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A new approach to evaluate methane production from *in vitro* rumen fermentation of concentrates

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Introduction

Such effects are more pronounced when highly fermentable substrates (i.e. concentrates) are incubated

Gas samples are often collected from headspace of closed bottles where gas is accumulated without being vented

High pressures are generated into the bottles

A given proportion of gas (especially CO_2) is solubilized in the fermentation fluid
(Tagliapietra et al., 2010)

Gas composition in the headspace is altered

Aim

To compare values of gas (GP) and methane production provided by two *in vitro* techniques

- Closed bottles
- Vented bottles connected to gas collection bags

Material and methods

Feeds

Three concentrates were selected to cover a large variability in chemical composition

Source of starch = **CORN GRAIN**

Source of digestible fibre = **DRY SUGAR BEET PULP**

Source of fat and protein = **FLAXSEED EXPELLER**

Automated GP system

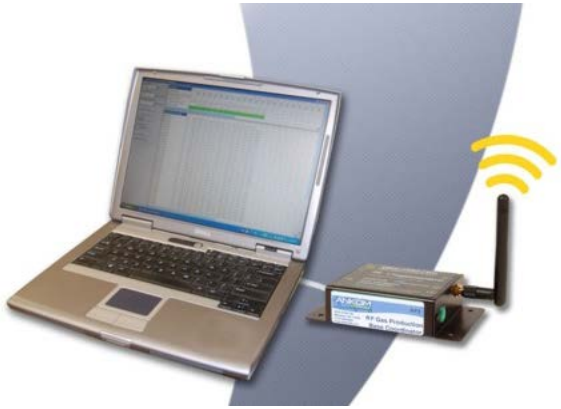
Kit of bottles (317 ml) equipped with:

- a pressure detector
- an open-closed valve for gas venting

Gas can be vented at a given threshold pressure

All bottles are wireless connected to a PC

Pressure values inside the bottles are recorded by PC every minute and converted into gas volumes (ml)



Methane measurements

CLOSED BOTTLES

Bottles were not vented and gas was accumulated in the headspace of bottles (257 ml) over the whole period of incubation

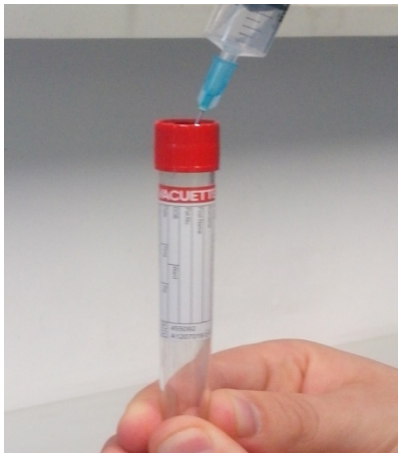
Fermentation of corn meal (0.4 g DM) produces a pressure of about 60 kPa in 24 h of incubation

VENTED BOTTLES

Bottles were vented at a fixed pressure (6.8 kPa) and the vented gas was collected into gas collection bags (capacity: 1 liter)

Gas sampling from closed bottles

10 ml

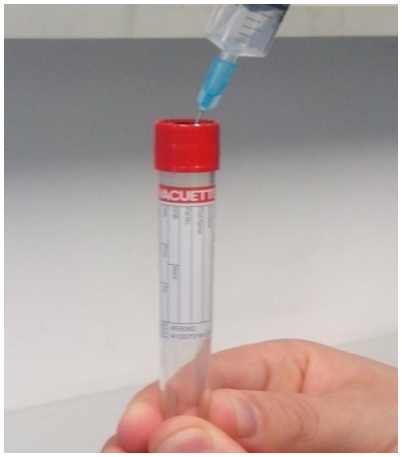


2 µl

GC

Gas sampling from vented bottles

10 ml



2 µl

GC

2 µl

GC

Experimental design

Incubation procedures	In each bottle:
Feed sample	0.40 g
Rumen fluid	20 ml
Buffer	40 ml
Bottle headspace	257 ml
Incubation time	24 h

