

Challenges and opportunities in the field of improvement of forage crops in Europe

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Improvement of forage crops

■ Challenges

- EU policies, enlargement of EU, biobased economy

■ Opportunities

- Increase efficiency: Nitrogen Use Efficiency & Feeding value
- Examples of genetic studies: maize & *Lolium*
- New chances:
 - Genetic tools
 - Grass biorefinery



Challenges

- EU policies
 - EU Nitrate directive: reduce mineral losses
 - EU Farm support: area based not production based
 - EU Bioenergy directives: land required for biomass
 - EU enlargement: increased feed/food production
 - Multifunctional agriculture: nature conservation & grassland production
- Climate change



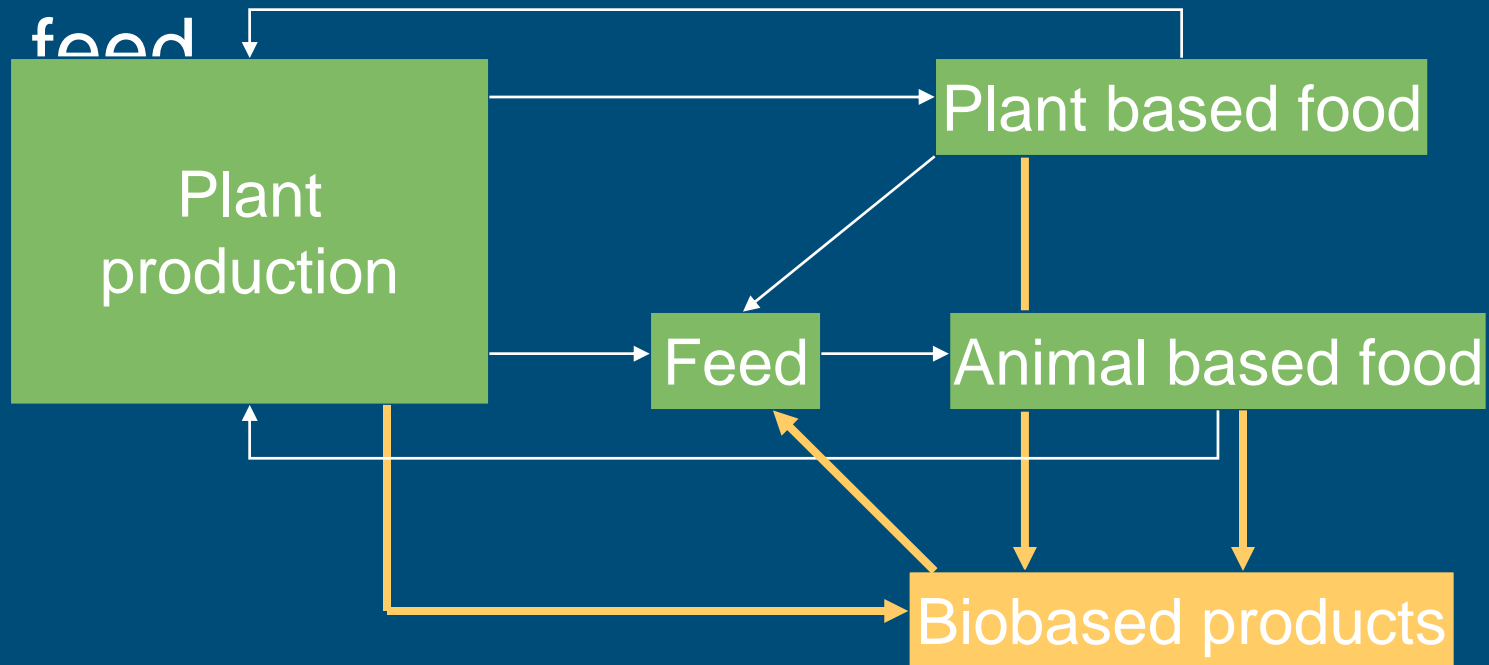
Effects of EU-policies

- Reduced N&P-input: lower production
- Underutilisation of grassland
- Low prices for forage feedstocks
- Co-fermentation of forage towards biogas:
Energy maize & 'nature' grass
- Lower profitability of grass & forage maize
breeding



