Effects of forage type and maturity on cell wall characteristics

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Cell walls

- Cellulose
- Hemicellulose
- Lignin
- Pectin
- Protein

NDF

- Cellulose
- Hemicellulose
- Lignin

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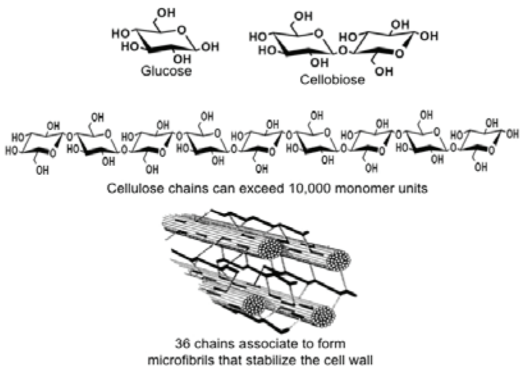

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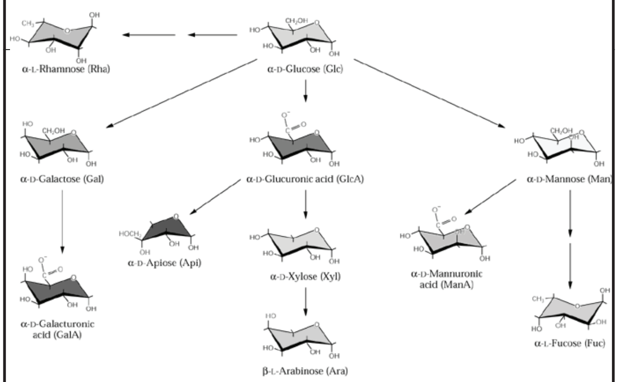
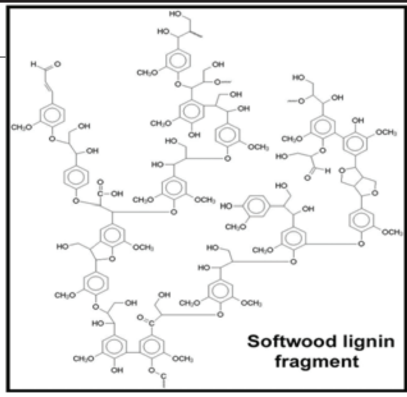
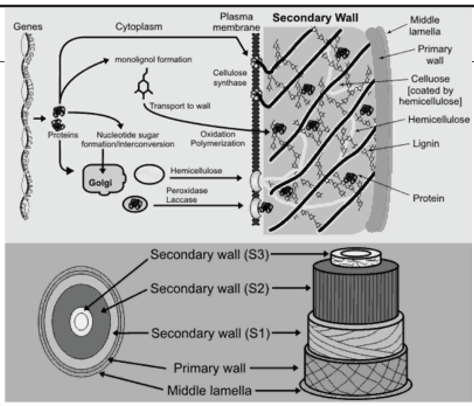
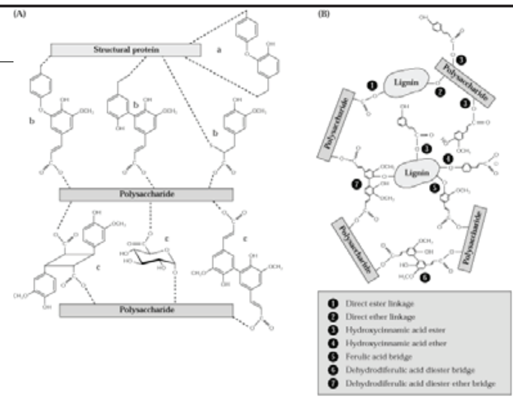


Lignin



Cone en Engels, 1993





Maize silages

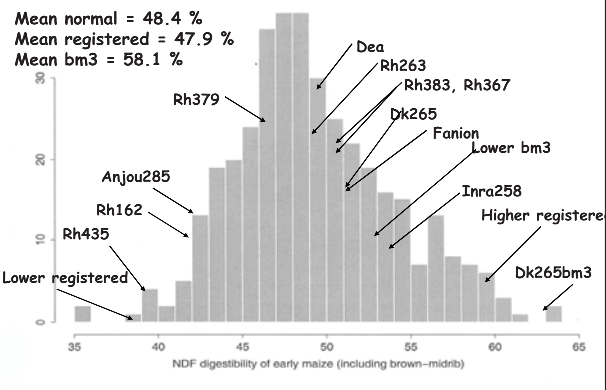
- Starch (350 – 400 g/kg DM)
- NDF (350 – 450 g/kg DM)
- Lignin (35 – 60 g/kg NDF)
- In vitro degradation OM, T&T (65 – 78 %)
- Cell wall degradation (40 - 60 %)

Grass silages

- NDF (400 – 600 g/kg DM)
- Lignin (30 - 65 g/kg NDF)
- In vitro degradation OM, T&T (70 – 82 %)
- Cell wall degradation (60 - 75 %)

What influences cell wall digestibility?

- Genotype
- Chemical composition
- Tissue composition
- Physical properties
- Harvest date, maturity
- Growing conditions



Y. Barriere, 2002

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Lignin and degradability

- Grass
  - % degradation = 103.3 – 0.550 lignin
  - $r^2 = 0.93$
- Legumes
  - % degradation = 110.8 – 0.34 lignin
  - $r^2 = 0.84$

Buxton and Russell, 1988

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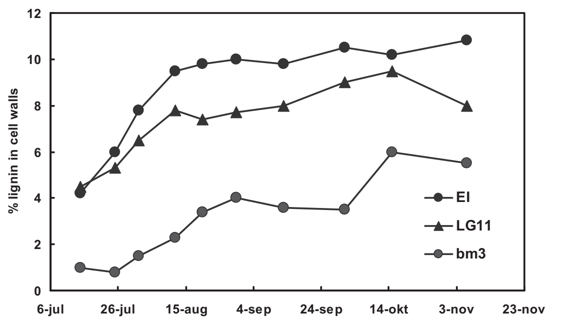
Lignin and degradability



Cone and Engels, 1993

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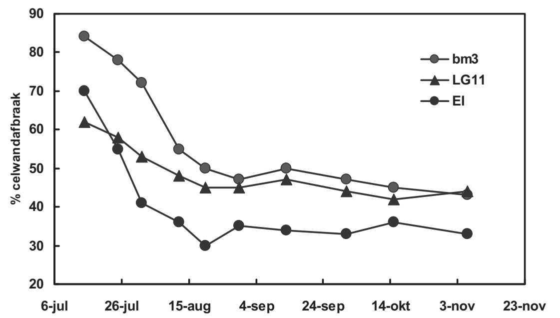
Lignin and digestibility



Cone and Engels, 1993

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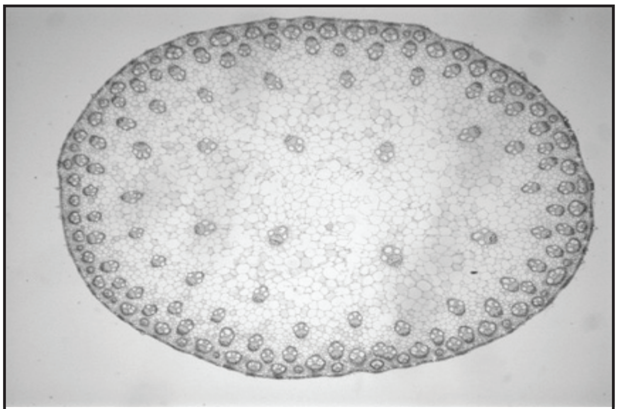

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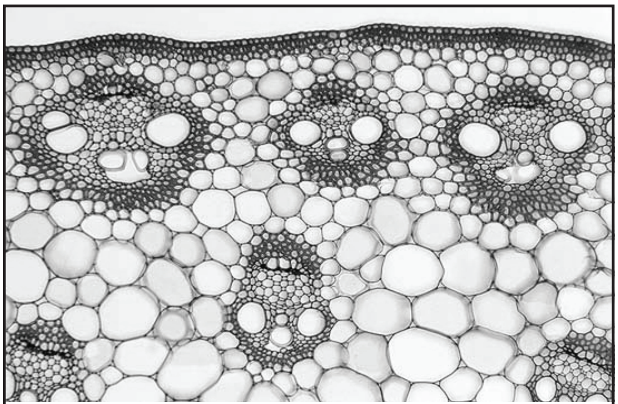

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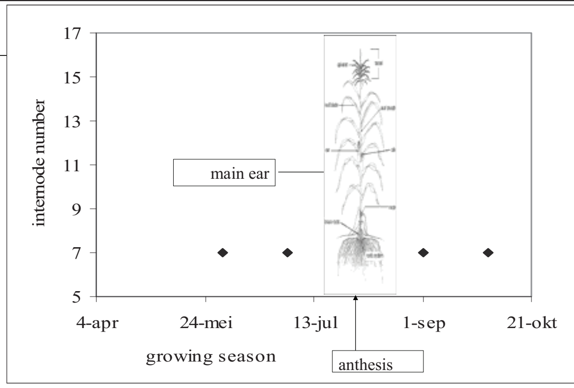
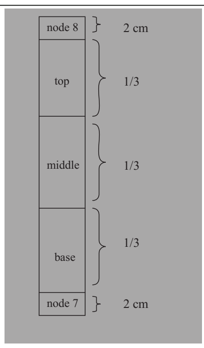
Boon et al.

- Systematic research in maize on differences in anatomy, chemical composition and fermentation characteristics
  - Within an internodium at anthesis
  - Between internodia at anthesis
  - In a specific internodium during the season
  - Between different types of tissue
  - Between different genotypes

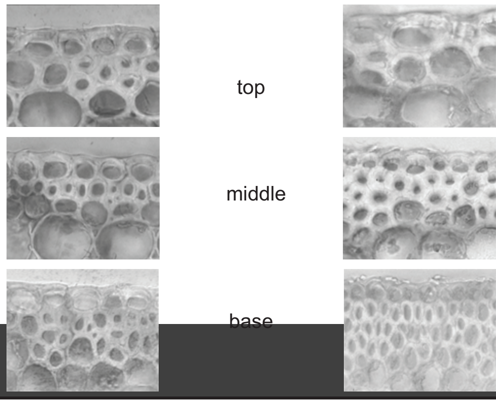


Maize

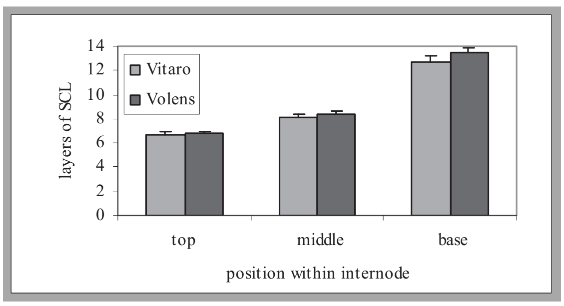


- Internodium 7 divided in 5 pieces
- Upper and lower pieces contain parts of the nodes

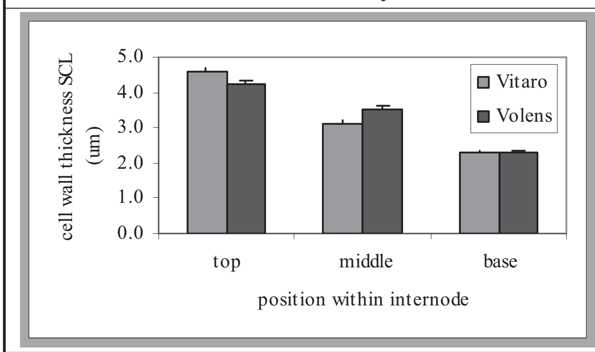
Vitaro | Volens



Sclerenchyma layers



Cell wall thickness sclerenchyma



Fermentation internode 7 at anthesis

a2 (ml g^{-1} OM) = NDF-degradation	Vitaro 1999	Volens 1999	Vitaro 2000	Volens 2000
top	118	107	148	140
middle	115	93	150	131
basis	110	93	144	119

Fermentation of maize stem slices

- 100 μm thin slices stick on microscope slides with double sided tape
- fermentation in buffered rumen fluid (12, 24 or 48 h)
- Measuring thickness of the cell walls

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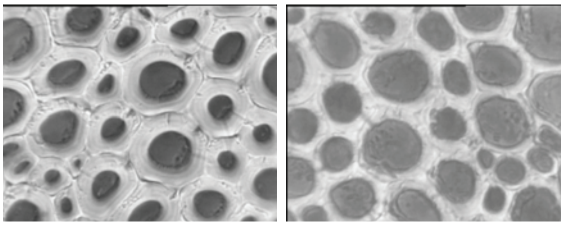
Vitaro top 2 august '99



control 48 h fermented

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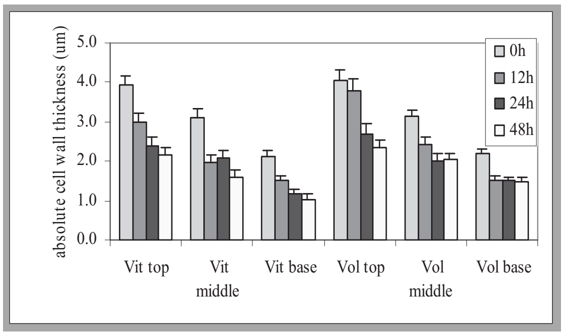
Cell wall thickness



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Anatomy within an internode

- Calculation of degradation rate

	Degradation rate 0-12 h (nm h^{-1})	Degradation during 48 h (μm)	Mean cell wall thickness sclerenchyma (μm)
top	76	3.6	4.4
middle	52	2.5	3.3
basis	49	2.4	2.3

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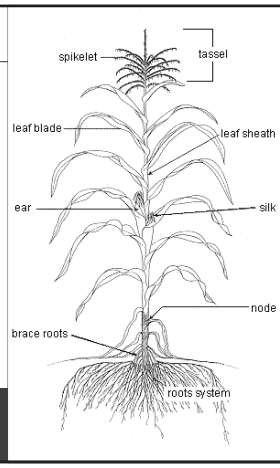
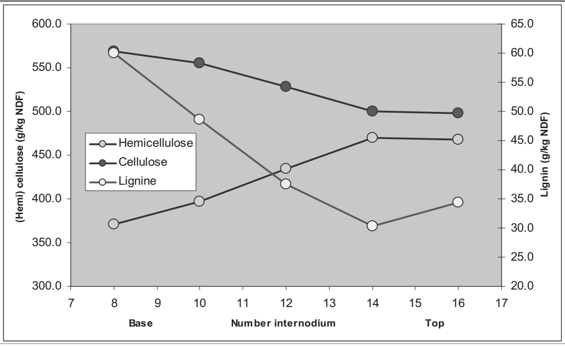
Differences in cell wall properties and degradation between internodes within a single plant





Vitaro

Volens

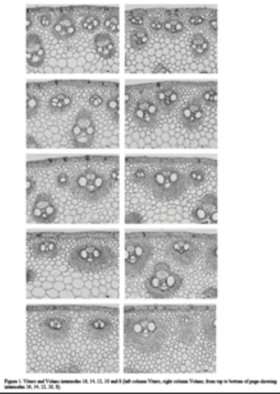
16

14

12

10

8



Vitaro

Volens

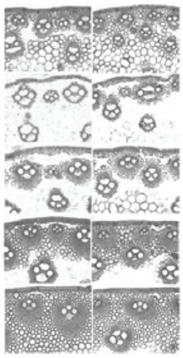
16

14

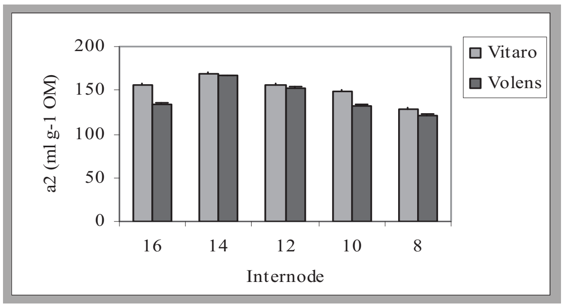
12

10

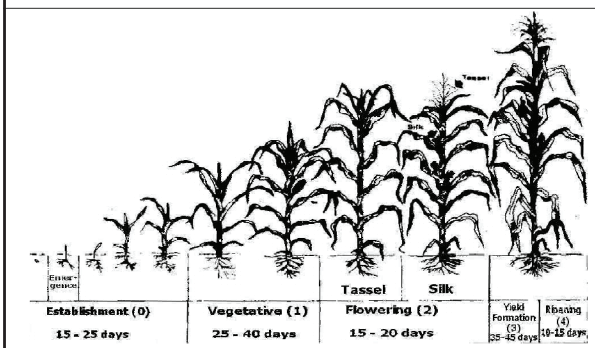
8

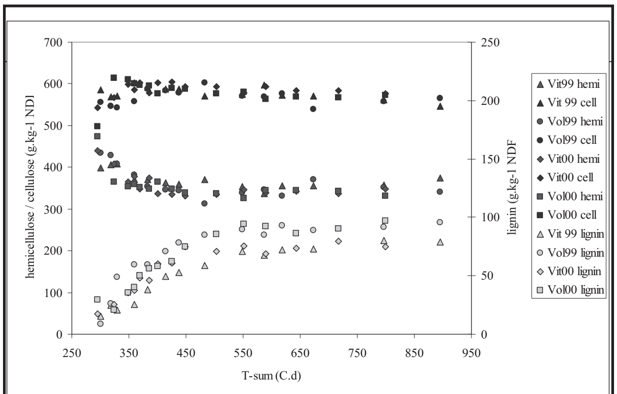


24 h in rumen fluid



Cell wall development during the growing season





Vitaro
12-7

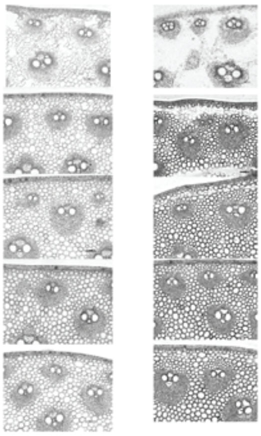
Control

19-7

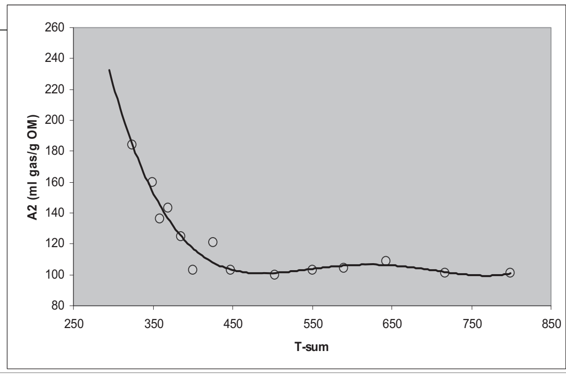
22-7

9-8

13-9



24 h in rumen fluid



Grass

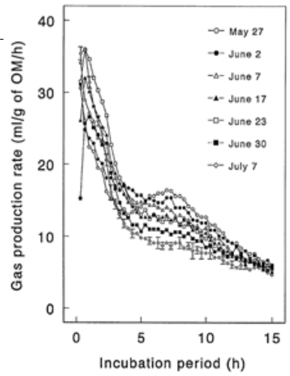


Figure 2. Calculated first derivative of the cumulative gas production profiles (n = 4) of the grass samples, representing the rate of gas production. The grass samples differed according to harvest date, as indicated, after a precut on April 27, 1993. For every two data points, one was omitted from the figure for clarity.