For each technique:	
Runs	2
Feeds	3
Replications	3
Bottles	18
Blanks	4
Total bottles	**22**

Computation of methane production

For closed bottles (Lopez et al., 2007)

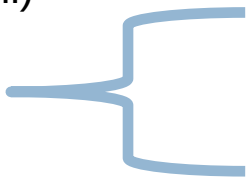
$$CH_4\ (ml) = CH_4\%_{headspace} \times (HS + GP)$$

For vented bottles

$$CH_4\ (ml) = (CH_4\%_{headspace} \times HS) + (CH_4\%_{bag} \times GP)$$

HS = bottle headspace (257 ml)

GP = gas production at 24 h



$GP_{measured}$

$GP_{adjusted}$

Data were expressed in terms of: *concentration* → *ml/100 ml GP*
production → *ml/g DM*

Adjustment of GP for solubilized gas

For closed bottles

At a constant temperature, amount of gas solubilized in a liquid is proportional to the pressure exerted by gas above the liquid

Solubilized gas (ml) = TOTAL GP × 0.147

0.147 = CO_2 constant solubility (Pell and Schofield, 1993)

For vented bottles

The pressure present in the headspace of bottles at the end of incubation was always very low thus any adjustment was required

$$GP_{adjusted}\ (ml) = GP_{measured} + GP_{solubilized}$$

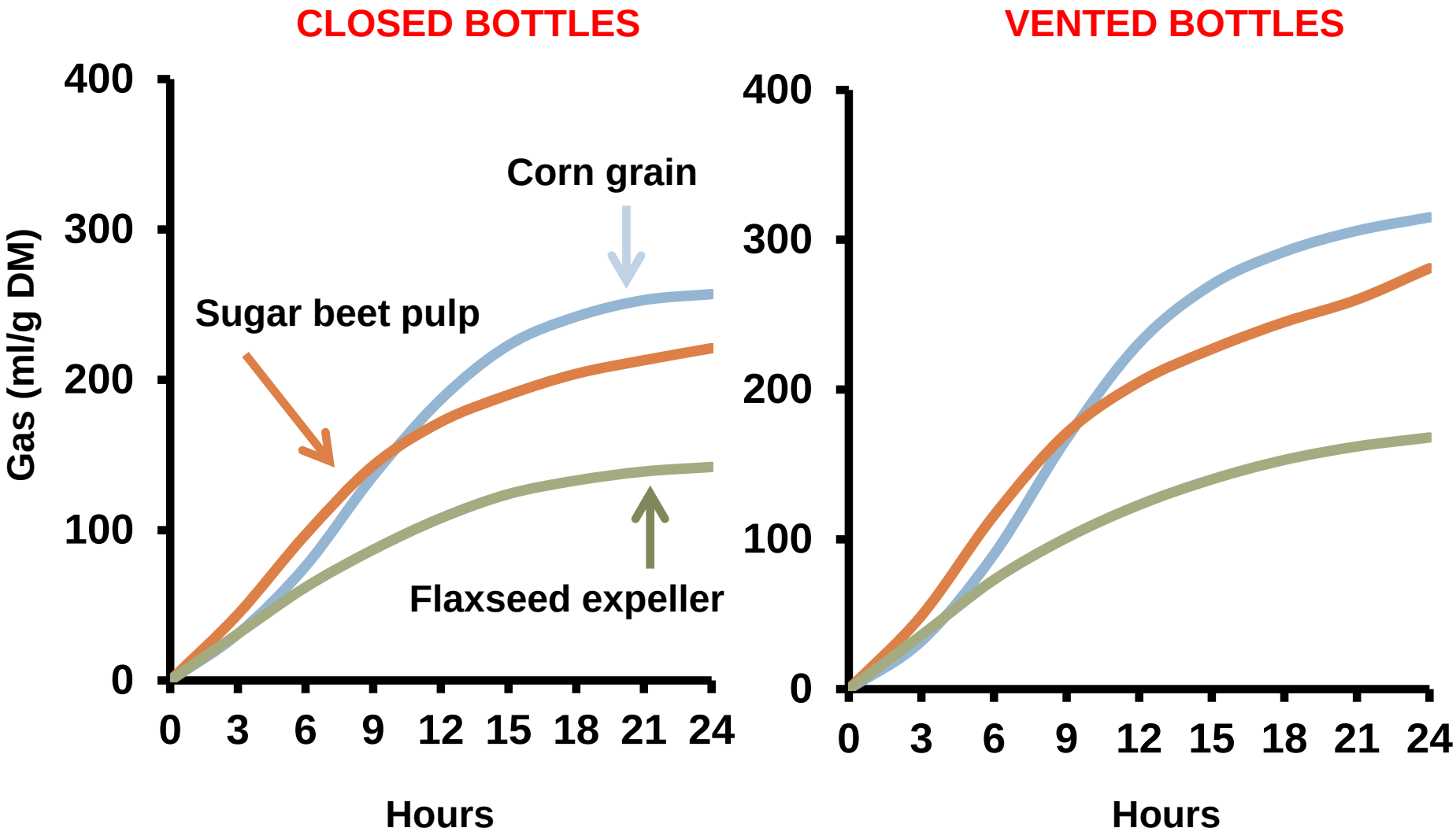
Statistical analysis

Model (Proc Mixed; SAS, 2005)

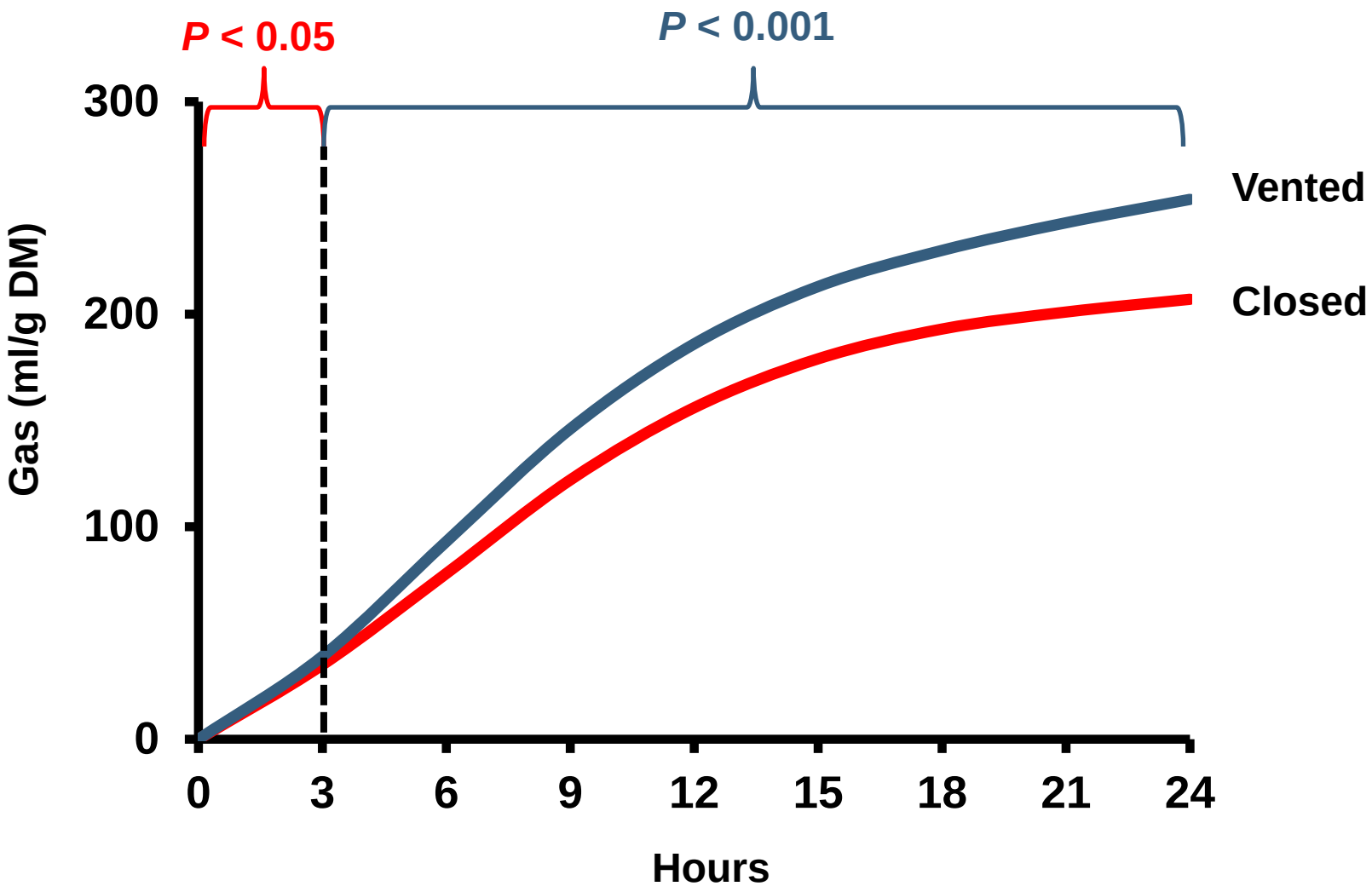
$$y_{ijkl} = \mu + F_i + T_j + (F \times T)_{ij} + R_k + \varepsilon_{ijkl}$$