New uses of plants and new pathways to

feed



Opportunities

- Higher demand for biomass/land for biobased products
- Increasing productivity becomes profitable again
 - Higher nutrient use efficiency
 - Higher energy content
 - Higher forage intake ← challenge!
- New tools available
 - Genetics/plant breeding (3 examples)
 - Technological treatments to improve feeding value (1 example)

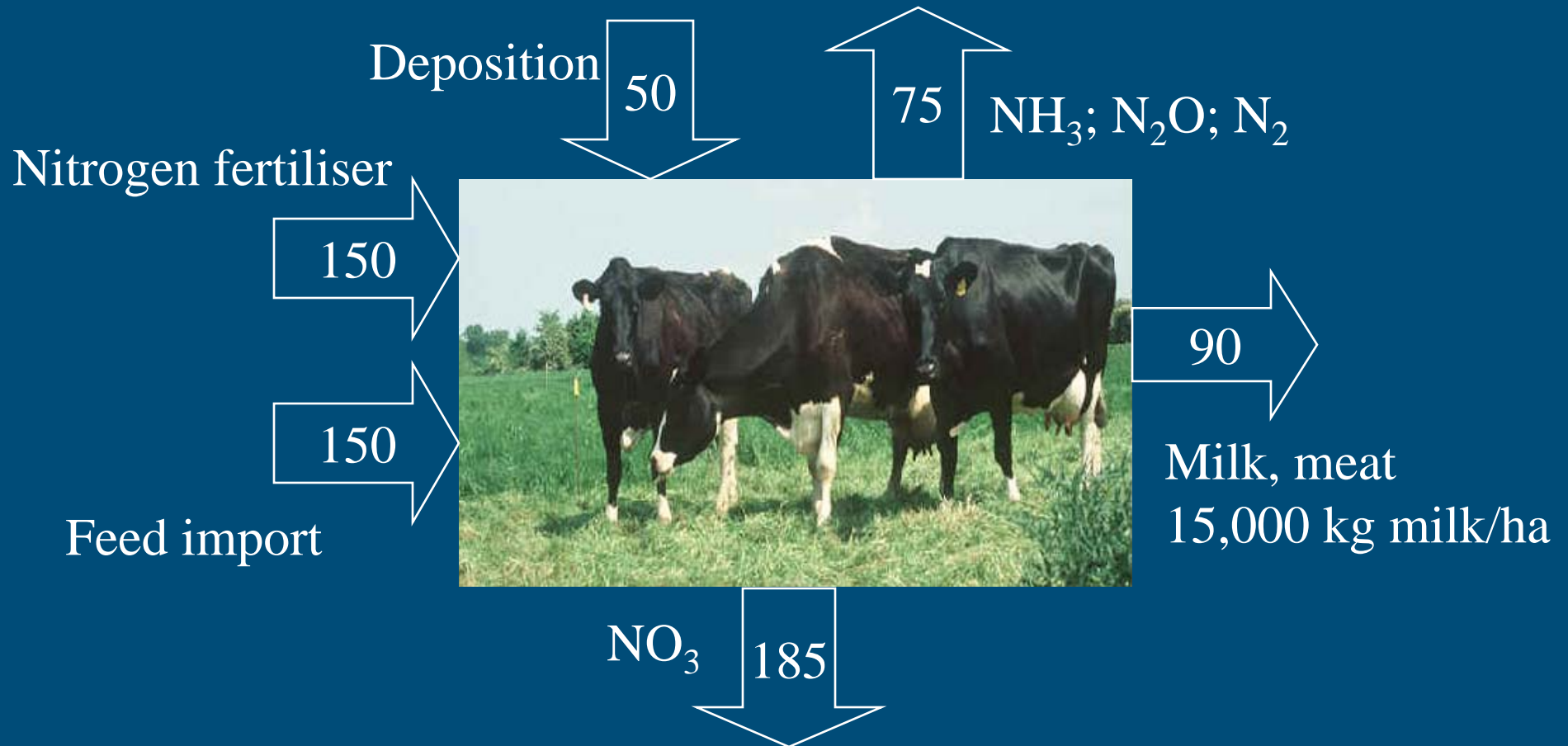


Some examples of progress in genetic tools

- Nitrogen use efficiency *Lolium*
- Rumen fermentation kinetics *Lolium*
- Cell wall digestibility forage maize