Journal of Dairy Science Vol. 82, No. 5, 1999

Cone et al, 1999

Conclusions

- Increasing cell wall degradability from bottom to top within internodes
- Increasing cell wall degradability from bottom to top between internodes in a single plant
- Decreasing cell wall degradability during the growing season
- High correlation between degradation and lignin
- Except lignin, more factors influence degradation
- Research on biochemistry of lignin and bondings with cell wall components

Research on different maize types

- Dry Down (DD) - early
- Stay Green (SG) - early
- Dry Down – late
- Stay Green – late

- Starchy genotypes
- Clay soil in Lelystad
- 2003

Maize plant without ear

		GP20	A1	A2	B2
		ml/g OM	ml/g OM	ml/g OM	h
20 August		210.0	52.1	157.9	8.47
16 September		193.0	38.3	154.8	9.05
3 October		190.7	43.3	147.4	9.40
LSD		*6.3*	*2.9*	*4.8*	*0.15*
DD-early		190.2	40.1	150.1	9.34
SG-early		193.2	39.2	154.0	8.96
DD-late		211.1	52.8	158.3	8.84
SG-late		197.0	46.1	151.0	8.76
LSD		*7.2*	*3.3*	*5.6*	*0.17*
Effect harvest date		***	***	**	***
Effect type		***	***	*	***
harvest date * type		NS	NS	#	#

Cone et al, 2008

Research on different maize types

- Cell wall degradation of Stay Green plants slightly faster than of Dry Down plants
- Cell wall degradation of late genotypes faster than that of early genotypes
- No systematic effect of maize type on cell wall degradation for whole plants

Research on different maize types

- Influence chop length (6 – 15 mm) on cell wall degradation
- Influence duration of ensiling on cell wall degradation
- 8 genotypes
- Harvest at ± 320 g DM/kg

chop length	cultivar	GP20	A1	A2	B2	starch degraded
		ml/g OM	ml/g OM	ml/g OM	h	g/kg starch
[redacted]						
15 mm	-	225	27.2	198	10.55	336
LSD		*21*	*1.0*	*21*	*0.20*	*68*
-	**cv1**	225	23.0	202	10.40	324
-	**cv2**	226	28.5	198	10.48	335
-	**cv3**	178	23.3	155	10.73	214
-	**cv4**	220	23.9	197	10.65	297
-	**cv5**	260	26.2	233	10.31	426
-	**cv6**	244	37.0	207	10.18	482
-	**cv7**	220	25.3	195	10.36	314
-	**cv8**	228	29.4	199	10.71	334
LSD		*43*	*2.1*	*42*	*0.40*	*118*
Effect chop length		NS	NS	NS	NS	NS
Effect cultivar		#	***	#	#	**
Chop length * cultivar		NS	NS	NS	*	NS

Cone et al, 2008

	A1	A2	B2
	ml/g OM	ml/g OM	h
[redacted]			
cv3	30.5[a]	226.2	7.31[b]
Harvest date			
02-09	32.9[a]	222.1[b]	7.32[b]
25-09	22.1b	233.1[a]	7.47[a]
Type of silo			
small	26.3	226.4	7.45
large	28.6	228.7	7.35
Ensiling period			
0 days	32.8[a]	232.2	7.41
14 days	26.5[b]	227.4	7.43
42 days	24.3[b]	225.2	7.45
180 days	26.3[b]	225.5	7.31
Effects			
cultivar	***	NS	**
harvest date	***	***	*
type of silo	#	NS	NS
ensiling period	***	NS	NS

Cone et al, 2008

Research on different maize types

- No effect of chop length
- No effect of duration of ensiling period

Improving cell wall degradability with fungal enzymes

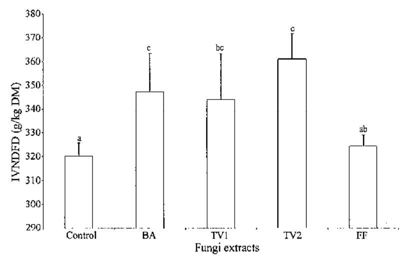


Fig. 3. Variation in the IVNDFD of wheat straw treated with enzymatic extracts. Different letters (a–c) denote a difference (P<0.05) with a one-way analysis of variance (S.E.M. = 5.12). [Vertical bars represent standard deviation.].

Rodrigues et al, 2008

Improving cell wall degradability with fungi

- Rice straw incubated with white rot fungi
  - *Pleurotis ostreatis*
  - *Volvariella volvacea*
- 0 – 35 days
- Drying and grinding
- Incubation with rumen fluid in the gas production technique

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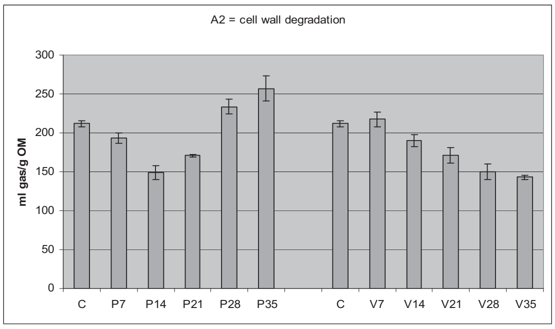
Improving cell wall degradability with fungi



Cone et al., 2010

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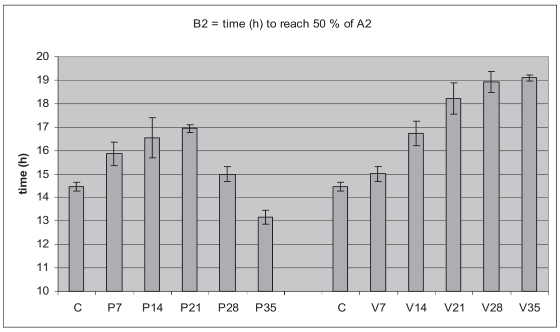
Improving cell wall degradability with fungi



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Improving cell wall degradability

- Conclusions
  - Lignin degrading enzymes can improve cell wall degradability
  - Some fungi specifically degrade lignin in cell walls
  - Fungi have potential to improve low quality feedstuffs

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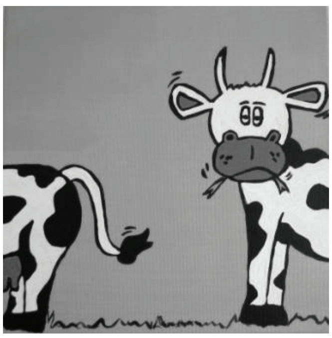
Thanks for your attention



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Feed intake regulation and cell wall digestion characteristics

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Introduction

Optimal forage allocation and supplementation strategies for dairy cattle must consider variation in forage quality characteristics as well as cow's responses to their diet throughout lactation. Forage quality characteristics can affect diet cost, energy intake and partitioning, feed conversion efficiency (FCE), and animal health. These characteristics include concentration and digestion characteristics of cell walls as well as particle size and fragility. Forages are unique among diet ingredients fed to ruminants because they provide fiber that is effective at retaining feed particles in the rumen, increasing their digestibility and increasing digesta mass and volume. Increased digesta mass in the rumen can reduce risk of ruminal acidosis and abomasal displacement but can also limit feed intake, depending upon the physiological state of animals. Eating is controlled by the integration of signals in brain feeding centers. Signals from ruminal distension can control feed intake when the drive to eat is high and metabolic control of feed intake is diminished (e.g. cows at peak lactation) while signals derived from metabolism of fuels dominate the control of feed intake when signals from distension decrease (e.g. cows in late lactation). This presentation will discuss the effects of cell wall concentration and digestion characteristics on feed intake of cows and how they change throughout the lactation cycle.

Filling effect of diets

The extent to which ruminal distention limits feed intake is positively related to milk yield. This was shown in two experiments from our laboratory in which groups of cows with a wide range of milk yield were offered diets differing in rumen fill. The first experiment compared diets differing in forage to concentrate ratio (Voelker and Allen, 2000). Diets contained either 44% forage (24.3% NDF) or 67% forage (30.7% NDF). Response in DMI to the lower forage diet increased linearly (up to ~4.5 kg/d for the highest producing cows) and FCM yield increased ~2.2 kg per kg increase in DMI for cows producing over ~40 kg FCM/d. However, cows producing less than ~40 kg/d had similar FCM for the two treatments. The second study compared brown midrib corn silage to control corn silage (Oba and Allen, 1999a). The two silages had similar DM and NDF concentrations but in vitro NDF digestibility (30 h) was nearly 10 units higher for the low-lignin brown midrib corn silage. When both forages were offered to a group of cows with a wide range of milk yield, response in DMI and FCM to the brown midrib corn silage compared to the control, corn silage increased linearly with milk yield. While the lower producing cows (~30 kg/d) had similar DMI and FCM for the two silages, FCM increased ~8 kg/d for the highest producing cows (~60 kg/d).

High producing dairy cows should be fed diets with lower filling effect to maximize feed intake. The filling effect of a diet is determined primarily by its filling effect over time in the rumen. The overall filling effect is determined by forage NDF content, forage particle size, fragility of forage NDF determined by forage type (legumes, perennial grasses, annual grasses), and NDF digestibility within a forage family (Allen, 2000). Forage NDF is less dense initially, digests more slowly, and is retained in the rumen longer than other diet components. Increasing diet forage NDF concentration can dramatically reduce feed intake of high producing cows. Many studies in the literature reported a decrease in DMI of up to 3 kg/d when diet NDF content was increased from 25 to 35% by substituting forages for concentrates (Allen, 2000). Although most studies reported a significant decrease in DMI as forage NDF increased, the DMI response was variable, depending upon the degree to which intake was limited by ruminal fill. Higher producing cows are limited by fill to the greatest extent and the filling effect of forage fiber varies depending upon particle size and fermentation characteristics.

Experiments that have evaluated effects of forage particle size have generally shown small effects on DMI (Allen, 2000). However, one experiment showed little effect of particle size of alfalfa silage when fed in high grain diets but a large reduction in DMI for the diet containing longer alfalfa silage when fed in a high forage diet (Beauchemin et al., 1994). Feed intake might have only been limited by ruminal fill in the high forage diet, which could explain the interaction observed.

Increasing diet NDF content by substituting non-forage fiber sources (NFFS) for concentrate feeds has shown little effect on DMI in studies reported in the literature (Allen, 2000). Non-forage fiber sources include byproduct feeds with significant concentrations of NDF such as soyhulls, beet pulp, cottonseeds, corn gluten feed, almond hulls and distiller's grains. Fiber in NFFS is probably much less filling than forage NDF because it is less filling both initially (smaller particle size) and over time in the rumen because it digests and passes from the rumen more quickly.

Forage NDF has a much longer ruminal retention time than other major dietary components. Retention time in the rumen is longer because of longer initial particle size, and greater buoyancy in the rumen over time, which differs greatly across forages. As forages mature, the NDF fraction generally becomes more lignified. Lignin is a component of plant cell walls that helps stiffen the plant and prevent lodging. It is also essentially indigestible by ruminal microbes and limits fermentation of cellulose and hemicellulose. Within a forage type, the degree to which NDF is lignified is related to the filling effects of the NDF. Fiber that is less lignified clears from the rumen faster, allowing more space for the next meal. However, ruminal retention time of NDF from perennial grasses is generally longer than for legume NDF despite being less lignified (Oba and Allen, 1999b; Voelker Linton and Allen, 2008). Because of this, it is more filling and should not be included in high concentrations in diets of cows for which feed intake is limited by ruminal fill, unless it is of exceptionally high quality. Corn is an annual grass, and corn silage NDF digests and passes from the rumen quickly compared to perennial grasses and can be an excellent source of forage NDF for high producing cows.

Importance of maintaining ruminal fill

While ruminal distention becomes a primary limitation to feed intake as milk yield increases, it likely has little effect on feed intake when it is controlled primarily by oxidation of mobilized fatty acids in the liver during the transition period (Allen et al., 2009). Glucose demand of fresh cows is high when glucose utilization for milk production outpaces gluconeogenesis by the liver. While cows require diets with adequate glucose precursors (i.e. starch from grains), it is important to also maintain rumen fill. Formulating diets to maintain rumen fill with ingredients that are retained in the rumen longer, and have moderate rates of fermentation and high ruminal digestibility will likely benefit transition cows several ways. Increased ruminal digesta mass can provide more energy over time when feed intake decreases at calving or from metabolic disorders, mastitis or infectious disease. This will help maintain plasma glucose and prevent even more rapid mobilization of body reserves compared to when diets are formulated with ingredients that disappear from the rumen quickly. Ruminal digesta is very important to buffer fermentation acids and buffering capacity is directly related to the amount of digesta in the rumen. Therefore, diets formulated with ingredients that increase the amount of digesta in the rumen will have greater buffering capacity and will maintain buffer capacity longer if feed intake decreases. Inadequate buffering can result in low ruminal pH, decreasing fiber digestibility and acetate production, and increasing propionate production, possibly stimulating oxidation in the liver and decreasing feed intake. Low ruminal pH also increases risk of health problems such as ruminal ulcers, liver abscess, and laminitis, and causes stress, likely increasing mobilization of body reserves even further. Diets formulated with ingredients that maintain digesta in the rumen longer when feed intake decreases will likely decrease risk of abomasal displacement.

Physically effective fiber

Optimum particle length of individual forages depends upon several factors including forage type, silo type (if ensiled), other forage(s) in the diet, the characteristics of the cow consuming the forage, stocking density/competition for feed, and diet fermentability. An adequate concentration of long particles is required to form a rumen mat to retain small particles that would otherwise escape, increasing diet digestibility, rumen fill, and buffering capacity. Some forages that are particularly fragile such as brown midrib corn silage benefit by chopping longer. Forages that are resilient to packing might have to be chopped shorter, particularly where packing is difficult (e.g. upright silos). Forages lacking physically effective fiber must be limited in diets and combined with forages with adequate particle length. When overcrowding causes competition at the feed bunk and slug feeding, diets with more physically effective fiber can limit rate of eating, decreasing risk of low ruminal pH, especially for highly fermentable diets.

Energy partitioning

Energy partitioning between milk production and body condition varies as physiological state changes throughout lactation. As lactation proceeds past peak, insulin concentration and sensitivity of tissues increase and energy is increasingly partitioned to body condition, sometimes at the expense of milk yield. While high-starch diets can increase milk yield of high producing cows, they can result in excessive gain in body condition as milk yield declines and insulin sensitivity of tissues increase. We showed that a 69% forage diet (0% corn grain) containing brown midrib corn silage increased energy partitioned to milk, decreasing body weight gain while maintaining yield of milk compared to a 40% forage diet (29 % corn grain) containing control corn silage (Oba and Allen, 2003). In vitro NDF digestibility of the brown midrib corn silage was ~20% higher (55.9 vs 46.5%) than the control corn silage. In contrast, DMI and milk yield was reduced when the control corn silage was fed in the higher forage diets. We also showed that beet pulp decreased BCS without decreasing yields of milk or milk fat when substituted for high-moisture corn up to 12% of diet DM (Voelker and Allen, 2003). Similarly, a recent experiment conducted with cows in the last 2 months of lactation showed that substitution of beet pulp for barley grain linearly decreased body condition score, maintained milk yield and linearly increased milk fat yield (Mahjoubi et al., 2009). Decreased body condition score and increased milk fat yield might have been because of a linear decrease in plasma insulin concentration which linearly increased plasma NEFA concentration. However, lower ruminal pH was reported as starch concentration of the diet increased, which might have caused the milk fat depression through CLA production in the rumen (not measured). Harvatine et al. (2009) reported that CLA-induced milk fat depression decreased gene expression for enzymes and regulators of fat synthesis in adipose tissue. Decreasing fermentability of diets by increasing fiber from forages or NFFS can maintain milk yield while decreasing gain in body condition.

Conclusions

The concentration and digestion characteristics of dietary fiber (cell walls) can affect energy intake and partitioning but responses are dependent upon the physiological state of cows that change through lactation. While more research is needed to better understand animal response to diets, these concepts will help to formulate diets to improve animal health and farm profitability.

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Feed intake regulation and cell wall digestion characteristics

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1

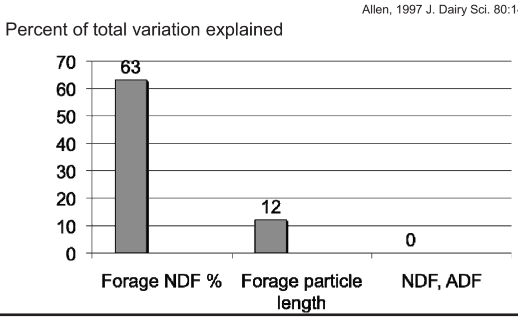
Forage fiber

- Slows rate of eating: prevent "slug" feeding
- Forms rumen mat, entraps small particles
  - Increases digestibility: increasing feed conversion efficency
  - Increases digesta mass and volume
    - greater distention decreases risk of displaced abomasum
    - increases buffering (3 ways: direct, increased chewing and saliva flow, increased mixing and absorption) – possibly increasing ruminal pH and fiber digestibility, and decreasing t10, c12 CLA and MFD
    - potentially decreasing feed intake

2

Variation in ruminal pH explained by diet characteristics

Allen, 1997 J. Dairy Sci. 80:1447



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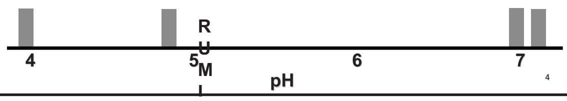
Buffer systems in the rumen

- Saliva
  - Bicarbonate, pK_a = 6.1, effective pK_a~ 7.0
    $H^+ + HCO_3^- \text{<->} H_2CO_3 \text{<->} H_2O + CO_2 \text{<->} CO_2$ (gas)
  - Hydrogen phosphate, pK_a = 7.2
    $H^+ + HPO_4^{-2} \text{<->} H_2PO_4^-$
- VFA, pKa = 4.7 - 4.8; lactate pKa = 3.8
- Digesta: BC of feedstuffs extremely variable (Jasaitis et al., 1987)
  - legume forages, high protein feeds > grass forages, low protein feeds > cereal grains
  - Most buffering is from pH 4 to 6 (Wohlt et al., 1987)



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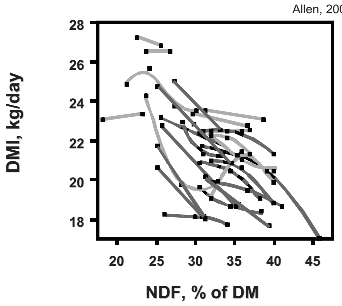
Feed intake decreases with increased diet forage NDF content

Allen, 2000, J. Dairy Sci. 83:1598



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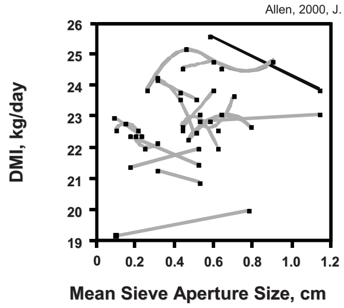
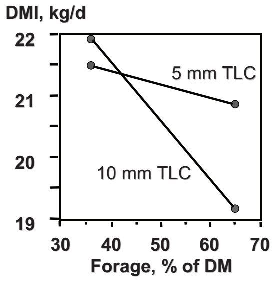
Forage particle size is not a major factor affecting feed intake (for corn silage and alfalfa)

Allen, 2000, J. Dairy Sci. 83:1598



Interaction of forage particle length and diet forage %

Beauchemin et al. 1994 J. Dairy Sci. 77:1326



Alfalfa silage

35% forage, 27.2% NDF

65% forage, 31.2% NDF

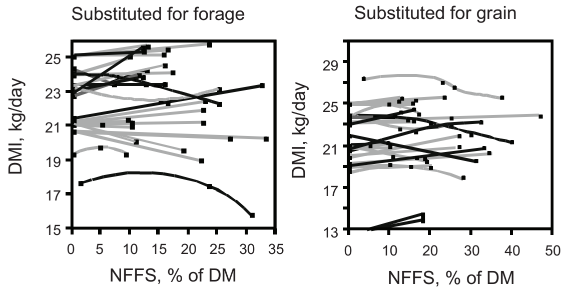
Forage: $P < 0.01$

Length: NS

Interaction: $P < 0.01$

Non-forage fiber sources have relatively little effect on feed intake

Allen, 2000, J. Dairy Sci. 83:1598



Forage: concentrate ratio

- Forages are more filling than concentrates
- Gut fill limits intake more as milk yield increases
- Grains provide more glucose precursors than forages
- Glucose precursors required increases with milk yield

9

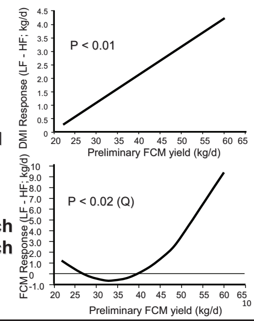
Production and DMI response to forage NDF

Voelker et al., 2002, J. Dairy Sci. 85:2650

32 cows, crossover design

Preliminary milk yield: ~20 – 60 kg/d
Intermediate diet

Treatments:
44% forage, 24.3% NDF, 33.8% starch
67% forage, 30.7% NDF, 23.1% starch



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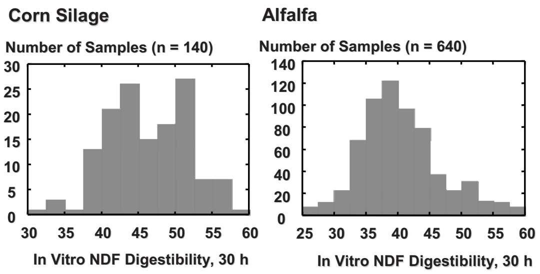
Variation in in vitro NDF digestibility of forages

Allen, 1993 NRAES-67



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DMI response to enhanced in vitro NDF digestibility

Extent to which feed intake is limited by ruminal distension

- – Appetite
- – Forage NDF concentration of diet
- – Forage NDF digestibility
- – Forage family

Quantification of the effect of forage NDF digestibility on DMI and milk yield of dairy cows

Oba and Allen, 1999, J. Dairy Sci. 82:589

Comparisons (n = 13) of forages with significant (P < 0.10) differences in forage NDFD (in vitro or in situ) and dietary NDF content.