y = experimental observation; μ = overall intercept of the model ; F_i = feed (fixed effect); T_j = gas sampling technique (fixed effect); (F

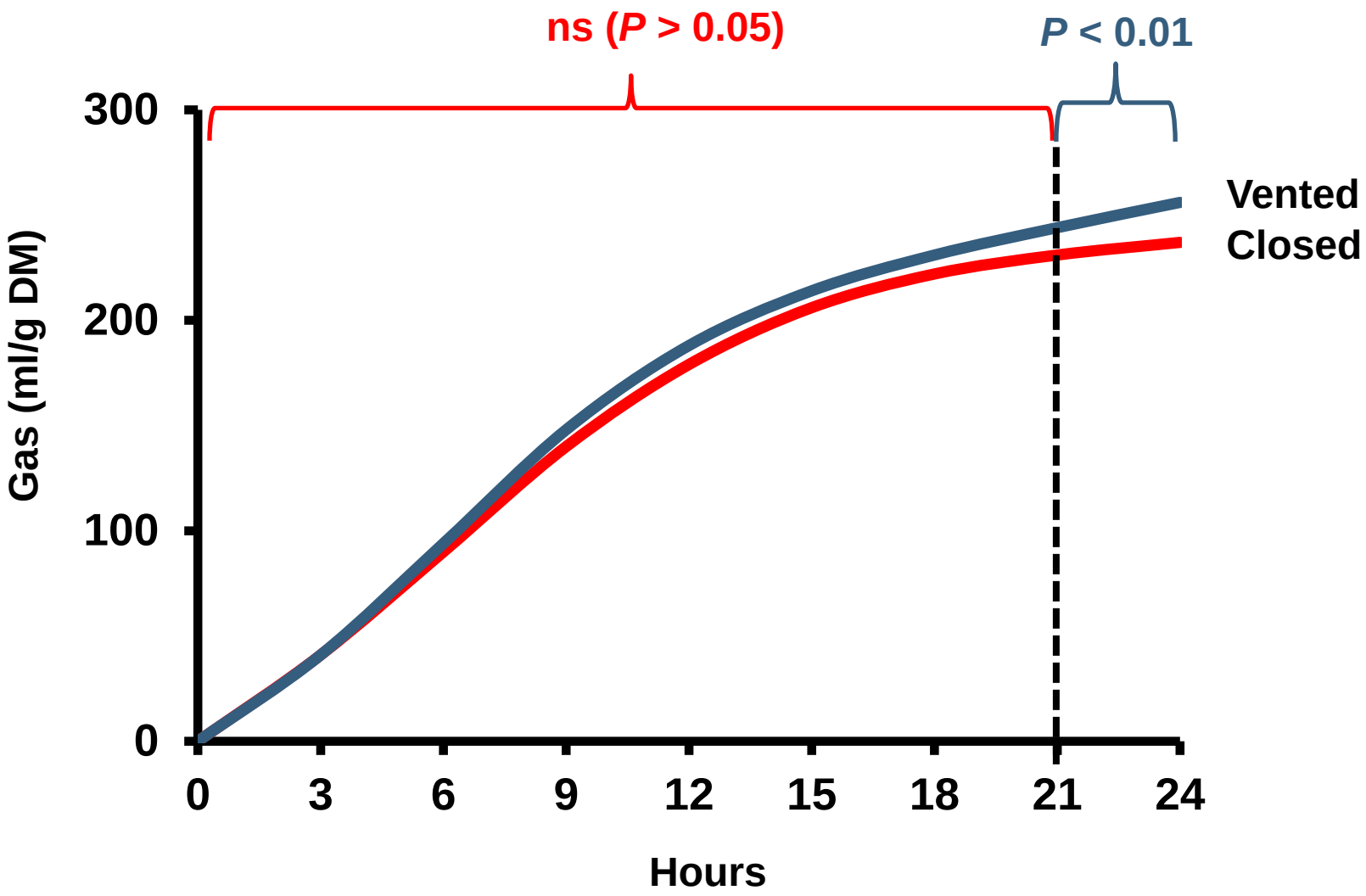
Kinetics of gas production of feeds with closed and vented bottles