N-use efficiency: the problem



Farm monitoring in the Netherlands, 2001



Effects of EU-nitrate directive on farmers

- Reduction of nitrogen fertilisation
- Lower grass production per ha
- Lower feeding value per kg grass
- Lower milk production per ha

- Higher cost price per kg milk



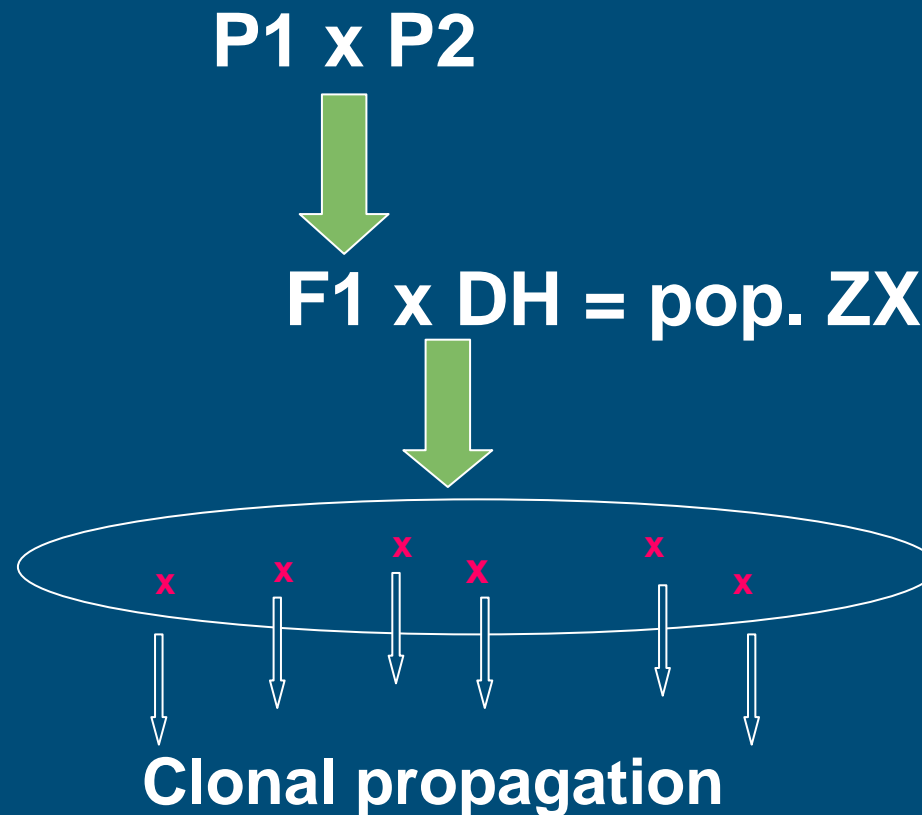
What can plant breeding offer farmers?

- 10 % higher productivity at low N-supply:
 - 35 % lower nitrogen surplus
 - +180 €/ha/year
- 5 % higher energy intake/ha:
 - +215 €/ha/year
- Economic benefits: farmers can reduce nitrogen inputs

Van Loo *et al.*, 1997



QTL-analysis of NUE in *Lolium perenne*



P1 = P510

P2 = P413

F1 = 1331

DH = DH875



PRI Mapping Populations



Testing Nitrogen-Use Efficiency



**Hydroponics
system**

Characteristics

- N-dosage system: 4 units
- light-interception model
- capacity: 1600 plants
- simulated crop conditions



Nitrogen-Use Efficiency (1)



1 week after
cutting

Traits:

- tiller number (TN)
- leaf length (LL)
- leaf width (LW)
- leaf area index increase

$dLAI =$

$LL * LW * TN / \text{'soil' area}$



Nitrogen-Use Efficiency (2)



4 weeks after
cutting

Traits:

- dry weight shoots DWS
- dry weight roots DWR
- dry weight total DWT
- leaf weight ratio LWR