$Y_{ijk} = \mu + Block_i + NDFD_j + NDF_{cov} + NDF^2_{cov} + F{:}C_{cov} + e_{ijk}$

	High	Low	NDFD	NDF
NDFD in vitro, in situ	62.9	54.5		
NDFD in vivo	54.8	51.5		
DMI, kg/d	23.2	21.8	0.001	0.04
4% FCM, kg/d	28.9	26.8	0.02	0.12

1 unit of NDFD (in vitro or in situ) = + 0.17 kg/d DMI
+ 0.25 kg/d 4% FCM

Enhanced intake and production of cows offered ensiled alfalfa with higher NDFD

Dado and Allen, 1996, J. Dairy Sci. 79:418

- **2 alfalfa silages, similar NDF concentrations, different NDFD**
- **Fed to lactating dairy cows, (13 DIM) at 83% of diet DM**

	Low	High	significance, *P*
DM,%	33	33	
NDF, % DM	40.4	40.1	
24-h IVNDFD, %	39.6	44.8	
NDF, % DM	40.6	38.8	
24-h IVNDFD, %	38.3	40.2	
DMI, kg/d	19.4	20.4	< 0.01
Milk Yield, kg/d	36.3	38.2	0.02

Effect of brown midrib corn silage on DMI of high producing dairy cows

Oba and Allen, 1999, J. Dairy Sci. 82:135

- Fed to lactating dairy cows, (89 DIM) at 45% of diet DM

	bm3	Normal	Signif, *P*
DM, %	31.7	32.6	
NDF, % DM	38.3	40.1	
Lignin, % DM	1.7	2.5	
Protein, % DM	8.7	8.4	
30-h IVNDFD	49.1	39.4	
DMI, kg/d	25.5	23.5	< 0.0001
Milk Yield, kg/d	41.7	38.9	< 0.0001
3.5% FCM, kg/d	41.0	38.4	< 0.0001
EB, Mcal NEL/d	2.8	0.7	< 0.01

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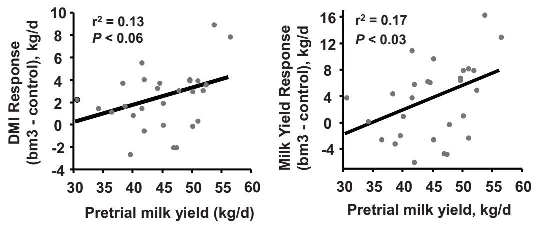
Feed intake and production response to bm3 corn silage increased with milk yield

Oba and Allen, 1999, J. Dairy Sci. 82:135



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Is there greater benefit of enhanced NDF digestibility for high forage diets?

Oba and Allen, 2000, J. Dairy Sci. 83:1333-1358

- **bm3 or isogenic normal corn silage**
- **Fed in high (66%) or low (42%) forage diets at 80% of forage DM**
  - **High forage diets: 38% NDF**
  - **Low forage diets: 29% NDF**

Effect of bm3 corn silage on intake and production of dairy cows fed low or high NDF diets

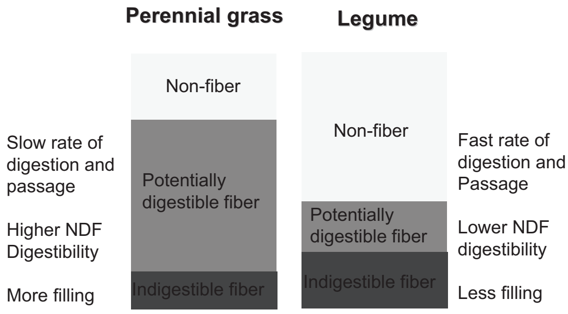
Oba and Allen, 2000, J. Dairy Sci. 83:1333

	29% NDF		38% NDF		Significance, *P*		
	bm3	control	bm3	control	NDF	CS	NDFxCS
Corn grain, % DM	26	29	0	5			
DMI, kg/d	24.7	23.9	22.9	21.5	<0.001	0.02	NS
3.5% FCM, kg/d	35.6	34.3	35.8	32.6	NS	0.06	NS
BW gain, kg/d	1.10	0.79	0.00	-0.02	< 0.01	NS	NS

Effect of forage NDF digestibility on DMI and milk yield of dairy cows

Oba and Allen, 1999, J. Dairy Sci. 82:589

- Analysis of treatment means from the literature
  - Different forage NDF digestibility (NDFD) in vivo, in vitro or in situ
  - NDFD classified as high or low
  - Blocked by experiment or treatment within experiment
- Across forage family:
  - 63 forage comparisons
  - Significant interaction of forage family (grass or legume) with NDFD
  - DMI and FCM positively related to NDFD within family
  - DMI and FCM negatively related to NDFD across family
- Within forage family:
  - 52 forage comparisons
  - DMI and FCM positively related to NDFD ($P < 0.001$)



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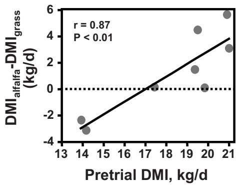
Grass vs. legume: response depends on pretrial DMI

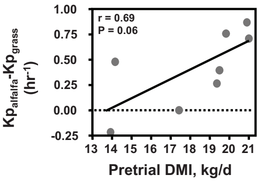
Linton and Allen, 2005, J. Dairy Sci. 88S:252

Diet had no effect on feed intake *averaged across cows*, but cows with greater drive to eat responded more positively to alfalfa over grass.

DMI response to alfalfa over grass



INDF passage rate response to alfalfa over grass



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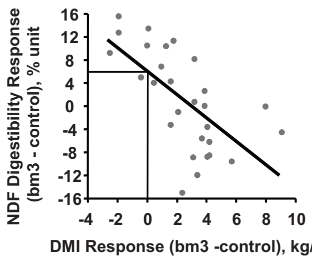
Relationship between DMI response and NDF digestibility response

Oba and Allen, 1999, J. Dairy Sci. 82:135

IVFD bm3 - control = 9.7 % units (49.1 - 39.4%)

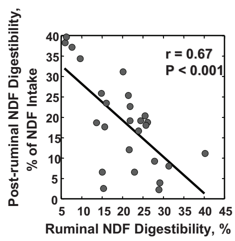


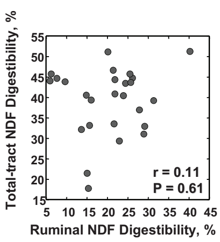
r = 0.64

***P* < 0.001**

Compensatory digestion (one example)

Oba and Allen, 2000, J. Dairy Sci. 83:1333-1358





Passage rate is affected by forage characteristics

Comparison of bm3 corn silage to isogenic control in 29% and 38% NDF diets (Oba and Allen, 2000, J. Dairy Sci. 83:1350)

	29% NDF		38% NDF		Significance, *P*		
	bm3	control	bm3	control	NDF	CS	NDFxCS
INDF kp, h^{-1}	3.73	3.13	3.55	3.27	0.81	0.0001	0.09

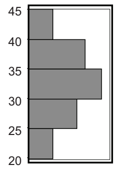
Comparison of alfalfa silage (43% NDF) to orchardgrass silage (48% NDF) (Voelker Linton and Allen, 2005)

	alfalfa	orchardgrass	Significance, *P*
INDF kp, h^{-1}	2.93	2.52	0.03

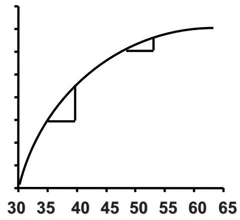
Factors besides DMI affect NDF digestibility in vivo

Oba and Allen, 1999, J. Dairy Sci. 82:135

- 28 cows fed a common diet
- Total tract NDF digestibility ranged from 21.6 to 42.2%
- DMI ranged from 21.3 to 32.1 kg/d
- <13% of variation explained by DMI as % of metabolic BW ($r^2 = 0.127$, $P = 0.06$, RMSE = 5.5)

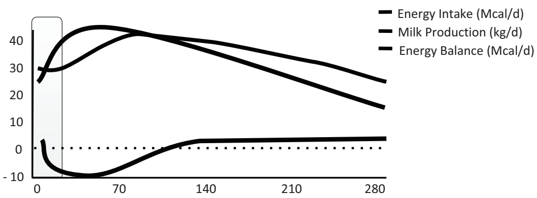


Response to enhanced IVFD likely dependent upon initial IVFD



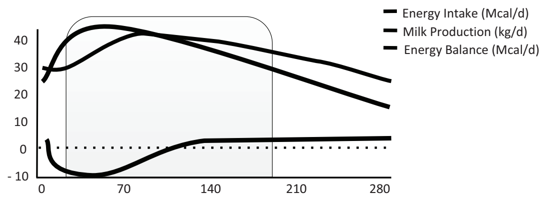
Fresh cows:

- low plasma glucose and insulin concentrations, insulin resistant
- mobilizing fat: elevated NEFA and ketones
- risk of DAs, acidosis, ketosis, fatty liver
- feed intake controlled by oxidation of fat in the liver
- forages with long retention time in rumen (e.g. grass hay or silage, cereal grain straws), limit finely chopped forages



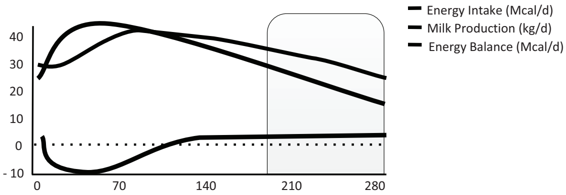
Early to mid-lactation cows:

- High glucose demand, low NEFA, ketones
- Need glucose precursors
- Rumen fill limits feed intake
- Feed higher starch, lower forage NDF diets
- Forages with short ruminal retention time i (e.g. alfalfa hay or silage; corn silage, especially low-lignin hybrids), limit grasses



Late lactation cows:

- plasma glucose, insulin and insulin sensitivity increased
- partitioning energy to body reserves
- intake less limited by distension and more by fermentation acids
- "flex-fuel" cows: lower glucose demand for milk production (need less starch)
- feed higher NDF diets with high NDF digestibility
- forages with longer ruminal retention time OK (e.g. grass hay or silage)



Symptoms of poor forage NDF digestibility

- Lower producing cows: DMI increased
- Higherproducing cows: DMI decreased
- Lower peak milk yield

Summary

- Concentration and digestibility of forage NDF and forage type (legume, perennial grass, annual grass) are the primary factors related to the filling effect of diets.
- Passage rate is affected by forage digestion characteristics as well as DMI.
- In vitro NDF digestibility is extremely variable and cannot be compared across forage type.
- Large variation in digestibility of NDF among cows fed the same diet.
- Benefits of reducing forage NDF concentration and increasing forage NDF digestibility are greater for high producing cows.

Theoretical impact of NDF quality on enteric methane production

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Current incentives to reduce anthropogenic greenhouse gas emissions are meant to prevent even more drastic climate changes. Ruminant production is noted as a main source of greenhouse gas emissions. This notion became more eminent after the claim by Steinfeld et al. (2006) that global livestock production is a source of greenhouse gas emissions of similar magnitude than global transport activities. In particular ruminant production contributes to greenhouse gas emission as a result of enteric production of methane (CH_4). Various measures have been proposed to mitigate enteric CH_4 emission. These measures address almost all nutritional factors also relevant for evaluation of feeding value and animal productivity, including feed intake, feed digestibility, carbohydrate composition, fat and crude protein content, resistance to rumen degradation, and so on. In this respect, evaluating the potential for CH_4 emission and feed evaluation have a lot in common, and are basically two sides of the same coin.

Ruminant diets normally have a main roughage component with a relatively high fibre content (analyzed as Neutral Detergent Fibre, NDF). Hence, components with a high NDF content are associated with enteric CH_4 emission by ruminants. Grass products are the main dietary component in temperate regions and their feeding value varies with grassland management and farming conditions (soil fertilization, grazing strategy, harvesting and conservation, feeding strategy, manure storage and manure application). The 'quality' of grass NDF reflects to a large extent grassland management and may have a significant impact on CH_4 emission. It will be evaluated here to what extent and how NDF 'quality' affects enteric CH_4 emission.

Principles of enteric methane production

Three causal factors can be identified that determine enteric CH4 emissions; 1) the amount of organic matter fermented in the rumen and large intestine, 2) the efficiency of microbial growth on substrates incorporated or fermented, and 3) the type of volatile fatty acid (VFA) produced from fermented organic matter (Bannink et al., 2005). Hydrogen is produced with the production of acetate and butyrate, whereas hydrogen is utilized with the production of propionate and other minor VFA such as branched chain VFA. Dependent on microbial growth on ammonia or protein as a nitrogen source, also a relatively minor amount of hydrogen is produced or utilized, respectively (Mills et al., 2001). The net balance of hydrogen production and utilizing processes in the rumen and the large intestine always results in a large net surplus of hydrogen which feeds methanogenesis and enteric CH4 emission.

One of the three causal factors, the type of VFA produced, appears to depend on pH with fermentation of rapidly degradable or fermentable carbohydrates (i.e. soluble carbohydrates and starch). A change of pH from 6.5 to 5.5 is associated with a drastic change in the type of VFA produced from these carbohydrates (less acetate, more propionate). Such changes in type of VFA produced affect the accompanying hydrogen yield. Empirical equations have been derived from in vivo data of rumen digestion trials in lactating cows, which identify clear differences in the amount and type of VFA produced, and in the production rate of hydrogen and CH_4, with varying pH. Compared to other substrates, most CH_4 is produced with fermented soluble carbohydrates. Only at low pH CH_4 production from fermented NDF seems to be higher (Dijkstra et al., 2007; Bannink et al., 2010). The lowest CH_4 emissions were established for fermented protein, and intermediate CH_4 emissions for fermented starch.

Because feed intake, type of carbohydrate and resistance to rumen fermentation all affect the three causal factors of enteric CH_4 emission identified above, they all need to be taken into account when quantifying enteric CH_4 emission. Predictive empirical equations do not take all of these factors into account and, hence, mechanistic approaches are to be preferred when the aim actually is to identify the underlying mechanisms of enteric CH4 emission (Ellis et al., 2008).

NDF quality

The 'quality' of NDF is a rather imprecisely defined term, but it may involve various NDF characteristics, such as the accessibility for microbial degradation, particle size, and some structural characteristics (affecting animal behaviour). These characteristics become apparent to a large extent from in situ incubations in the rumen under standardized animal and feeding conditions. These standardized in situ incubations reveal to us the so-called intrinsic degradation characteristics of NDF, which are presumed here as indicative of its 'quality'. However, the effective quality of this NDF will also depend on the fermentation conditions, affected by the level of feed intake and associated pH, retention time of NDF, as well as dynamics of digesta passage and saliva production. Also, the effective quality of NDF will be dependent on the non-NDF fractions in the diet (other carbohydrates, protein, fat, organic acids) that may also affect fibrolytic activity and enteric pool size of fibrolytic micro-organisms (Dijkstra et al., 1992). From this, it can be concluded that NDF quality cannot be treated independently from other nutritional factors.

NDF quality of grass herbage and enteric CH4 emission

For an accurate evaluation of the consequences for enteric CH_4 emission, it is prerequisite to take notice of the fact that variation in NDF quality can be accompanied by variation in feed intake, in the dietary content of other chemical fractions, in enteric fermentation conditions, in diet digestibility and in animal productivity. Systematic studies with in vivo observation in grass herbage-fed cattle appear lacking. For this reason a theoretical study was performed by Bannink et al. (2010). They used a dynamic, mechanistic simulation model of microbial fermentation processes in the rumen and large intestine of cows (Mills et al., 2001), which included an update of the representation of the stoichiometry of VFA production from fermented substrate. This stoichiometry was pH-dependent for fermented soluble carbohydrates and starch (Bannink et al., 2008; 2010). This updated version of the model is in current use as a 'Tier 3' approach to estimate enteric CH_4 emission in dairy cattle in the Dutch national inventory report of greenhouse gas emissions (Brandes et al., 2009).

Several qualities of grass herbage and grass silage were evaluated by adopting estimates of chemical composition and intrinsic degradation characteristics for starch (only relevant for the small concentrates supplementation), protein and NDF for each grass type that were derived by Reijs (2007). Variation in grass type involved: 1) high rate of N fertilization (350 kg N/ha/yr) vs. a low rate (150 kg N/ha/yr), 2) grass silage from early-cut grass (3000 kg DM/ha) vs. from late-cut grass (4500 kg DM/ha), and 3) grass herbage vs. grass silage. As a result, grass NDF quality varied by the following characteristics: content varied from 463 to 551 g/kg DM, NDF degradability from 60% to 91%, fractional degradation rate of degradable NDF from 0.72/d to 1.31/d, crude protein content from 118 to 246 g/kg DM, and soluble carbohydrate content from 50 to 162 g/kg DM.