Kinetics of gas production
Effect of technique on $GP_{measured}$



Kinetics of gas production
Effect of technique on $GP_{adjusted}$



Methane concentration and production

	Closed bottles	Vented bottles	SEM
Methane concentration			
- CH_4, ml/100 ml $GP_{measured}$	14.2^{A}	11.4^{B}	0.33
- CH_4, ml/100 ml $GP_{adjusted}$	12.3^{a}	11.2^{b}	0.29
Methane production			
- CH_4, ml/g DM^{1} $GP_{measured}$	27.8	28.6	0.64
- CH_4, ml/g DM^{1} $GP_{adjusted}$	28.8	28.7	0.65

[1] **g of incubated dry matter**

Repeatability of methane values provided by the two techniques

	Closed bottles	Vented bottles
Methane concentration		
- CH_4, ml/100 ml $GP_{measured}$	2.07	1.86
- CH_4, ml/100 ml $GP_{adjusted}$	1.93	1.83
Methane production		
- CH_4, ml/g DM^1 $GP_{measured}$	2.50	2.08
- CH_4, ml/g DM^1 $GP_{adjusted}$	2.54	2.09

[1] g of incubated dry matter

Conclusions

Vented bottles provided values of $GP_{measured}$ that were about 25% greater than those provided by closed bottles, but the ranking of feeds was the same

Values of $GP_{adjusted}$ did not differ between the two techniques, except at later phases of incubation (from 21 h to the end)

Methane concentration (ml/100 ml GP) DIFFERED between techniques, as result of differences in GP at 24 h of incubation

Methane production (ml/g DM) DID NOT DIFFER between techniques

An harmonization of these techniques is desirable to make easier the comparison between results of different experiments

Acknowledgments

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PROGRAMMA 2007 0 PSR 1 VEN 3 ETO SVILUPPO RURALE

REGIONE DEL VENETO

Thanks for your attention…

Chemical composition of feeds

	DM, %	NDF (% DM)	CP (% DM)	EE (% DM)	Ash (% DM)	NSC (% DM)
Corn grain	90.0	10.6	9.3	3.7	1.5	74.9
Dry sugar beet pulp	93.5	44.3	9.5	6.0	5.1	35.1
Flaxseed expeller	92.3	26.0	37.5	9.1	5.9	21.5

[1] Computed as difference: (100 – NDF – CP – EE- Ash)

Collection of rumen fluid

3 dry Holstein-Friesian intact cows as donor animals
Flexible oesophageal probe (Tagliapietra et al., 2012)