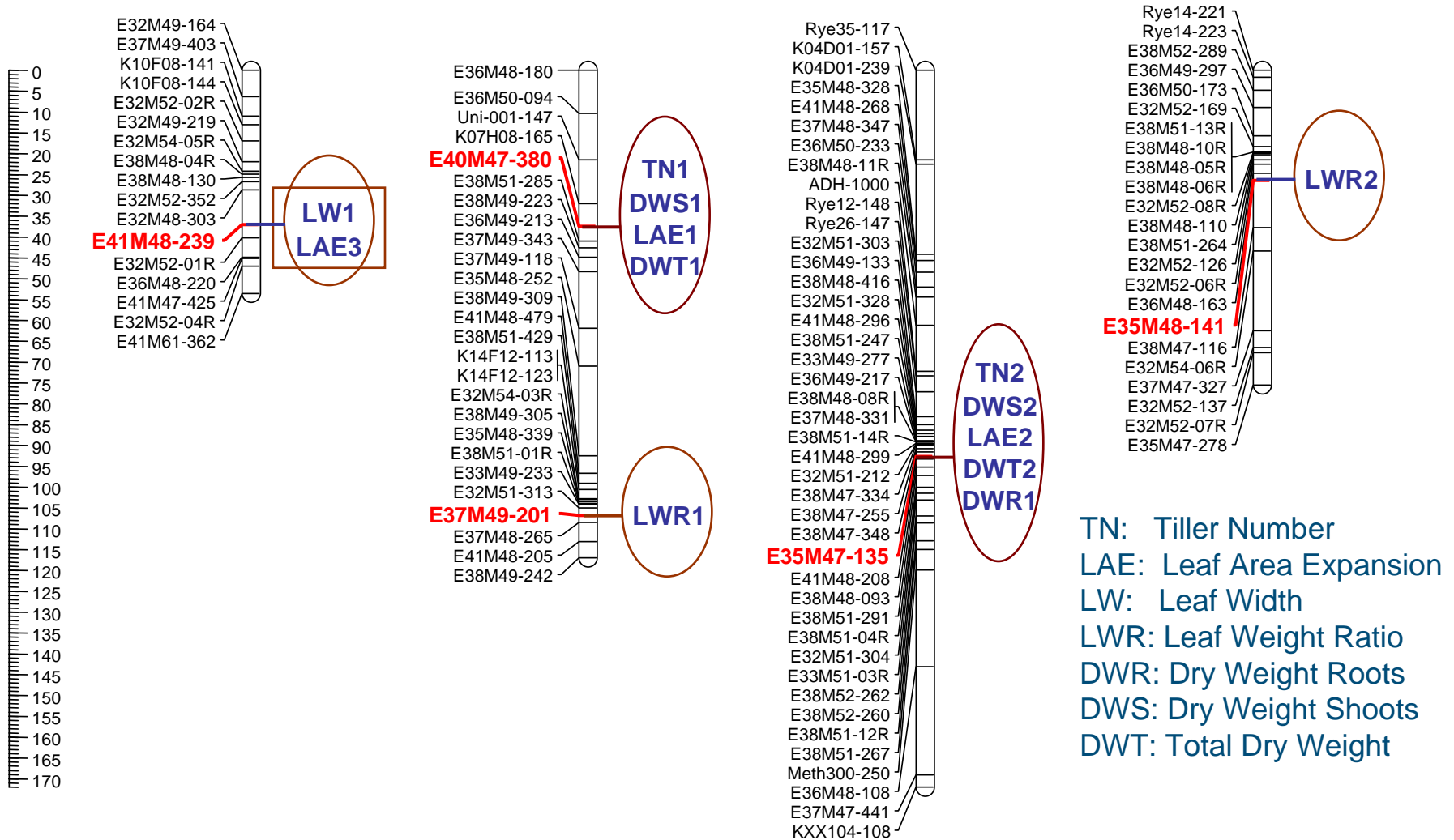
QTLs for NUE-related Traits

LG1

LG3

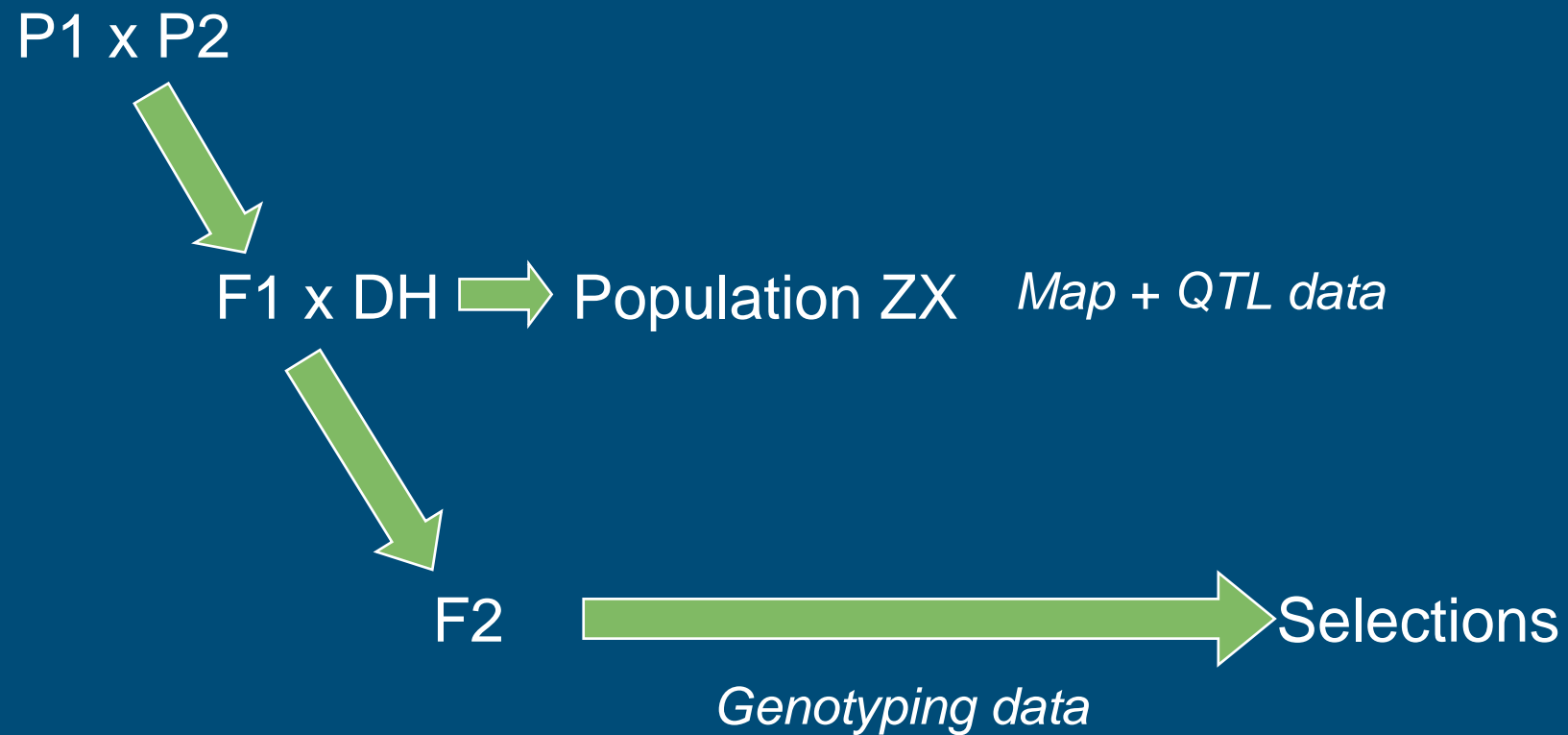
LG4

LG6



TN: Tiller Number
LAE: Leaf Area Expansion
LW: Leaf Width
LWR: Leaf Weight Ratio
DWR: Dry Weight Roots
DWS: Dry Weight Shoots
DWT: Total Dry Weight