At a feed intake level of 18 kg DM/d, including 90% grass product and 10% concentrates, the simulated amount of CH_4 emitted per kg feed DM was higher with low N fertilization compared high N fertilization, irrespective of the unit of expression used (g CH_4/d, g CH_4/kg DM or g CH_4/kg milk). A late-cut grass silage resulted in higher (with high N fertilization) or equal (with low N fertilization) CH_4 emission per kg DM. When expressed per kg milk, late-cut grass silage always gave higher CH4 emission estimates. Grass herbage had a higher CH_4 emission per kg DM, but equal CH_4 emission per kg of milk compared to early-cut grass silage.

When expressing CH_4 as a % of ingested gross energy (according to the 'Tier 2' approach in the IPCC guidelines; IPCC, 1997), estimates differed from the previous 6.0% previous default value and the more recently proposed 6.5% default value. The most extreme values in the simulation study differed by about 1%, which is more than 15% of the default IPCC estimate. Again, less N fertilization resulted in a higher estimate, whereas response to the moment of grass-cutting varied with N fertilization level.

It is concluded that with evaluation of CH_4 emission for a wide range of NDF qualities as a result of grassland and farm management, the IPCC default estimates probably are not very accurate in reproducing variation in CH_4 emission. Simulation results were also compared to some reported observations for cattle fed grass herbage. Inspection of trial results with lactating and non-lactating cattle indicates that similar responses in CH_4 emission have been observed as predicted by the simulation studies discussed here. Although some systematic bias between predicted and observed values remains, the observed and predicted trends correspond reasonably.

Conclusions

The quality of NDF varies with grassland management and farming conditions, and this variation may be accompanied with variation in contents of NDF, crude protein, soluble carbohydrate and fermentation products, and in NDF degradation characteristics. Although not shown in the present work, it may also be accompanied with variation in feed intake. This implies that, with the aim to relate NDF quality to CH_4 emission, NDF quality does not stand on its own and at least three principal factors need to be included in the evaluation: dry matter intake, feed digestibility and chemical composition of feeds. It is concluded that a higher grass NDF quality is associated with management practices that lead to grass with a higher feeding value, a lower NDF content and a higher crude protein content, and less CH_4 emission. A detailed evaluation of effects on CH_4 emission to be expected requires that also details about grass quality and nutrition are taken into account.

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Theoretical impact of NDF quality on enteric methane production

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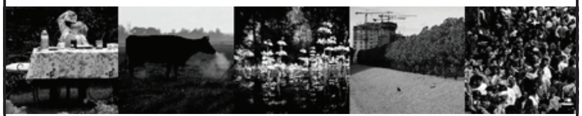
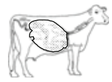


Two separate aspects to be evaluated?

- NDF quality
  - digestibility (rumen & large intestine)
  - depends on whole diet
  - depends on level of feed intake (animal)

- Enteric methane (CH_4) production
  - fermentation (rumen 90% & large intestine 10%)
  - yield of fermentation end-products, including gases
  - depends on whole diet
  - depends on level of feed intake (animal)

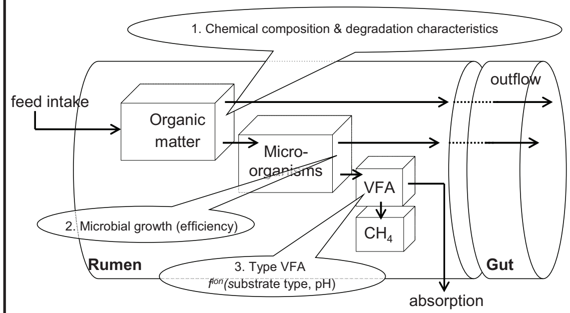


- Evaluation 'NDF quality & CH_4' ….two sides on same coin

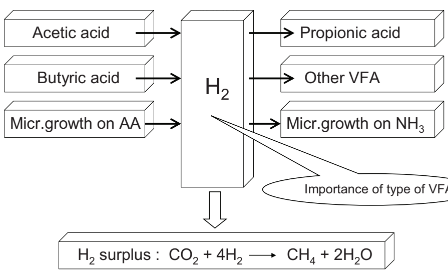
1. Enteric CH_4 production

- General nutritional factors driving CH_4 yield
  - feed intake
  - digestibility characteristics (digestion as outcome ?)
  - diet composition
- Represented in most empirical CH_4 prediction equations
- Rumen factors driving CH_4 yield
  - amount of fermented organic matter
  - type of substrate fermented and type of VFA formed
  - rumen fermentation 'conditions' (e.g. pH, fractional growth rate)
  - retention time / passage rate (resistance against rumen degradation)
- Representation
  - in few empirical prediction equations
  - rather in mechanistic approaches

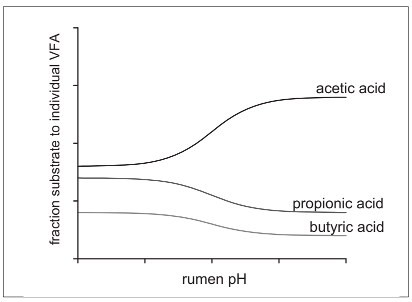
Rumen CH_4: 3 causal factors



Rumen CH_4 ≈ H_2-balance in rumen



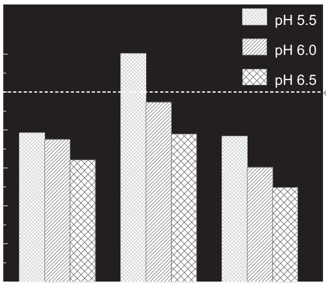
pH-dependency VFA from 'soluble carbohydrates'



In vivo data
rumen digestion
lactating cows

Analysis
substrate type
roughage vs. concentrates
efficiency microbial growth
kinetics VFA-absorption
pH

CH_4: differences between substrates



Dijkstra et al. (2007)
Bannink et al. (2010)

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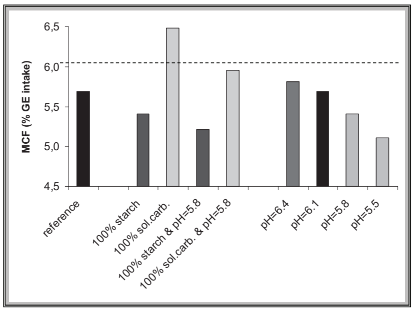
Sensitivity analysis: sugars *vs.* starch + pH



Reference
20 kg DM intake
42% grass herbage
9% grass silage
27% maize silage
23% concentrates
average pH = 6.1
6% sol. carbohydrates
18% starch
46% NDF
17% crude protein
4% crude fat
8% ash

Bannink et al., 2005

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Quantify effects on CH_4: in conclusion

- Account for confounding of various factors relevant for CH_4
  - types of fermentable substrates
  - level of feed intake
  - fermentation conditions (e.g. pH)
  - retention time (fract. passage rate)
- Need to evaluate CH_4 from perspective of whole diet
- Quantify CH_4 from fermentation
  - mechanistic approach
  - dietary & animal characteristics

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2. NDF quality

- NDF quality depends on NDF characteristics
  - accessibility, particle size, structural characteristics
  - intrinsic degradation characteristics (in situ incubations)
- Effective NDF quality depends on fermentation conditions
  - pH
  - retention time, rumen fill
  - level of feed intake (meal pattern)
  - saliva production
- Non-NDF fraction
  - other carbohydrates, protein, fat, organic acids
  - affects pH, passage of particles & fluid, fibrolytic acitivity (pH, fat)
  - affects pool size fibrolytic micro-organisms
    - substrate competition (at low CP%)
    - predation by protozoa

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Variation in NDF quality

- NDF quality varying with type of roughage / feedstuff
  - variation in digestibility
- NDF quality varying with farm management
  - grass / maize varieties
  - fertilization management
  - harvest management (stage of plant maturity at cut)
  - ensiling management
  - specific treatments (e.g. additives)
- But, variation in NDF quality always accompanied by
  - variation in other chemical fractions
  - variation in digestibility & feeding value
  - variation in feed intake & animal productivity
- Effects on CH_4 hence to be evaluated in this respect !

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3. Example varying NDF quality in grass diet

- Grass herbage (*GH*) *vs.* grass silage (*GS*)
- High N-fertilization (*HF*, 350 kg N/ha) *vs.* low N fertilization (*LF*, 150 kg N/ha)
- Early-cut (*EC*, 3000 kg DM/ha) *vs.* late-cut (*LC*, 4500 kg DM/ha)
- Varying DM intake (14 – 17 kg DM/d; 90% grass, 10% concentrates)

Reijs et al., (2007)
Bannink et al. (2010)

Assumptions on NDF quality

	NEL *(MJ/kg DM)*	***NDF*** *(g/kg DM)*	***U-NDF*** *(%)*	***D-NDF*** *(%)*	***kd-NDF*** *(/d)*	***SU*** *(g/kg DM)*	***org.acids*** *(g/kg DM)*	***CP*** *(g/kg DM)*
GH - HF	6.8	493	*9*	*91*	*1.31*	110	0	246
GS - HF-EC	6.3	463	*10*	*90*	*1.08*	50	102	233
GS - HF-LC	5.8	539	*25*	*75*	*0.86*	100	58	177
GH - LF	6.3	515	*11*	*89*	*0.99*	162	0	169
GS - LF-EC	6.0	470	*100*	*60*	*0.86*	110	109	156
GS - LF-LC	5.7	551	*25*	*75*	*0.72*	130	71	118

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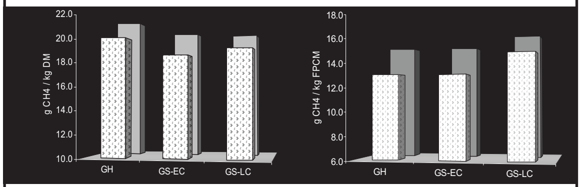
Grass NDF quality & CH_4

Bannink et al., 2010

18 kg DM/d (90% grass & 10% concentrates)



GH = grass herbage; **GS** = grass silage
EC = early cut ; **LC** = late cut
= high N-fertilization = low N-fertilization

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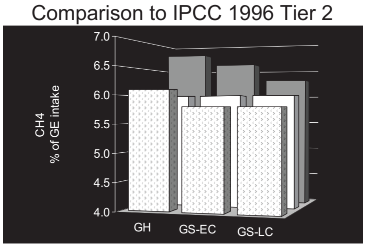
Grass NDF quality & CH_4

Bannink et al., 2010

Comparison to IPCC 1996 Tier 2



GH = grass herbage; **GS** = grass silage
EC = early cut ; **LC** = late cut
= high N-fertilization = low N-fertilization = IPCC Tier 2

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Variation in NDF quality: in conclusion

- NDF quality varies with
  - grassland management
  - fertilization management
  - harvest / conservation method
- Variations in grass NDF quality associated with
  - variation content NDF
  - variation content crude protein
  - variation content soluble carbohydrates
  - variation content fermentation products
  - variation in feed intake (not shown) & milk yield
- Hence,
  - variation NDF quality does not stand on its own
  - evaluate effects of NDF quality in this manner !

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Concluding

- Principal factors affecting CH_4 emissions
  - 1st dry matter intake
  - 2nd digestibility
  - 3rd chemical composition
- Higher grass NDF quality
  - DM intake (not shown) ↑ g CH_4/kg DM ↓ g CH_4/kg milk ↓ %GE ↓
  - NDF digestibility ↑ g CH_4/kg DM ↑ g CH_4/kg milk ↓ %GE ↑
  - NDF content ↓ g CH_4/kg DM ↓ g CH_4/kg milk ↓ %GE ↓
  - sol.carb. content ↓ g CH_4/kg DM ↓ g CH_4/kg milk ↓ %GE ↓
  - protein content ↑ g CH_4/kg DM ↓ g CH_4/kg milk ↓ %GE ↓
  - ferm.prod. content ↑ g CH_4/kg DM ↓ g CH_4/kg milk ↓ %GE ↓
- In general, higher grass NDF quality ……
  - lower CH_4 emission
  - but may be equal / higher % GE intake (= IPCC Tier 2 approach !)
  - quantifying CH_4 =
    considering simultaneous effects of all chemical fractions & DMI

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Thank you for your attention

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Financial support by the
- Dutch Product Board Animal Feed
- Ministry of Agriculture, Nature & Food Quality

is gratefully acknowledged

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Effects of *Saccharomyces cerevisiae* yeast (CNCM I-1077) on ruminal fermentation and fibre degradation

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Introduction

The ban on antibiotics has stimulated renewed interest in non-antibiotic alternatives to manipulate rumen fermentation. Yeast cultures, mainly from strains of *Saccharomyces cerevisiae* (SC), have been the alternatives most studied and used in ruminants. Some consistent effects were observed at animal level (e.g. increase in DM intake and milk production), which seems to be related to the stimulation of cellulolytic bacteria (Newbold et al., 1996) and, thereby, increasing the potential to digest fibre in the rumen. Such benefits in microbial population arise from the ability of SC to prevent a decline in rumen pH by decreasing lactate production and/or increasing utilization of lactic acid by some bacteria, oxygen scavenging and supply of growth factors (Jouany, 2006). Experimental support for the effects of SC on rumen fibre degradation, regardless of the digestibility of the basal diet, is inconsistent (Roa et al., 1997; Krehbiel et al., 2006). The two experiments (Exp) described here were designed to examine the effects of a live yeast strain of SC (CNCM I-1077) on *in situ* ruminal degradation of 40 maize (MS; Exp. 1) and 66 grass silages (GS; Exp. 2). Within each type of silage, silages differed in chemical composition and *in situ* ruminal degradation. In both Exp, ruminal activity was also assessed through pH, volatile fatty acids (VFA), lactate and ammonia N (NH_3-N) measurements.

Materials and methods

Animals, diets and management. Three rumen-fistulated, non-lactating Holstein cows, housed in individual tie stalls, were used in each Exp. In Exp1, cows were fed MS, concentrate feed and hay (48:42:10; DM basis) supplemented with 0 (SC0) and $1x10^{10}$ cfu (SC1) of SC/day. The SC was offered in the morning feed dosed directly into the rumen. In Exp 2, cows were fed GS and concentrate feed (60:40, DM basis) supplemented daily with 0 (Yeast-) and $1x10^{10}$ cfu of SC (Yeast+) mixed with the concentrate. Animals were fed twice daily and fresh water was offered *ad libitum*. Well-fair was respected according to the Portuguese law (Port. nº 1005/02).

Forage samples. The forage samples (40 MS (Exp1) and 66 GS (Exp. 2)) were obtained from Segalab (AGROS, Portugal). Samples of all forages were dried in a forced-air oven at 65ºC for 48 h and ground through a 4-mm screen. Each type of silage was divided in 2 groups (low (LFD) and high neutral-detergent fibre (NDF) degradation (HFD)), according to its *in sacco* degradation (preliminary results obtained for each experiment with cows fed with the basal diet). For MS (Exp. 1), NDF degradation (NDFdeg) varied between 20 and 30% for LFD group (*n* = 20) and between 35 and 45% for the HFD group (*n* = 20). For GS (Exp. 2), NDFdeg was <50% (n = 38) for the LFD and > 50% (n = 28) for the HFD group.

Rumen fluid collection. Rumen fluid was collected from each cow on 2 non-consecutive days, during the incubation period, before feeding (0h) and at 2, 4 and 8 h after feeding. The pH values of the rumen fluid were measured immediately with a digital pH meter (WTW, pH 530, Weilheim, Germany). Thereafter, samples were kept frozen at • 20ºC for NH_3-N, VFA and lactate analyses.

Degradation studies. Samples were incubated in nylon bags (Nybolt PA 40/30, Zurich, Switzerland) (methodology described by Guedes and Dias-da-Silva 1994) 2h after the morning feeding and withdrawn after 36 h. Incubations were repeated once in each cow making, in total, 6 replications/sample. After removal from the rumen, bags were washed with tap water in a washing machine, and dried at 65ºC for at least 24 h in a forced-air oven. Bags were weighed and residues analysed for NDF.

Laboratory analyses. Dry and ground (1-mm) silage samples and residues from incubated samples were analysed for ash (AOAC 1990), crude protein as 6.25 x N (Kjeldahl method, AOAC, 1990) and starch (only maize silages; Salomonsson et al. 1984). The procedures of Van Soest et al. (1991) were used to determine NDF which was expressed on an ash-free basis. For MS samples, -amylase was used prior to NDF analysis. Acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed according to Robertson and Van Soest (1981). NDF expressed with residual ash was used for the determination of NDFdeg. Samples of rumen fluid were analysed for NH_3-N (Novozamsky et al. 1974), VFA by gas-liquid chromatography (Shimadzu GC-141 B, Kyoto, Japan; Czerkawski, 1976), and lactate by an enzymatic assay procedure (K-DLate 02/06, Megazyme, Ireland).

Statistical analyses. Statistical analyses used SAS (1990) software. Fermentation data were analysed using MIXED procedure and repeated measures with the model $Y_{ijk} = \mu + D_i + T_j + (D \times T)_{ij} + A_{ik} + {}_{ijk}$, where μ is overall mean, D_i is the fixed effect of diet, T_j is the fixed effect of time after feeding, $(D \times T)_{ij}$ is the fixed interaction effect of D_j and T_j, A_{ik} is the random effect of animal within D_i, and ${}_{ijk}$ is the random error. Time after feeding was used as repeated measure. The NDFdeg data were analysed using MIXED procedure with the model $Y_{ijk} = \mu + D_i + SQ_j + (D \times SQ)_{ij} + A_{ik} + {}_{ijk}$ were SQ is the fixed effect of silage quality. Differences between treatment means were determined by Student's multiple range *t*-test.

Results and discussion

Chemical composition of incubated feeds. Among the available silage samples, we observed a wide range of variation in the chemical components of the incubated feeds as shown in Table 1.

Table 1 Mean, minimum and maximum values and standard deviation (SD) of the dry matter content (DM; %) and chemical composition (% of DM) of incubated samples.

			Mean	Minimum	Maximum	SD
Experiment 1						
Maize silages (n=40)	DM		32.6	27.0	42.9	3.9
		Ash	4.5	3.9	6.1	0.6
		CP	8.1	7.0	9.6	0.7
		NDF	47.9	38.5	58.2	4.7
		ADF	26.6	21.1	30.6	2.4
		ADL	3.1	2.2	3.8	0.4
		Starch	25.1	17.0	37.8	4.6
Experiment 2						
Grass silages (n=66)	DM		41.4	15.7	70.1	13.5
		Ash	10.5	6.4	16.8	2.2
		CP	11.3	5.4	17.9	2.3
		NDF	55.1	43.0	66.9	5.6
		ADF	37.2	26.6	46.5	4.0
		ADL	3.9	2.0	8.0	1.2

CP, crude protein; NDF, neutral-detergent fibre; ADF, acid-detergent fibre; ADL, acid-detergent lignin; SD, standard deviation.

Effect of SC on rumen fermentation characteristics, Exp.1. Inclusion of SC in the diet based on MS led to higher ruminal pH, (*P*<0.001). SC also reduced pH variation during the day (Fig. 1A). Concentration of lactate was reduced (*P*<0.001) by SC supplementation (Fig. 1B). The pattern of diurnal fluctuation of ruminal NH_3-N was similar among diets. Inclusion of SC in the diet increased acetate (*P*<0.05), propionate (*P*<0.001) and butyrate (*P*<0.05) concentrations and acetate:propionate ratio (*P*<0.001) (Fig. 1C).

