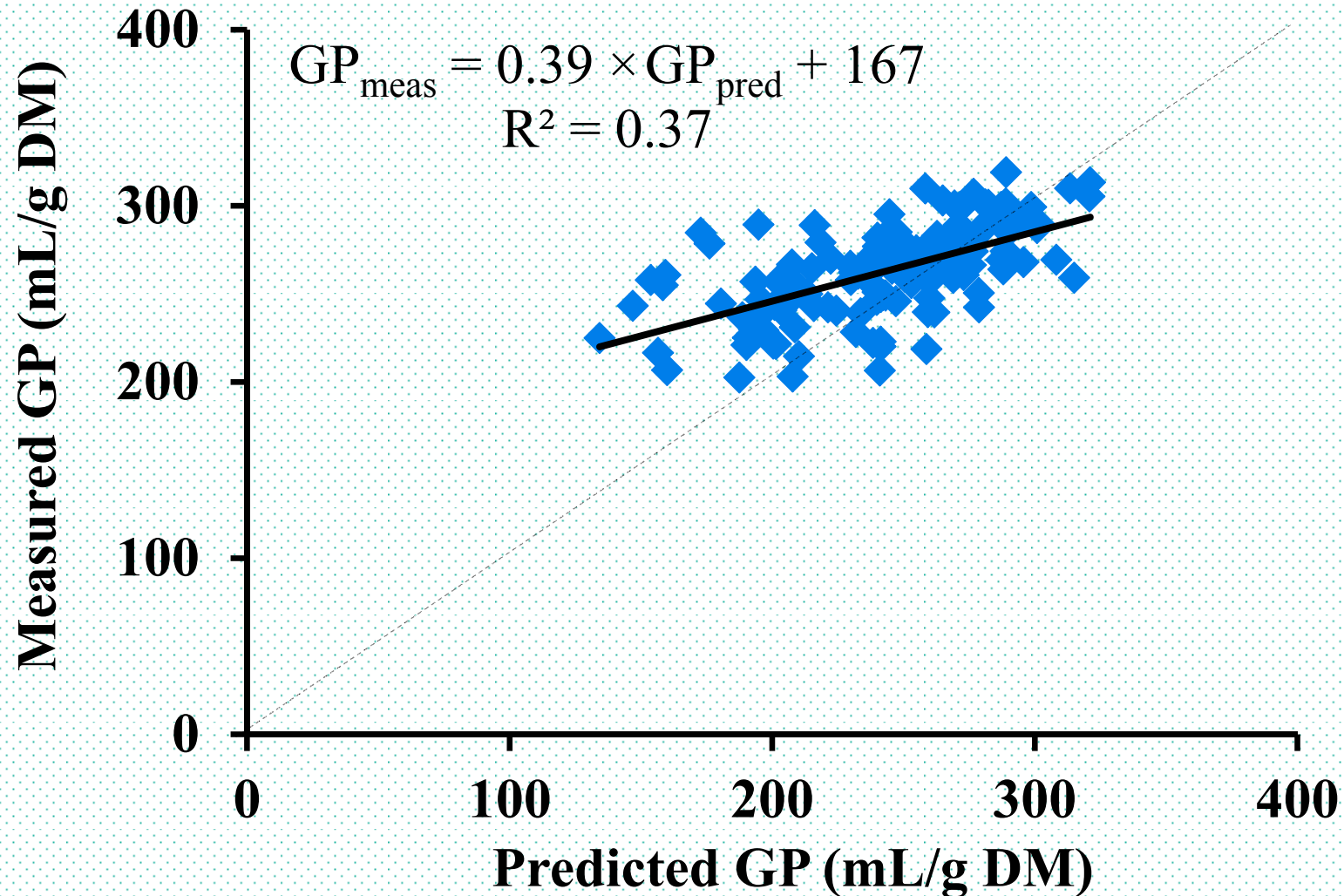
Diet	Gas production (mL/g DM)		
	Predicted	Measured	Pred./meas.
Reference diet	248	273	0.91
Low protein	250	277	0.90
High protein	234	255	0.92
Low fibre	260	287	0.90
High fibre	218	228	0.96
Low lipid	252	265	0.95
High lipid, fat	232	258	0.89
High lipid, oilseeds	237	252	0.94
SEM	7.2	2.4	0.027
P values			
Diet	0.001	<0.001	0.61