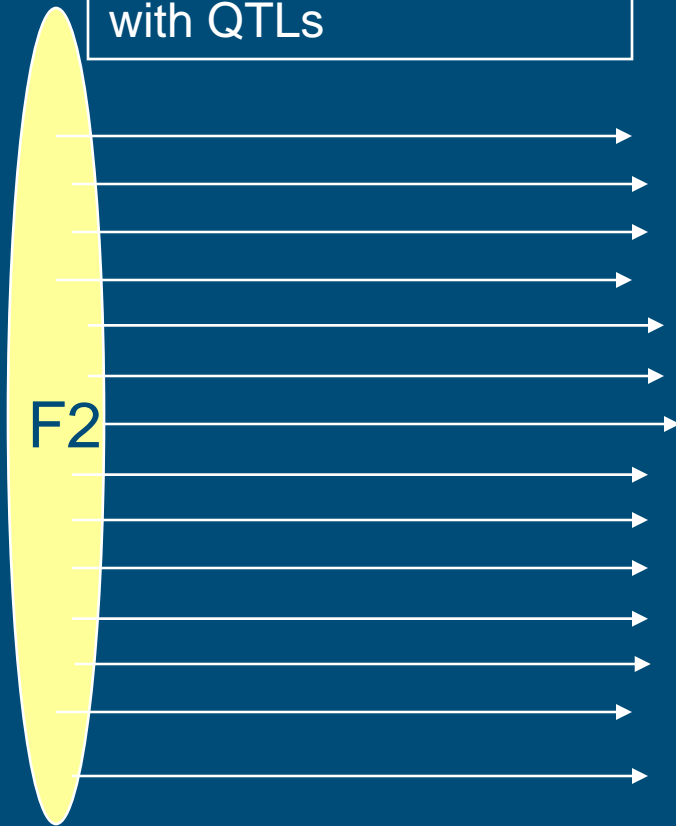
Marker Selection for NUE



Marker Selection Strategy

Codominant scoring of markers associated with QTLs

Selection Criterion



Genotype	Number of Plus Alleles					Sum
	QTL1	QTL2	QTL3	QTL4	QTL5	
1	2	1	0	0	2	5
2	1	1	0	0	2	4
3	0	2	2	1	1	6
4	2	2	2	1	1	8
5	2	2	1	1	1	7
.						
.						
.						
195	1	1	2	2	2	8
196	1	1	2	0	2	6
197	0	2	0	1	2	5
198	0	2	0	1	1	4
199	2	2	1	1	1	7
200	2	1	2	2	1	8



Response to marker selection

High NUE selection

Low NUE selection

Hydroponics

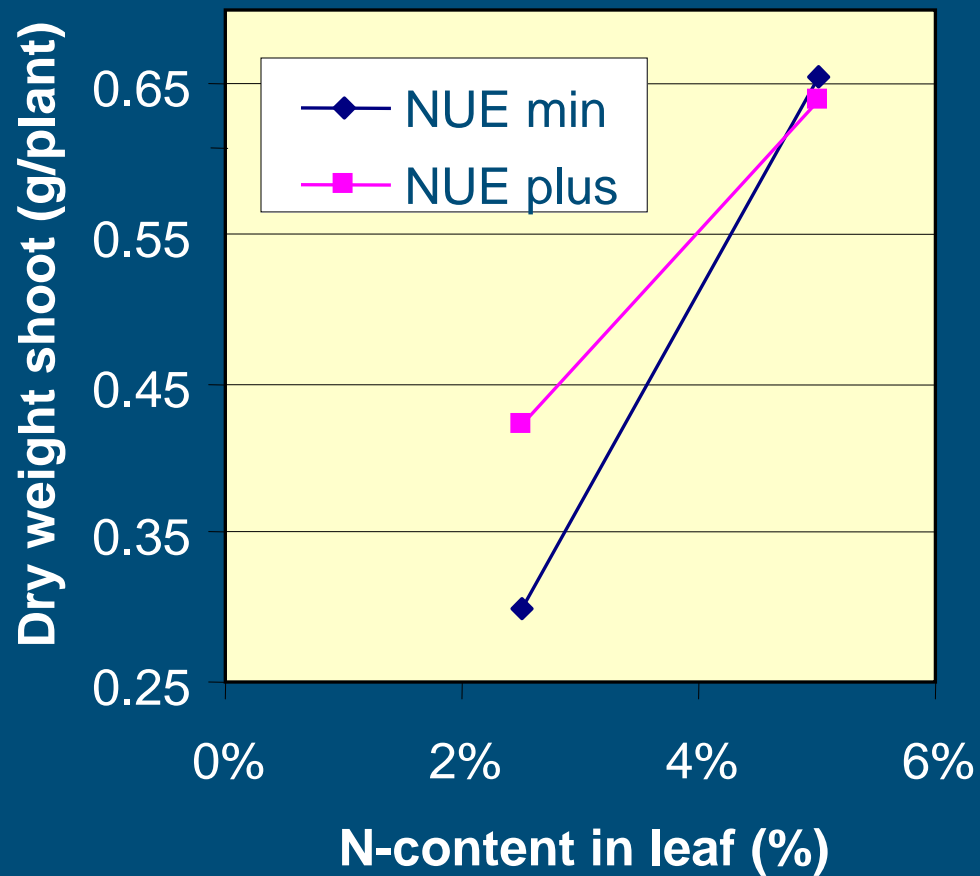
- extensive evaluation
- only results of 2 final cuts
- 2 pretreatments
- 2 selections
- 2 evaluation conditions