Exp.2. A tendency (P=0.099) was observed for higher pH values (Fig. 2A) with SC supplementation compared to no SC supplementation (6.38 *vs.* 6.55); pH was not affected by time after feeding (P> 0.05). Supplementation with SC affected the lactate concentration (P<0.05; Fig 2B) with the highest values found for diet without SC (0.99 *vs.* 0.78 mmol/L), as observed in Exp1 with MS. Lactate fluctuation after feeding was lower for Yeast+ than for Yeast- diet, which explains the observed interaction between time and diet (P<0.05). The NH_3-N concentration was affected (P<0.05) by diet, increasing from Yeast• diet (257 mg/L) to Yeast+ diet (286 mg/L).

It has been suggested that the effect of SC on ruminal pH may be due to reduced lactate concentrations in the rumen (Williams et al., 1991), through the increase of activity of lactate-utilising bacteria such as *Selenomonas ruminantium* (Nisbet and Martin, 1991; Callaway and Martin, 1997) or *Megasphaera elsdenii* (Chaucheyras et al., 1996; Callaway and Martin, 1997) and/or through the decrease of activity of lactate producing bacteria (Martin and Nisbet, 1992). In our study, the decrease in lactate concentration was observed in both experiments, the effect being more evident in Exp1 (MS diet). Variations in pH observed in our study due to SC inclusion are consistent with the lactate concentrations.

A B C

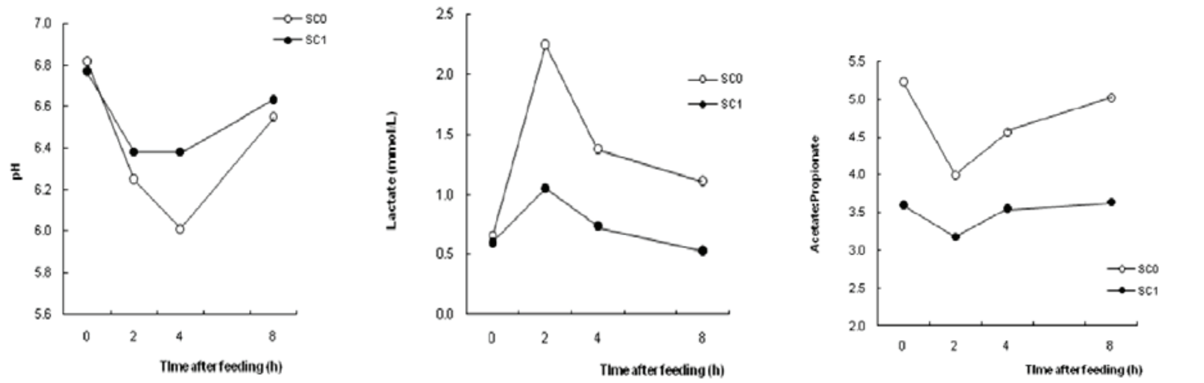

Figure 1 Effects of supplementation of maize silage based diet with 1x10[10] cfu *Saccharomyces cerevisiae* per day on pH (A), lactate concentration (B) and acetate:propionate ratio (C).

A B C

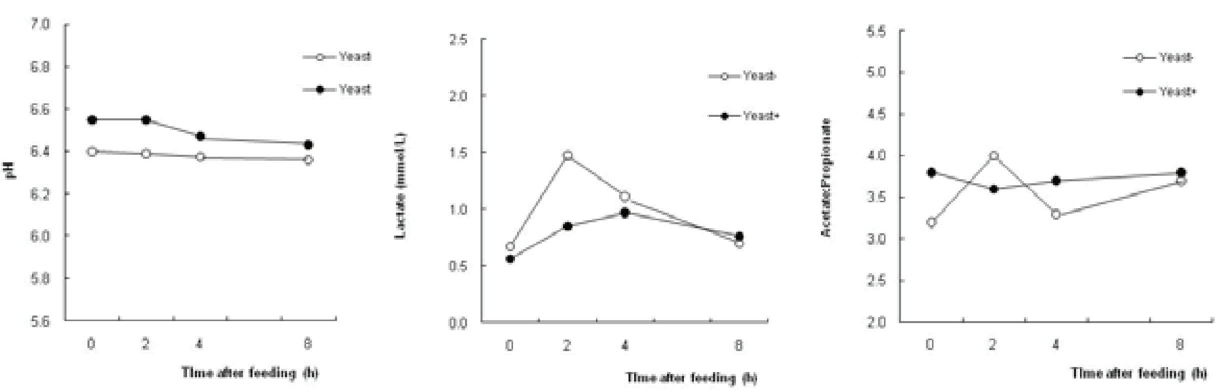

Figure 2 Effects of supplementation of grass silage based diet with 1x10[10] cfu *Saccharomyces cerevisiae* per day on pH (A), lactate concentration (B) and acetate:propionate ratio (C).

Effect of SC on fibre degradation of silage samples. Effect of SC supplementation on NDFdeg of silages is presented in Table 2. Supplementing SC had a positive effect on NDFdeg of both silages (P<0.0001 for MS and GS). In both Exp an interaction effect was observed between silage quality and diet (SC supplementation), showing that SC effect on NDFdeg depends on silage quality.

The most original aspect of the research presented here is the fact that we verify that the extension of the effects of SC inclusion in the diet depended on the initial NDFdeg of the silages (Figure 3). Whereas, in Exp1, SC supplementation at a level of $1x10^{10}$ cfu/day increased (*P*<0.05) NDFdeg of the maize silages in the LFD group, it had no significant effect on the HFD group (Table 2). In Exp. 2, both groups were positively affected by SC supplementation, but the effect was more pronounced with LDF GS than with HDF GS(+11.5 vs. 8.9 percent units).

Table 2 Effects of *Saccharomyces cerevisiae* supplementation and silage quality on neutral detergent fibre degradation (NDFdeg) of silages incubated in the rumen

Exp. 1	Maize silage based diets							
	SC0		SC1			Probability		
	LFD (n=20)	HFD (n=20)	LFD (n=20)	HFD (n=20)	SE	Diet	Silage quality	Interaction
NDFdeg	24.7[a]	39.3[c]	30.6[b]	41.0[c]	0.001	<0.0001	<0.0001	0.0082

Exp. 2	Gras silage based diets							
	Yeast-		Yeast+			Probability		
	LFD (n=38)	HFD (n=28)	LFD (n=38)	HFD (n=28)	SE	Diet	Silage quality	Interaction
NDFdeg	43.2[a]	55.0[c]	51.2[b]	60.1[d]	0.001	<0.0001	<0.0001	0.0025

SC0 and Yeast- basal diet without *Saccharomyces cerevisiae* (SC), SC1 and Yeast+ basal diet with $1x10^{10}$ cfu SC/day; HFD group, high fibre degradability group; [c]LFD group, low fibre degradability group. SE - pooled standard error; Values in the same row, within the same effect, with different letters differ according to the Student's *t*-test (*P*<0.05).

Conclusions

The supplementation of maize and grass silages with SC showed beneficial effects on the ruminal environment and on the fibre degradability of maize and grass silages. The extension of this latest effect was higher when the forage fibre quality was lower. The improvement of the fibre degradability was observed even under non-acidogenic conditions. The evidence of an interaction between forage quality and SC supplementation found in this study might explain some lack of consistency between previous reports on SC effect.

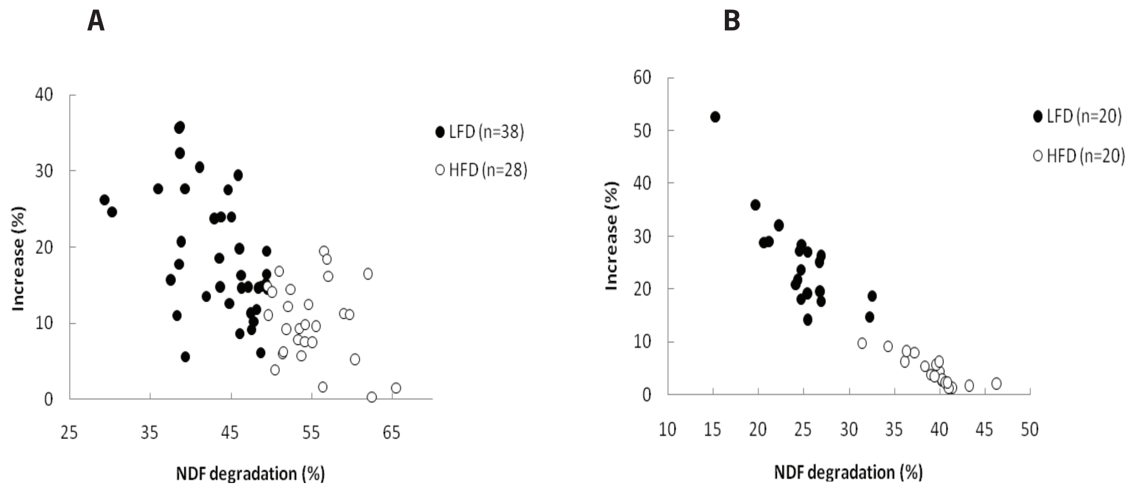

Figure 3 Effects of supplementation with $1x10^{10}$ cfu *Saccharomyces cerevisiae* per day on fibre (NDF) degradation of maize silages (A) and grass silages (B) after 36 h of incubation in the rumen of cows: HFD, high fibre degradation groups and LFD, low fibre degradation groups.

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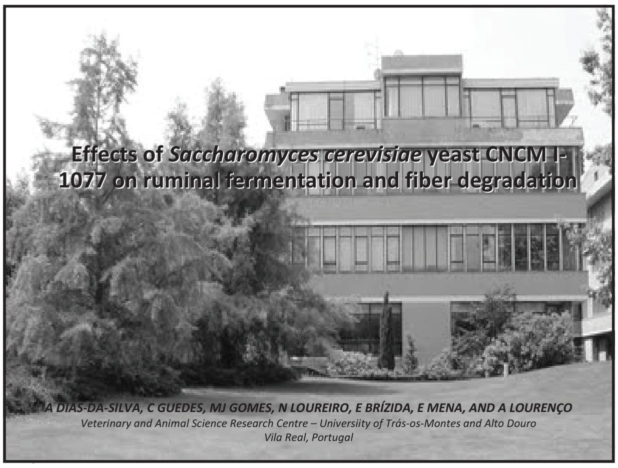

Effects of *Saccharomyces cerevisiae* yeast CNCM I-1077 on ruminal fermentation and fiber degradation

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BACKGROUND

RESEARCH PRIORITY OF NUTRITIONISTS

↓

IMPROVE RUMEN FUNCTION

REDUCE ENERGY AND NUTRIENT LOSSES → INCREASE PRODUCTION EFFICIENCY OF DOMESTIC RUMINANTS

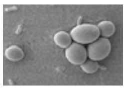
BACKGROUND

BAN OF ANTIBIOTICS

↓

RENEWED INTEREST IN ADITIVES TO MANIPULATE RUMEN FUNCTION

Saccaromyces cerevisiae *(SC)*



MOST STUDIED ALTERNATIVE IN RUMINANTS

BACKGROUND

RUMEN LEVEL

BACKGROUND

Yeast preparation

- O_2 uptake → ↓ Redox potential
- Micronutrient supply → ↑ Total bacteria, celulolytic bacteria (protozoa, fungi)
- Glucose uptake → pH stabilization
- Lactic acid metabolism → pH stabilization

pH stabilization → ↑ Total bacteria, celulolytic bacteria (protozoa, fungi)

↓

↑ Polysaccharide depolymerases

↓

↑ Rate of OM digestion

Jouany, J.-P., 2006. *Animal Reproduction Science*, 96:250-264.

BACKGROUND

pH stabilization

↓

↑ Total bacteria, cellulolytic bacteria (protozoa, fungi)

↓

↑ Polysaccharide depolymerases

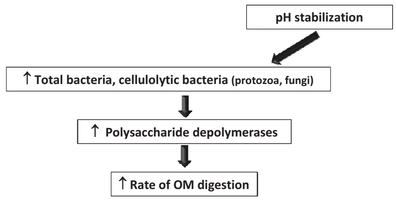
↓

↑ Rate of OM digestion

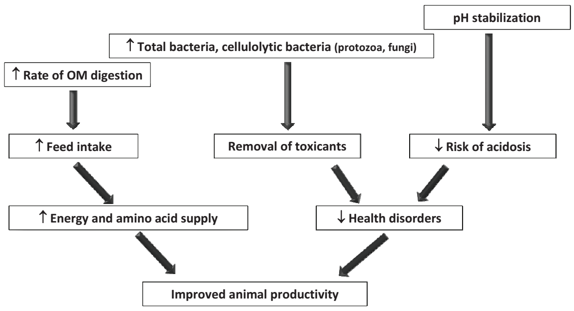
BACKGROUND

ANIMAL LEVEL

BACKGROUND



BACKGROUND



Jouany, J.-P., 2006. *Animal Reproduction Science*, 96:250-264.

BACKGROUND

Effects of SC on animal productivity

Item	n exp	Treatment		P-value
		Control	Yeast	
DMI (g/kg BW)	47	34.6	35.0	*
Milk yield (g/kg BW)	59	46.5	47.7	***
Milk fat content %	57	3.80	3.85	†
Milk protein content %	52	3.20	3.19	NS

(Desnoyers et al., 2009. J. Dairy Sci. 92:1620-1632)

BACKGROUND

Effects of SC on rumen parameters

Item	n exp	Treatment		P-value
		Control	Yeast	
Rumen pH	97	6.31	6.34	*
VFA (*mM*)	77	95.2	97.3	**
Lactate (mg/L)	16	1.21	1.13	†
OM digestibility (%)	45	70.2	71.0	**

(Desnoyers et al., 2009. J. Dairy Sci. 92:1620-1632)

BACKGROUND

The effect of SC on fibre degradation are less well studied and the results are not consistent among authors

Aim

To study the effects of the CNCM I - 1077 (Levucell SC®) yeast on *in situ* ruminal fibre degradation of :

- maize silages (**MS**) - Experiment 1 (**Exp. 1**)
- grass silages (**GS**) - Experiment 2 (**Exp. 2**)

MATERIAL AND METHODS

Animals

- 3 Holstein cows
- Non-lactating
- Fistulated in the rumen

MATERIAL AND METHODS

Diets

Experiment 1

SC0 (basal diet)
maize silage + concentrate feed + hay (48:42:10, DM basis)

SC1
basal diet + $1x10^{10}$ cfu of SC/day

Experiment 2

Yeast - (basal diet)
grass silage + concentrate feed (60:40, DM basis)

Yeast +
basal diet + $1x10^{10}$ cfu of SC/day

MATERIAL AND METHODS

Management

- Housed in individual tie stalls
- Fed 2 x per day (08:00 and 16:00 h)
- Intake level = 1.2 maintenance ME **(AFRC, 1993)**
- Water *ad libitum*
- Welfare conditions according to the Portuguese law (Port. nº 1005/02)

MATERIAL AND METHODS

Samples

Donated by Segalab (Agros, Portugal)

- Collected in dairy farms in the Northeast region of Portugal
- Non identified varieties of corn **(Exp. 1)**
- ryegrass based grass silages **(Exp. 2)**
- Ground to a 4 mm particle size for *in sacco* incubations

MATERIAL AND METHODS

Rumen fluid collection and measurements

- Collected during 2 non-consecutive days of the incubation period
- 0 h (before feeding) and 2, 4 and 8 h after feeding
- pH values measured immediately
- Samples were kept frozen at −20ºC for ammonia-N, VFA and lactate assay

MATERIAL AND METHODS

Degradability measurements

- Nylon bags Nybolt PA 40/30 (Zurich, Switzerland)
- The bags were introduced in the rumen 2 h after the morning meal and removed 36 h later
- The bags were washed in a washing machine, dried at 65ºC, during 24 h, weighed and the NDF of the residues was determined

MATERIAL AND METHODS

Laboratory analysis

Feed samples and incubated silages (1 mm)

- Ash, nitrogen **(AOAC, 1990)**
- Starch **(Salomonsson et al., 1984)**
- NDF, ADF and ADL **(AOAC, 1990, Van Soest et al., 1991)**

MATERIAL AND METHODS

Laboratory analysis

Rumen fluid

- NH_3-N **(Novazamsky et al., 1974)**
- VFAs **(Czerkawski, 1976)**
- Lactate **(K-DLate 02/06, Megazyme, Ireland)**

MATERIAL AND METHODS

Statistical analysis

- pH, NH_3-N, VFAs and lactate **(SAS, 1990)**

$$Yijk = \mu + Di + Tj + (D \times T)ij + Aik + \varepsilon ijk$$

- NDFdeg

$$Yijk = \mu + Di + SQj + (D \times SQ)ij + Aik + \varepsilon ijk$$

- Treatment means were compared by multiple range *t*-test

Results

Chemical composition of basal diets

Item	Basal diets	
	Exp 1	Exp 2
DM (%)	62.8	62.2
Chemical composition (% DM)		
Ash	6.7	9.7
Crude Protein	13.4	16.7
NDF	**37.4**	**46.7**
ADF	20.2	29.4
ADL	2.9	3.9
Starch*	**22.2**	**13.6**

Results

Chemical composition of Maize silages (n=40)

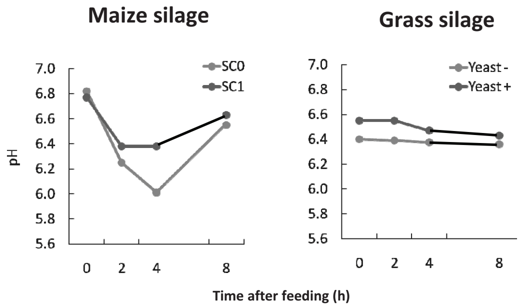
Item	Mean	Minimum	Maximum	SD
DM (%)	32.6	27.0	42.9	38.6
Chemical Composition (% DM)				
Ash	4.5	3.9	6.1	0.55
Crude Protein	8.1	7.0	9.6	0.66
NDF	47.9	38.5	58.2	4.73
ADF	26.6	21.1	30.6	2.40
ADL	3.1	2.2	3.8	0.39
Starch	25.1	17.0	37.8	4.56

Results

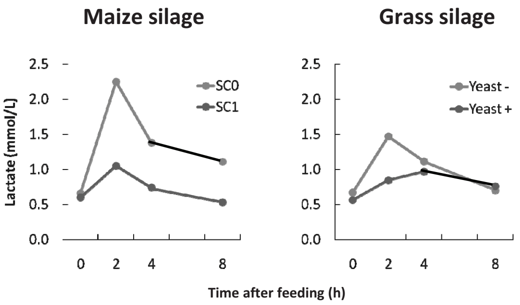
Chemical composition of Grass silages (n=66)

Item	Mean	Minimum	Maximum	SD
DM (%)	41.4	15.7	70.1	13.5
Chemical Composition (% DM)				
Ash	10.5	66.4	16.8	2.2
Crude Protein	11.3	5.4	17.9	2.3
NDF	55.1	43.0	66.9	5.6
ADF	37.2	26.7	46.5	4.0
ADL	3.9	2.0	8.0	1.2

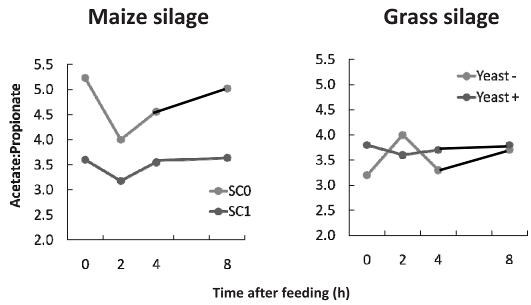
Results



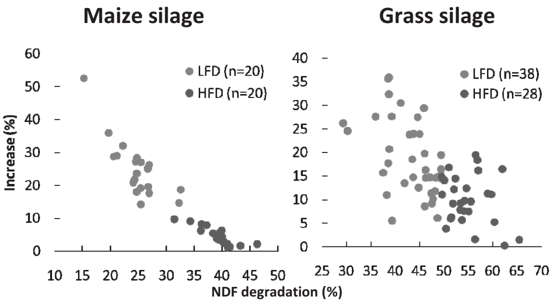
Results



Results



Results



Conclusions

Supplementation with CNCM I-1077

- Beneficial effects on the ruminal environment
- Increase of fibre degradability of maize and grass silages
- Increase of fibre degradability observed with non-acidogenic conditions
- Higher fibre degradability improvement in low quality silage which might explain some lack of consistency between previous reports on SC effect

Implications

Supplementation with CNCM I-1077

- Potential to prevent sub-acute acidosis in dairy cows
- Improve the glucogenic potential of maize silage diets
- Improve the ME of silages

Effect of NDF digestibility on Diet Formulation and Animal Performance

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Introduction

The work of Dr. Mike Allen, Michigan State in the late 80's, measuring NDF digestibility of different corn silage hybrids, initially resulted in much controversy. There was much resistance to the concept that differences in fiber digestibility in corn silage could have an impact on animal performance. He proceeded to do studies with BMR hybrids as well as with conventional hybrids both in lactating cow studies as well as a demonstration of a wide range of digestibility's among hybrids and over years.