Predicted and measured CH₄ production of eight diets

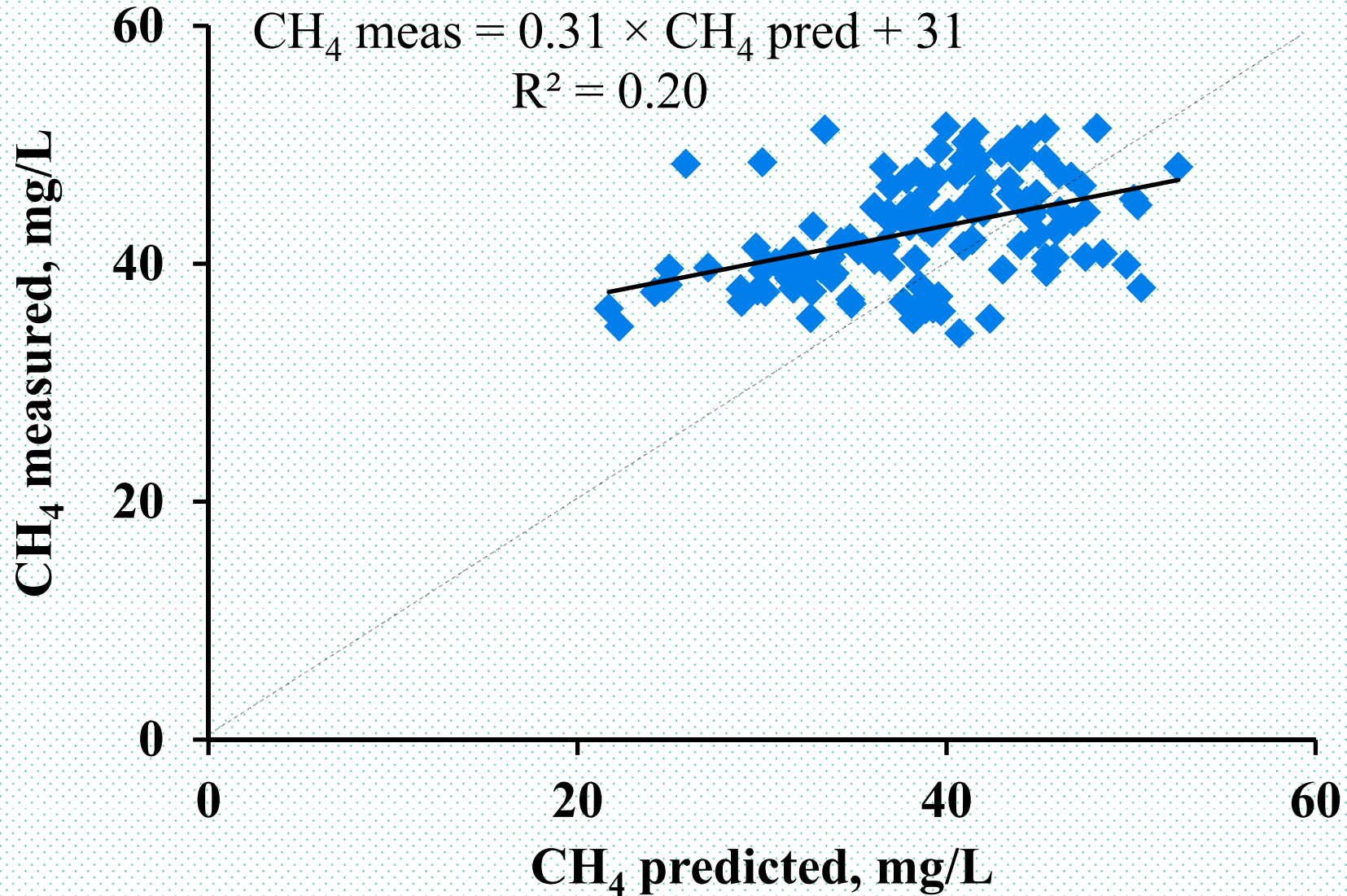
Diet	CH ₄ production (mL/g DM)		
	Predicted	Measured	Pred./meas.
Reference diet	39.4	43.5	0.91
Low protein	39.2	41.0	0.96
High protein	37.8	43.9	0.86
Low fibre	41.2	44.6	0.92
High fibre	35.3	40.7	0.87
Low lipid	39.8	42.9	0.93
High lipid, fat	36.8	42.2	0.87
High lipid, oilseeds	37.4	40.5	0.93
SEM	1.28	0.43	0.030
P values			
Diet	0.05	<0.001	0.25

RESULTS &
DISCUSSION

Linear regression between predicted and measured values of gas production



Linear regression between predicted and measured values of CH₄ production



Take home messages

Chemical composition of diets changed relations among *in vitro* gas, CH₄, and VFA

Microbial lysis occurring *in vitro* at prolonged incubation times (24 or 48 h) could have partially altered such relations

Stoichiometrical equations are debatable, as do not consider

- *possible effects of protein (and lipid) on in vitro GP*
- *microbial mass as end-product of rumen fermentation*

In vitro GP and CH₄ should be measured, to ensure a greater reliability of results

Accuracy of predictions could improve at shorter incubation times

Acknowledgments

The present study was financed by “ARCHAEA” Project:
“Feeding strategies to reduce methane emissions from
dairy cows”, Veneto Region Rural Development
Programme (RDP) 2007-2013





**THANKS FOR YOUR
ATTENTION**

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Sample collection

At the beginning of 4 runs ($t = 0$)

At the end of 4 runs (24 or 48 h)



An aliquot (5 ml) of fermentation fluid was sampled from each bottle and analyzed for VFA by gas-chromatography