The argument was made that there was more variation over years than there was among hybrids. However, he demonstrated that within any year there were hybrids that always were on the top in digestibility and those that were always on the bottom. He then extended this work to forages other than corn silage, essentially demonstrating the same concept.

Today, in the USA, NDF digestibility has become a routine measurement both as an *in vitro* measurement as well as with the use of NIR prediction.

NDF Digestibility Measurements

The method for the measurement of NDF digestibility has not been without controversy. NRC suggested that the appropriate measurement was to do a 48 hour Tilley Terry *in vitro*. Dr. Allen suggested that a 30 hour *in vitro* was much more sensitive in terms of animal response, which was based more on a consideration of rumen retention time and rumen turnover kinetics of a lactating cow. The 48 hour measurement is more suitable to providing a prediction of TDN at maintenance which then could be converted to a NE_L estimate. The 30 hour measurement came closer to being sensitive to intake and the prediction of performance of a cow. The 30 hour measurement was quickly adopted by forage labs in the East and then used as a means to adjust rations. The Midwest continued to use the 48 hour measurement as a method of ranking forages, however, currently Midwestern forage labs are now doing 30 hours measurements as well.

There has been the introduction of the use of the Ankom system to measure fiber digestion. This is attractive because many samples can be analyzed at one time relatively quickly at very reasonable costs. There appear to be some issues with this approach. There appears to be more variation among duplicates which can be due, in part, by bag pore size with sample loss and less uniformity within the fermenter jar system. The Tilley Terry system appears to give more uniform results.

The challenge with the *in vitro* system is rumen fluid preparation, buffer type, and fiber and lignin analyses. The amount of lag before fermentation actually occurs is actually quite variable among labs. Lag needs to be determined. If lag exceeds 3 hours, methodology needs to really be examined closely. With long lag times, measurements of NDFd become problematical at 24 and 30 hours, even if we correct for the lag. This is an *in vitro* procedure and requires good technique.

The use of NDF Digestibility

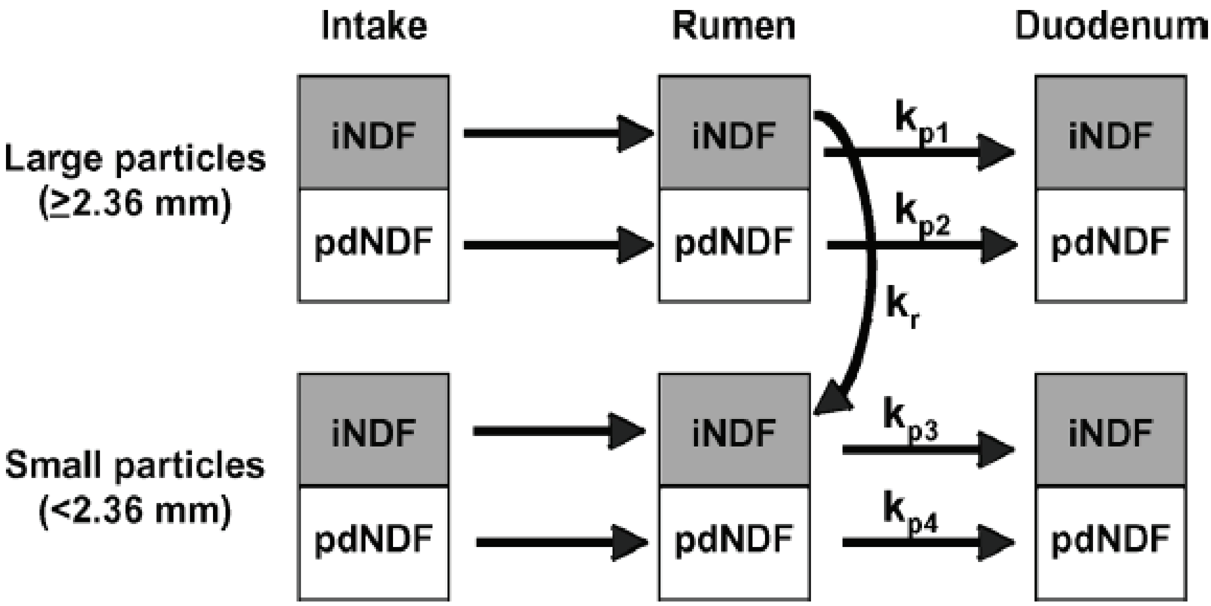
The major problem with the NDF digestibility measurement was how to incorporate the information into a ration program. The 48-hour measurement could be used in the NRC 2001 estimate for net energy, using the Weiss equations, however not many people were using NRC 2001. Van Amburgh, Van Soest and colleagues developed a spreadsheet to estimate the rate of digestion of the available pool. Below is an example of a high protein ryegrass with a 24 hour NDF digestibility.

Values in red are inputs					
Feed name	**Rygrass Silage 21% CP**				
NDF, %DM	**43.70**				
lignin, %DM	**2.40**				
hour	**NDF digestibility**	**A**		**lag, h**	**kd, %/h**
0	**0.00**	**0.8682**	**Unitized A**		
24	**60.50**		**1.0000**	**2.50**	**5.55**
Unavailable NDF, %NDF	**0.13**		**0.3031**		
k1 pool NDF kd, %/h	**9.50**	**Equation 6**			
k2 pool NDF kd, %/h	**0.91**	**Equation 7**			
Normalized P1	**77.87**	**Equation 8**			
NDF pool 1, % NDF	**67.61**				
NDF pool 2, % NDF	**19.21**				
Unavailable NDF, % NDF	**13.18**				

There is an estimate of the unavailable pool of 2.4*lignin. This has been questioned by several researchers and recently, the Cornell group, reevaluating this has found that the unavailable pool might be different among forage types and maturities. A rate of digestion (Kd) for one pool is predicted, with a correction for lag, that is a weighted value which is now used in the CNCPS based models, assuming one pool and has worked well in the field. Of interest is the prediction of the two pools and their associated Kd's. The concept of two pools does make biological sense. In current work with different hybrids in corn, it has been interesting to note that there is significant genetic diversity relative to these pools. The question yet to be answered is, will having two pools enhance our ability to predict performance in cattle?

Dr Allen in a series of papers presents an integrative model which uses two pools to describe fiber dynamics in a cow. Below is the most recent model describing these dynamics. The major factor is particle size with particle size reduction rates (Kr). The iNDF is the indigestible pool and the pdNDF is the potentially digestible pool. There are 4 passage rates, describing that the particles from the particle pools larger than 2.36mm moves at different rates than the particle pools less than 2.36mm, with different Kp's for the pdNDF in the two pools This model does not provide the disappearance rate of the pdNDF from the two particle pools however this is detailed in other models. If one were to expand on the model below to incorporate a division of the pdNDF pool into fast and slow pools, this might provide some enhancement to this model. We have forages like BMR corn and BMR sorghum as well as new varieties of grasses with large fast pools that allow us to suggest this approach.

We have found that the prediction and use of the NDF Kd's has significantly improved our ability to predict animal performance. Our goal, of course, is to better predict the upper and lower limits of forage that can be included in a ration. The lower limit to keep the cow healthy and the upper limit to be able to have high productivity at lower cost. The integrative model above provides direction on how this might occur.



The Future

Forages are absolutely needed at a minimum level to provide for normal rumen function. The particle size distribution of the forage is critical to the proper formulation. We routinely make the recommendation to increase the % forage in the ration when BMR corn silage is fed because the forage has a large fast rate digestibility pool as well as a very fast rate (Kr) of particle size reduction. Miner Institute has been measuring the rate of particle size reduction with a ball mill and has found that this rate is well correlated with the NDF digestibility.

We need to improve the methodology for measuring NDFd. The data support that there are two pools of fiber. Work at Miner Institute is examining the impact of the ester and ether bonds to the core lignin on fiber digestibility and its potential link to fragility of the particles. This will help us in refining our prediction of fiber digestion, gut fill, particle size reduction rates, and animal productivity.

There has been extensive work done with enzymes, yeasts and other rumen additives as a means to improve fiber digestion. The enzyme approach has seen variable results. The use of yeast may be indirect through pH control but has shown consistent positive results with certain live yeasts.

With our continued work with improving plant genetics for fiber digestion, understanding of soil and environmental management practices that influence fiber digestion, we will improve animal productivity and efficiency. With the understanding of the variation in fiber digestion, we will be able to better use different products to reduce the variation in fiber digestion.

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Effect of NDF digestibility on Diet Formulation and Animal Performance

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Introduction

- Feed efficiency in ruminants is a difficult measurement and concept to think about
- The swine and poultry industry have long looked at efficiency as an important measurement relative to their profitability

Introduction

- The simplest approach is the one put forth by Dr. Hutjens, U of Ill.
  - Milk produced/DMI
  - This might seem simple, but to have a measure of milk produced and DM intake per cow for a group can be difficult
- More complicated is the same measure but the milk is fat corrected milk, which puts efficiency more on an energy basis

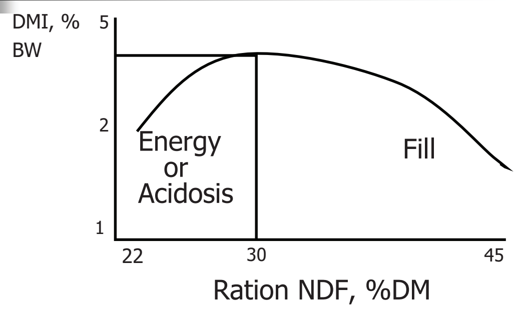
Feed efficiency in the dairy cow

- There can be 3 components that we can consider to influence feed efficiency
  - Gut fill and particle flow through the gut
  - Ruminal fermentation
  - Animal metabolism

Gut fill

- Much focus has been placed on gut fill over the last many years
- Mertens many years ago expanded on the Ohio work and looked at ration NDF as a criterion of formulation
- This concept moved us ahead in ration formulation

Intake and NDF - Mertens



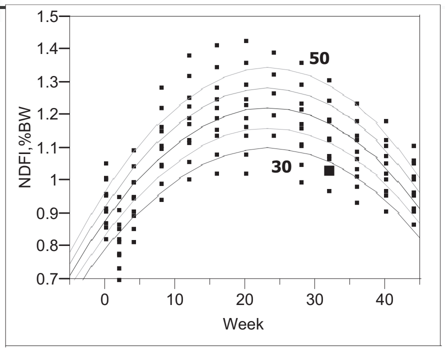
The effect of particle size and fiber digestibility

- In the dairy animal, our desire is to feed forage close to the ability of cows to reduce the coarse particles to enhance digestion and passage of the indigestible particle mass
- There has been a significant amount of work over the last several decades in this area.

Fiber digestion and gut fill

- Mertens provided data that suggested that gut fill was a part of intake regulation.
- His data demonstrated that not only was intake affected by NDF concentration but also by digestibility
- Of interest is the change in intake as a function of days in milk

Effect of Fiber Digestion on NDF Intake – Mertens, 98



Physically effective fiber (peNDF)

- Mertens in a paper in 1997 suggested that we could measure the particles retained on a 1.18mm screen from vertical dry sieving to define physically effective fiber
  - Basic assumption is that particles greater than 1.18mm will be retained in the rumen

A field tool to measure effective fiber

- A need to measure particle differences in the field
- The Penn State Particle Separator was developed based on Wisconsin work
  - Sieve hole diameter and hole thickness
    - Diameter to separate particles by size
    - Hole thickness to prevent, reduce particles greater than the diameter to move through the hole
- A excellent qualitative tool

Particle effectiveness and nutrition models

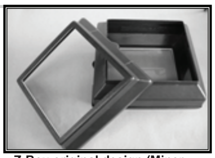
- There was a need to have a field tool to measure particle effectiveness so that the data could be used in nutrition models
- Miner research has suggested that the PSPS is not satisfactory

A different approach

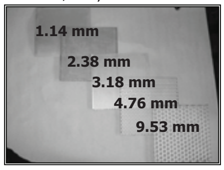
- Miner Institute developed the Z-Box, based initially on the years of work by Dr. James Welch, University of Vermont
  - Particle size
  - Particle density
  - Particle shape
- The Z-Box has been found, in controlled studies at Miner and recently, Formigoni, at the U of Bologna, to correlate well with the vertical dry sieving method of Mertens

Development of new tool to measure peNDF: "Z Box"

- **Miner Institute & ZenNoh**
- **One solid clear plastic side, one open side for interchangeable sieves**
- **2 sieves:**
  - **3.18-mm for corn silage & TMR**
  - **4.76-mm for hay/haylage**



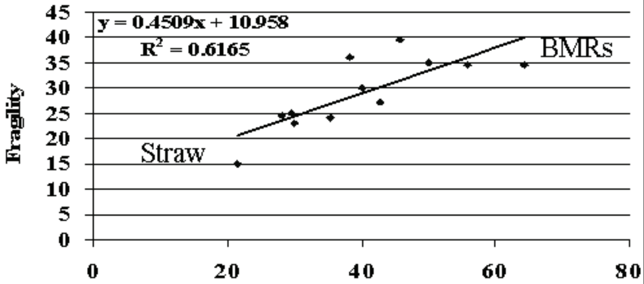
Z-Box original design (Miner Institute, 2000)



A problem

- The Z-Box does a nice job of measuring particle size
- It does not relate well to the fragility of the particle
  - The minutes to chew a kg of alfalfa NDF is very different than that required for a kg of NDF from straw
- A need to develop a new approach

Measuring fragility, chewing and rumination with Ball Milling



Grant & Cotanch, 2009

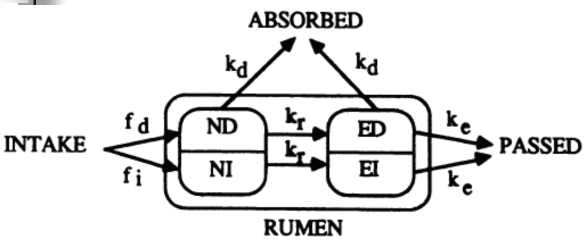
The potential

- Use the 15 minute ball milling method to
  - Adjust the peNDF measurements
  - Develop rates of particle size reduction in the rumen
  - Potentially modify the chewing time per kg of NDF consumed

Particle breakdown in the rumen

- Allen and Mertens developed a more sophisticated model which incorporated
  - Particle size
  - Rates of particle reduction
  - Rates of digestion
  - Rates of passage

Allen & Mertens, 1988

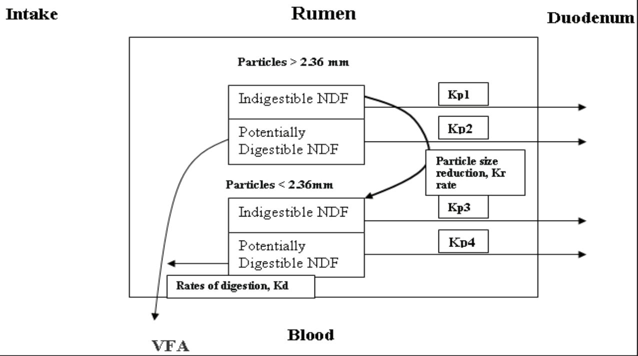


N = Non escapable, E = Escapable, D = Potentially digestible
I = indigestible, Kd = digestion rate, Kr = particle size reduction rate
Ke = Rate of passage

A more sophisticated model

- Allen built on the earlier model of Allen and Mertens to develop a model that suggested there are differential rates of passage from the different particle pools.

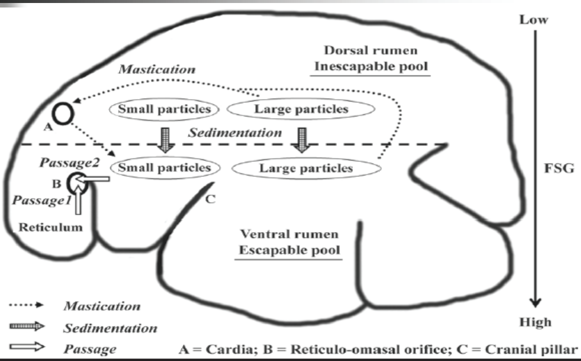
NDF Model, Allen



A more sophisticated model Seo et al

- Seo and his colleagues developed a model which incorporated
  - Particle size
  - Particle specific gravity
  - Rumination
  - Sedimentation and passage
  - Rumen location

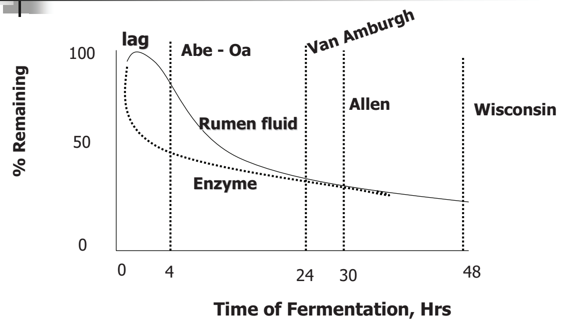
Particle Flow Kinetics Seo et al - 2009



Measurement of NDF digestibility

- This has been an area of controversy
- There have been two approaches used
  - Enzymatic – Abe, Japan
    - OCW – Oa (4 hour enzymatic) and Ob Residue from 4 h
    - Used in Japan on a relatively limited basis
  - Invitro – Tilly Terry and Daisy – Ankom
    - This is the major method used today

NDF Digestibility Measurements



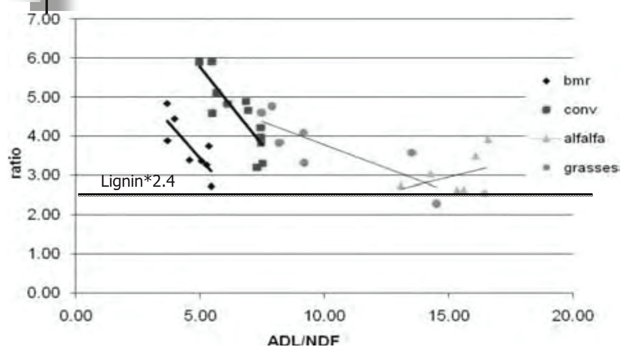
The key factors in measuring fiber digestibility

- The proper assay of fiber
  - NDF
  - ADF
  - Lignin
- Proper filter method
  - Recommend the use of a 1.5 micron micro fiber filter in a 40 micron Gooch crucible
- Proper in vitro procedures to reduce lag to less than 3 hours

iNDF

- In the CNCPS system 2.4*lignin has been used across forage species and maturities
- With the improved recovery of lignin and measurement of NDF (Raffrenato et al, 2009 Proc CNC)
  - Determined that iNDF is variable among species and possibly maturities

iNDF ratio as related to ADL as %NDF, CNC, 2009



NDF digestibility and linkages

- The core lignin is linked to the hemicellulose through ester and ether linkages to the arabinose/xylose complex
  - P-coumaric and ferulic acids.
- These linkages will provide us with better information on estimating iNDF in the future

Translating fiber digestibility into a model

- We need to translate the measurement of NDF digestion into our nutrition models
- Van Amburgh, Van Soest and colleagues have given us a starting point
  - With assumptions for unavailable fiber and a two pool concept with a weighted one pool with a Kd.
    - iNDF will be improved going forward

NDF digestion rates

Values in red are inputs					
Feed name	Rygrass Silage 21% CP				
NDF, %DM	43.70				
lignin, %DM	2.40				
hour	NDF digestibility	A		lag, h	kd, %/h
0	0.00	0.8682	Unitized A		
24	60.50		1.0000	2.50	5.55
Unavailable NDF, %NDF	0.13		0.3031		
k1 pool NDF kd, %/h	9.50	Equation 6			
k2 pool NDF kd, %/h	0.91	Equation 7			
Normalized P1	77.87	Equation 8			
NDF pool 1, % NDF	67.61				
NDF pool 2, % NDF	19.21				
Unavailable NDF, % NDF	13.18				

Inclusion within a model by RU.M. & N., S.a.s., Italy

- NDS, Professional, has within the platform, the ability to do the calculations for the rate of digestion
- Also provides the pools and their potential digestibility

Corn Silage

Restores NDF Digestibility Starch Digestibilty

	% DM		% NDF		Rates (Kd %/hr)		Int.Dig. (% escape)	
Total CHO	84.580							
NDF	41.510							
ADF	25.070		60.395					
ADL	2.850		6.866					
peNDF	33.208		80.000	80.000				
CHO B3	34.670		83.522		3.100		20.000	
CHO C	6.840		16.478					

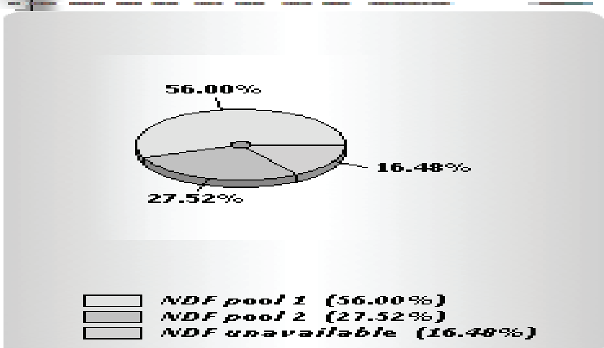
Corn Silage

Hours	NDF Digestibility	ND residues	Unitized A	lag time hr	Kd %/hr
0	0,00		1,0000		
6					
12					
24	40.600	0.5940	0.5139	2.50	3.10
30					
48					
iNDF %NDF	0.16				
			average lag h	2.50	
			average Kd %/hr		3.10

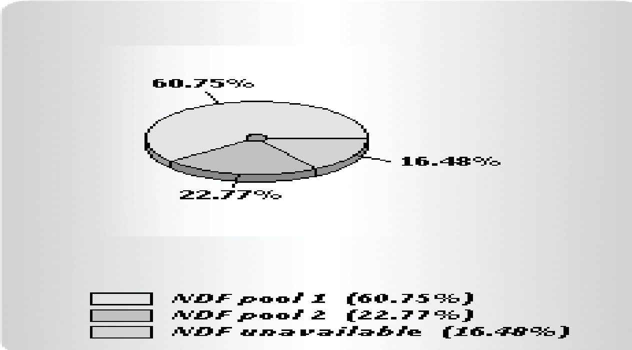
Corn Silage

k1 pool NDF kd	6.37	% hour
k2 pool NDF kd	0.64	% hour
NDF pool 1	56.00	% NDF
NDF pool 2	27.52	% NDF
NDF unavailable	16.48	% NDF
NDF available	83.52	% NDF
NDF digested	40.23	% NDF
Passage rate - Kp	4.60	%/hour

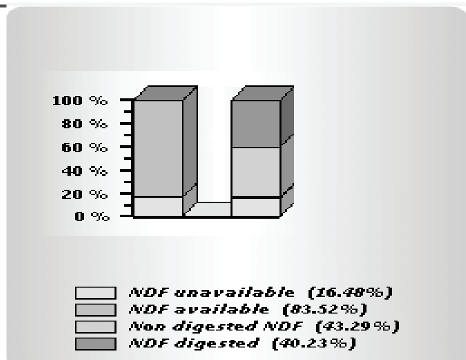
Corn Silage NDFd-24 40.6%



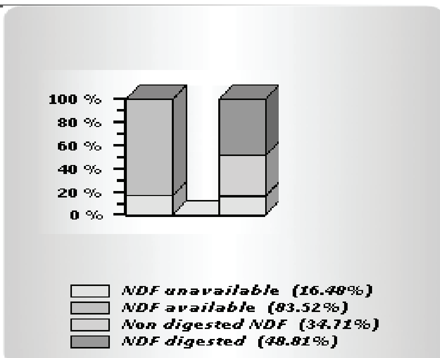
Corn Silage NDFd-24 51%



Corn Silage NDFd-24 40.6%



Corn Silage NDFd-24 51%

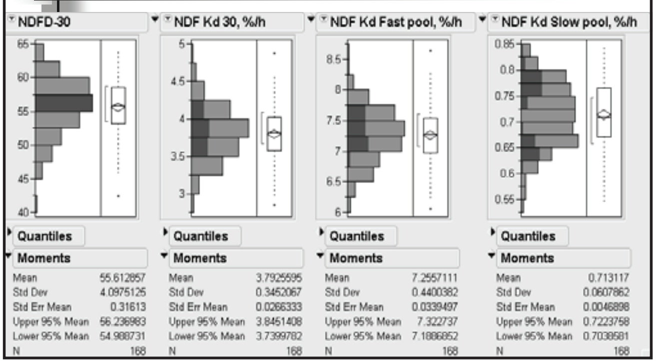


Effect of Corn Silage NDFd on animal performance – 2400 g avail NDF pool

Nutrient, % Pool	**NDFd 51.8, Kd = 4.5**	**NDFd 34.7, Kd = 2.5**
Ruminal Digested	37	32.1
Ruminal Escape	63	67.9
Intestinal Digestion	12.6	13.6
Fecal Loss	50.4	54.3
ME Allowable Milk	46.2	45.1
MP Allowable Milk	46.4	44.8

From NDS Professional

NDFd-30 and associated Kd's for Northeast US Corn silages

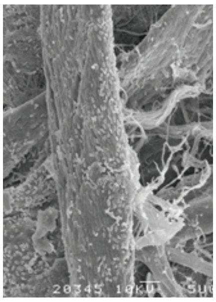


Can we enhance fiber digestion?

- There has been a lot of work done over the last decade in attempting to enhance fiber digestion
  - Enzymes
  - Microbial additives
  - Co-factors
  - Yeasts

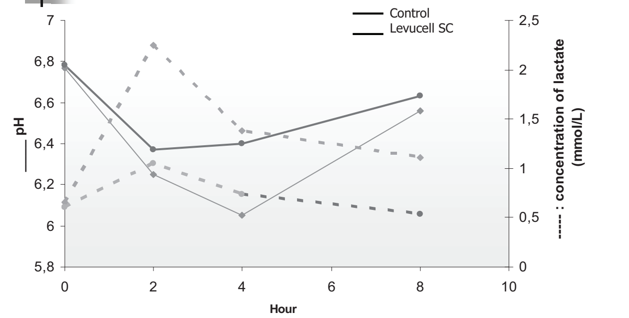
Levucell SC: improving fibre digestion

- **MODE OF ACTION:**
  - oxygen scavenging
  - improved growth of cellulolytic organisms.
  - stabilisation and elevation of rumen pH.



Levucell SC maintains high rumen pH even in diet rich in fiber.

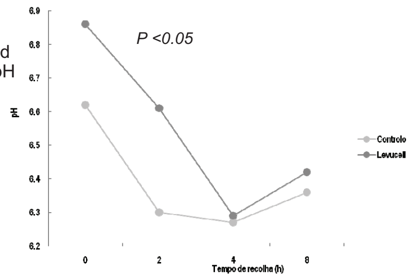
Guedes and al., 2007.



Effect of Levucel on rumen pH

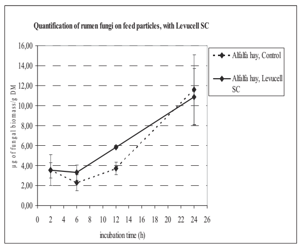
Effect on ruminal pH

- When animals were fed Levucell SC, ruminal pH was higher.
- Less time spent at the lower pH, and faster recovery in Levucell treated animals

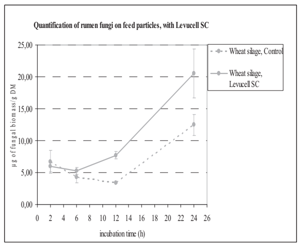


Microbial analyses: organisms attached to fibre – anaerobic fungi

Alfalfa hay



Wheat silage

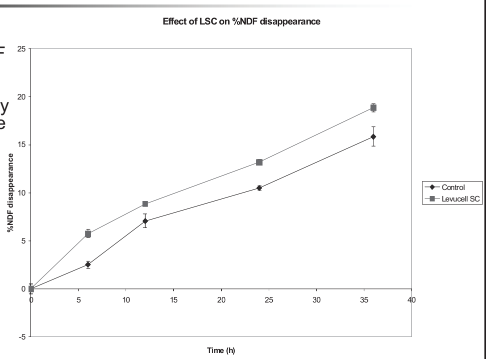


Substrate colonisation by fungi increased with Levucell SC on alfalfa hay from 6h to 12 h and on wheat silage from 12h to 24h

Effect of Levucell SC on fibre digestion

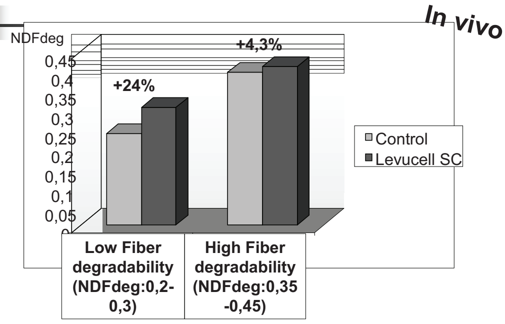
NDF = 466 g/kg DM

- The rate and extent of %NDF disappearance of corn silage was significantly increased in the presence of Levucell SC.



Effect of Levucell SC on the NDF degradability of 40 corn silage

In vivo



Effect on the energetic value of the ration: NEl

Some guidelines

- The goal is to obtain high productivity in a herd, maintaining or improving milk components
- Achieving this goal means doing so
  - With healthy rations
  - With rations that are efficient (kg FCM milk:kg DMI)

Forage Quality

- Growing and storing high quality forages
  - Astute purchasing of high quality forage
  - High quality also means the forage fits the replacements, dry and lactating cows being fed

A Balanced Rumen Ecology

- There is a need to balance
  - The effective fiber for a good rumen mat and saliva flow
  - The fermentation of the carbohydrates
  - The feeding behavior
- This allows the control of ruminal pH & the balance of the microbial ecology

Microbial dynamics

- There are major microbial niches in the rumen that are responsive to
  - Nutrients available
    - Both directly from the feeds consumed and indirectly from the products produced by microbes
  - The balance of the ration
  - The cow's feeding behavior

Microbe	Primary Substrate	Optimum Rumen pH	Primary requirement	Main fermentation products	Doubling Times
Bacteria	**About 630 different bacteria (50% of microbial mass)**				
Fiber & pectin	Fiber & pectin	6.3 to 6.8	NH_3 , isoacids	Acetate	8 – 10 h
Protein	Protein	6 to 7	Protein, peptides, NH_3	NH_3, Isoacids	4 – 8 h
Allisonella histaminiformans	Histidine	4.5 to 6.5	Histidine, peptides from silage	Histamine	Rapid
Starch	Starch & sugars	5.5 to 6.5	Peptides, AA, NH_3	Propionic, Lactic	15m – 30m
Secondary - M. Elsdenni, Methanogens	Lactic, H_2	6 to 6.8	Peptides, AA, malic	Propionic, CH_4	2 – 4 h
Protozoa	**About 30 different protozoa (40 – 45% microbial mass)**				
	Starch, sugars	6.3 to 7.0	Peptides, AA, Bacteria	Propionic, H_2	15 – 24 h
Fungi	**About 14-15 types of fungi (3 – 8% microbial mass)**				
	Fiber	6 to 7	NH_3, AA, sugars	lactic, acetic, H_2,	15 - 24h
Bacterial viruses (5 –7 types & .0000001% TMM) Yeasts (0.1 – 0.2%TMM)					

Feeding Fiber

- We are finding that having 24 hour NDFd's has significantly improved our ability to formulate rations
- The effective fiber is a continuous challenge
  - We do not have good means to measure
    - The Penn State box does provide a qualitative value
    - The Miner Institute Z Box provides a quantitative assessment.
  - However we need a measurement that provides a rate of particle breakdown in the rumen
- With highly fermentable fiber, the recommendations could be changed
  - Non irrigated alfalfa and grasses
  - Corn hybrids with high digestibility characteristics

Fiber Guidelines for the Early Lactation Cow

Nutrient	Kg	% Fraction	Fiber Quality		
			Poor	Average	Good
Dry Matter Intake	24.5				
Fermentable DM	10.5	43	41	43	45
Total NDF	7.4		28	30	33
Forage NDF			20	22	24
peNDF	5.5	76.6	22	23	24
Lignin	0.9	11.7	3.0	3.5	4.0
Fermentable NDF	2.6	>35	9.5	10.5	12

The fermentation of CHO's will change with different levels of intake Because of changes in rates of passage.

Summary

- Remember that the NDF represents 28 to 45% of a dairy animals dietary intake
- The digestibility of the fiber impacts
  - Fragility and effective fiber
    - Rumen mat
    - Passage kinetics
  - DMI and feed efficiency
  - Microbial yield and balance in ecology
  - Animal health

Summary

- In order to obtain yield, components and efficiency we need to start with a quality forage program
  - The right forage varieties, hybrids to fit the farm and animal groups on the farm
  - A good harvesting and storage system
  - A good feed out system
- We have moved dairies ahead with the knowledge and application of fiber digestibility in our models
- We still have a ways to go in refining the methodology and improving our nutrition models

De Marke

Farm Facts

SITE

- Light sandy soil
- Precipitation surplus 300 mm/yr
- Organic matter content 4.5 %

Roessinkweg 2, 7255 PC Hengelo, The Netherlands
+31 575 467 323
www.livestockresearch.wur.nl

Farm

- Total area: 55 ha
- Permanent grassland: 11 ha
- Crop rotation grass-clover – maize – barley: 44 ha
- Catch crop in maize: Italian ryegrass (under-sow)
- Milking cows: 75
- Young stock: 6/10 milking cows
- Milk production per cow: 8,500 kg/yr
- Production intensity: 12,000 kg milk/ha
- Grazing period: May – September
- Grazing hours per day: 4

Nitrogen inputs

- Chemical fertilizer: 0 kg/ha
- N binding clover: 11 kg/ha
- Purchased feeds: 92 kg/ha
- N surplus: 109 kg/ha

Phosphorus inputs

- Chemical fertilizer: 0 kg/ha
- Purchased feeds: 13 kg/ha
- P surplus: 0 kg/ha

Manure management

- Fermented slurry: 37 tons per ha
- Separated liquid fraction: 19 tons per ha
- Separated solid fraction: 2 tons per ha

DAIRYMAN project secretariat
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DAIRYMAN

for vital rural communities!

This project has received European Regional Development Funding through INTERREG IV B.

INTERREG IVB

Investing in opportunities

November 2009

Strict manure regulations

The legislation on manure application aims at achieving a balance between the needs of crops and the amounts of fertiliser applied. In practice this means a reduction in the quantities of nitrogen and phosphate. On Wageningen University's experimental farm, De Marke in Hengelo, the Netherlands, where the proposed fertilisation standards for the year 2015 are already being applied, the lower limit seems to have been reached now as the quality of the grass has declined too much.

ing. Geesje Rotgers

The conditions at De Marke Experimental Farm are demanding. Strict standards are applied: no chemical fertiliser is used and the crops have to make do with the nutrients applied with the farm's animal manure.
In addition, the amount of nutrients in this manure has decreased as the cattle rations at De Marke are very 'close'. Approximately 200 kg N/ha is applied. Moreover the farm is located on dry sandy soil that is susceptible to leaching, which is the most difficult type of soil as regards mineral management to be found in the Netherlands. One of the research projects at De Marke examines how far the minerals supply (fertiliser supply and purchase of feed) can be reduced, before its effects on the herd are felt. "We think we have reached the lower limit of what is safe", says Léon Šebek, researcher with Wageningen UR Livestock Research.

Problem at De Marke

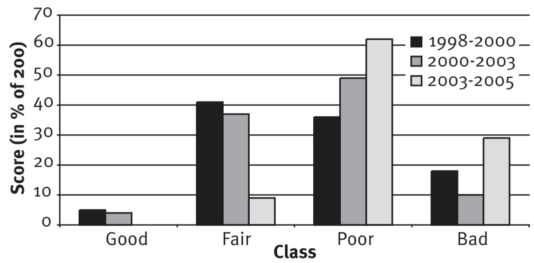
"If you aim at less excretion, you will almost automatically affect the diet of dairy cattle", says Šebek. High-starch products replace protein-containing products, which changes the protein energy ratio in the diet. As a result, the rumen environment will also change. The pH value will fall slightly and the micro-organisms are becoming more attuned to starch digestion than cell wall digestion. When grass is the main source of protein, adequate cell wall digestion is highly important. "This does not need to be a problem: as long as the grass is of good quality, it will be digested well." At De Marke, however, crops are only fertilised with farm-produced animal manure. As a consequence, the quality of grass has gradually deteriorated in the past decade. In combination with the changed rumen environment this has adverse effects on the digestion of the grass and consequently on the protein supply to the cow. "In the cow's faeces we can find many undigested feed particles", observes Šebek. "This has been noticed for some years now, and therefore soybeans extracted were added to improve the diet at De Marke with rapidly degradable carbon hydrates. While maintaining the microbial protein formation, this 'speeds up' the diet. This has resulted in a fine performance of the herd." Unfortunately, this is contrary to the wish to reduce excretion and to make the best possible use of the farm-grown forage or to purchase as little feed with minerals as possible.

Analyse results inadequate

It is remarkable that the feed value analyses on De Marke Experimental Farm do not indicate a worsening grass quality as a result of the smaller fertiliser doses. It can even be stated that, apart from a decreasing crude protein content, there were only minor changes in the results over the past ten years. Digestibility of organic matter and non-detergent fibre has remained fairly constant. It would seem there is no reason for concern, but that appears to be wrong. The use of forage certainly gives reason for concern, as shown by the many undigested feed particles in the faeces *(figure 1)* and the degradation characteristics of the grass silage in the rumen *(figure 2)*.

Figure 1

Faeces score of the dairy herd at De Marke in the period from 1998 to 2005 (in % of 100).



The faeces score is a measure for the digestion of the forage. Consequently, it is a major indicator for a farm that intends to bring in the smallest amount of minerals through concentrate feed from outside.

reduce grass quality

The solutions
New grass mixtures were considered that produce an easily digestible crop, even when less fertiliser is applied. “But this line of approach has not (yet) produced”, Šebek says. The application of artificial fertiliser to grassland is also an option but this is less desirable at De Marke because of the increase in N surplus, that results from it. The third option is one we have already adopted, by adding another concentrate pellet. But that boosts the minerals supply once more when, in fact, a reduction was intended..

“Alternatives?”
“For the moment we are doing nothing yet”, says Šebek. “First of all we want to repeat the experiment once more so that we can confirm the results. The final conclusion might be that, in the present layout, De Marke has reached the lower limit of the minerals issue.”

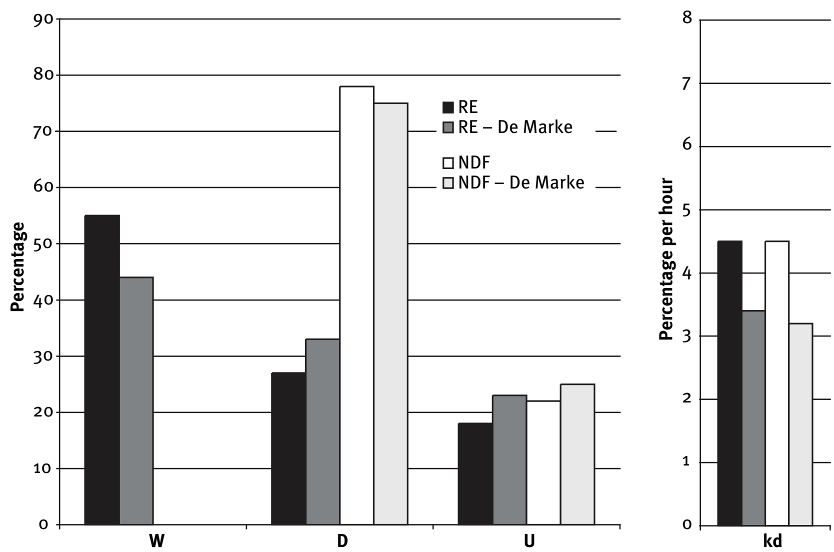
THE QUALITY OF MANURE

On De Marke Experimental Farm, there have been too many undigested feed particles in the faeces for several years.

Picture: ASG

Figure 2

Degradation characteristics of normal grass silage in the Netherlands and grass silage of De Marke (in %). (W = washable fraction, D = potentially degradable fraction, U = undegradable fraction, kd = fractional degradable rate per hour).



Degradation of the grass silage of De Marke is less complete than a normal grass silage in the Netherlands. The grass silage of De Marke has smaller D and W fractions that can be degraded in the rumen. Furthermore, the degradable D fraction appears to be degraded more slowly than in the normal grass silages

This article is a full copy of the article ‘Strenge mestregels nadelig voor gras’, first published in the Dutch magazine V-focus, published by AgriMedia, Wageningen

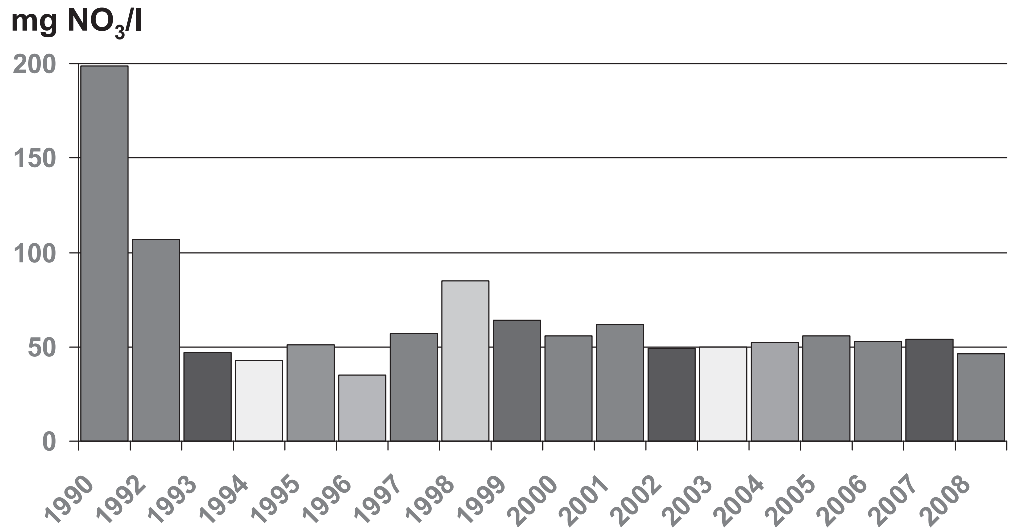
Dairy Farming in NL and experimental farm De Marke

Feed

	NL average	De Marke
Milk quota *(*1000 kg)*	609	665
Dairy cows	77	81
Young stock	7,5	7,8
Milk yield *(kg/yr)*	7980	7850
Grassland *(ha)*	*36,4*	*31,5*
Maize/crops *(ha)*	*9*	*23,8*
% grassland	*80%*	*57%*
Milk per ha *(kg)*	13480	11200
Concentrate *(kg/cow/yr)*	2180	1820
Grazing system	daytime	4h/day
Soil type	divers	poor sand
Fertiliser *(kg N/ha)*	125	0
Milking system	divers (most herringbone)	AMS

Objectives De Marke:

- **Phosphate surplus = 0 kg/ha**
- **NH_3 emission < 30 kg N/ha**
- **EU nitrate directive (max. 50 mg NO_3/l)**

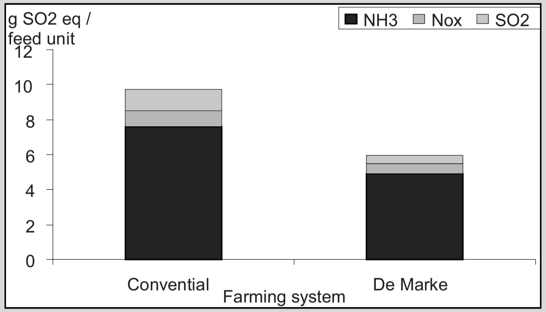


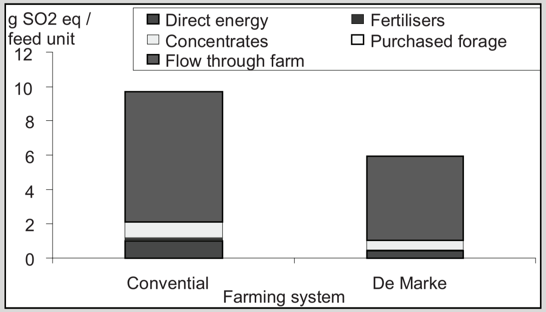
Ecological footprint of De Marke

In a life-cycle analyses, Iepema and Pijnenburg compared De Marke with a conventional farm representative for Dutch dairy farms on sandy soil

Acidification

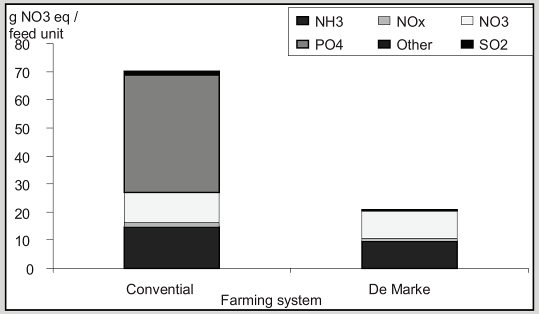
Emission of NH_3, NO_x and SO_2 on De Marke was 40% lower than on the conventional farm

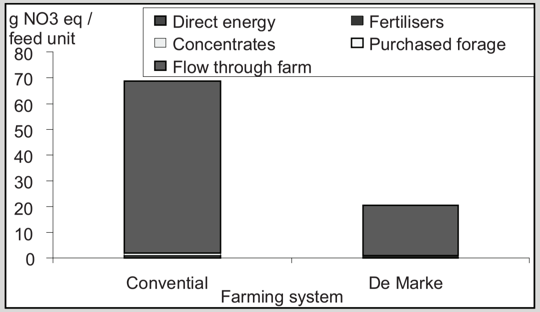




Eutrophication

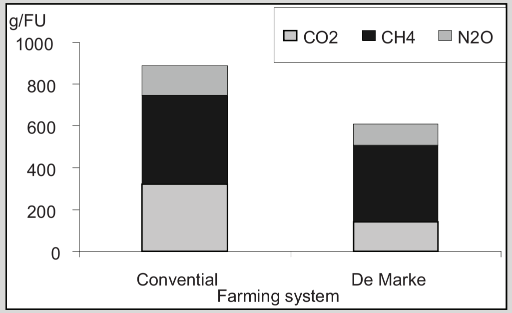
Emission of NH_3, NO_x, NO_3, and PO_4 on De Marke was 70% lower than on the conventional farm

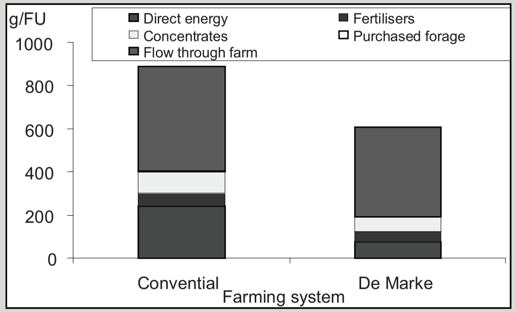




Greenhouse gasses

Emission of CO_2, CH_4 and N_2O on De Marke was 30&% lower than on the conventional farm





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Iepema, G., Pijnenburg, J., 2001. Conventional versus Organic Dairy Farming. A Comparison of three Experimental Farms on Environmental Impact, Animal Health and Animal Welfare. MSc thesis Animal Production Systems Group, Wageningen University, Wageningen, The Netherlands

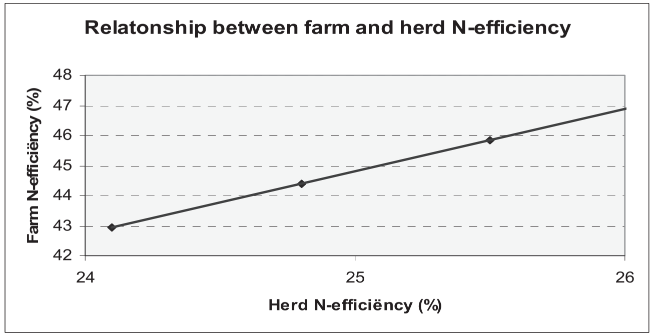
Dairy farming	Land use	**Feeds & feeding**	Manure	Life cycle analyses

De Marke

- Environmental EU-directives challenge dairy farms (above 10.000 kg milk per ha) to increase N and P efficiency at farm level by reducing farm inputs. How does that affect feeds and feeding?

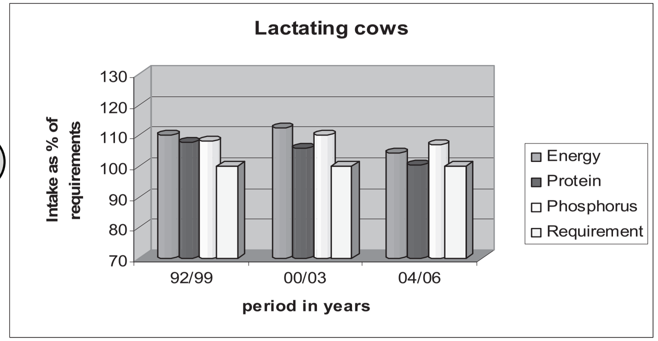
- Herd efficiency is important at farm level

1



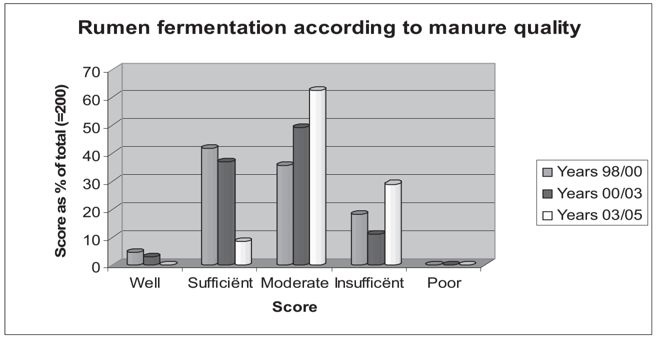
- Effect of reduced fertilizers on crop and feeding efficiency

2



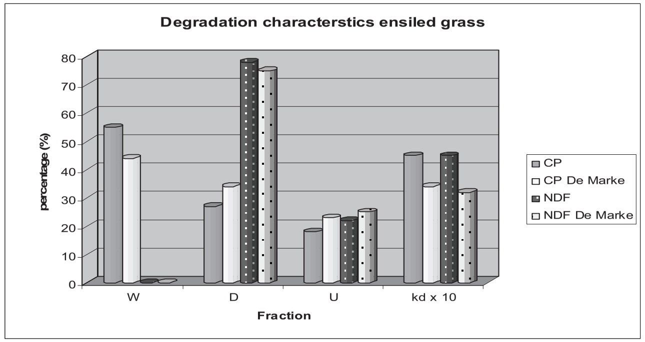
- Sub-optimal functioning of rumen fermentation

3



- Rumen degradation rates decrease without warning

4



- **Future importance of additives to enhance rumen degradation of cell walls (NDF)?**

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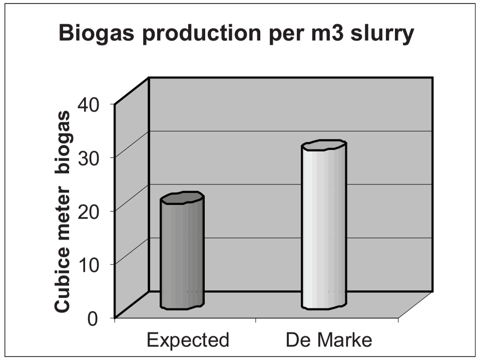
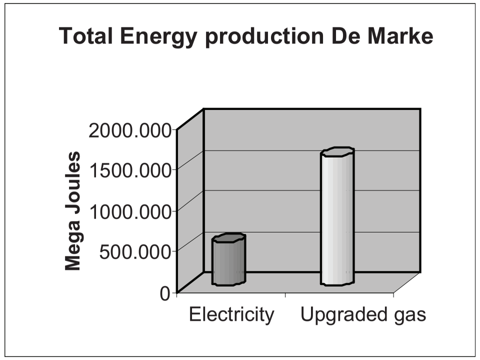
Dairy farming | Land use | Feeds & feeding | **Manure** | Life cycle analyses

De Marke

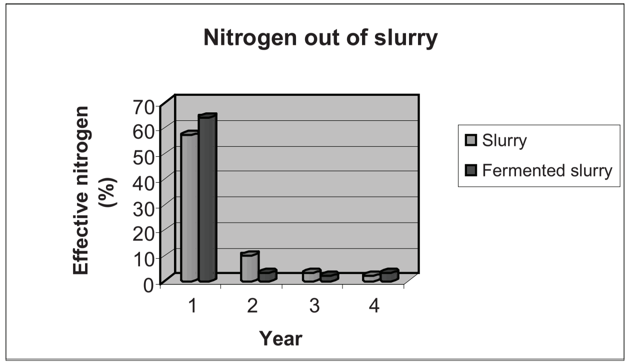
Results of slurry fermentation

Energy production

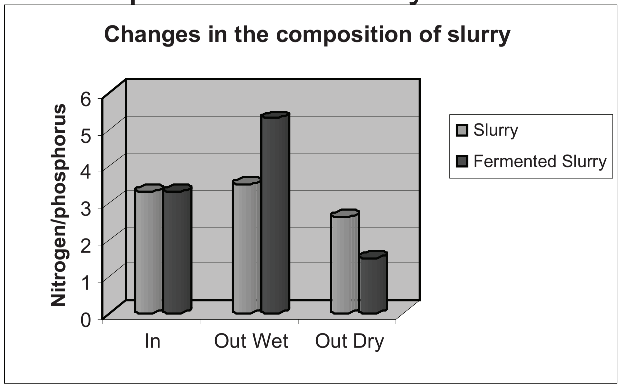




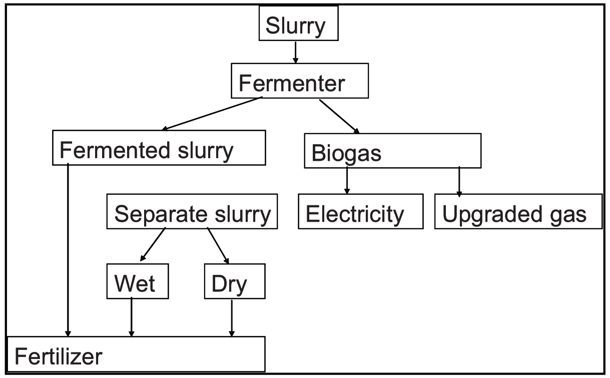
Fertilizing efficiency



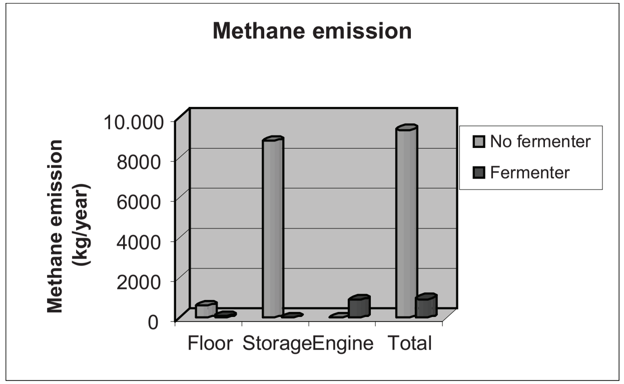
Separation of slurry



Process



Greenhouse gasses



Is farming without artificial N and external energy possible by fermenting slurry?

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De Marke

Intelligent crop rotation plan

Crop selection

- 33 ha grass - 11 ha permanent
  22 ha temporary
- 16 ha maize - 10 ha silage maize
  6 ha maize cob silage
- 6 ha grain barley

Manure management (tons per ha)

		Separated slurry		
	Fermented slurry	Liquid fraction	Solid fraction	Total
Grass	50	32	1	83
Maize	8	3	4	15
Barley/grass	45	0	5	50
Total area	37	19	2	58

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