Field Evaluations

- 3 sites (UK, DE, NL)
- 3 replicates
- 2 N-levels



Effect marker selection on N-response

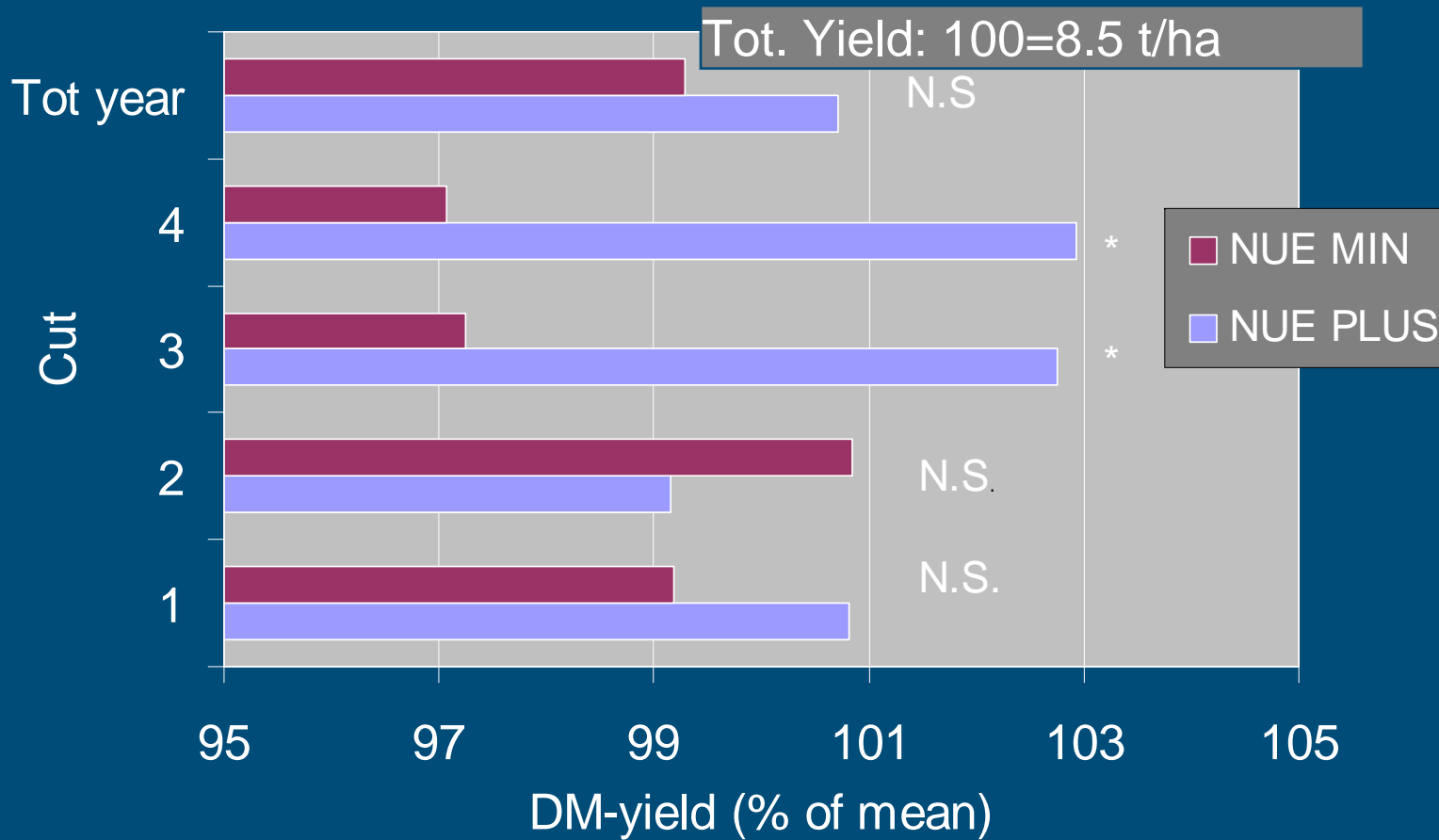


In hydroponics experiment!!



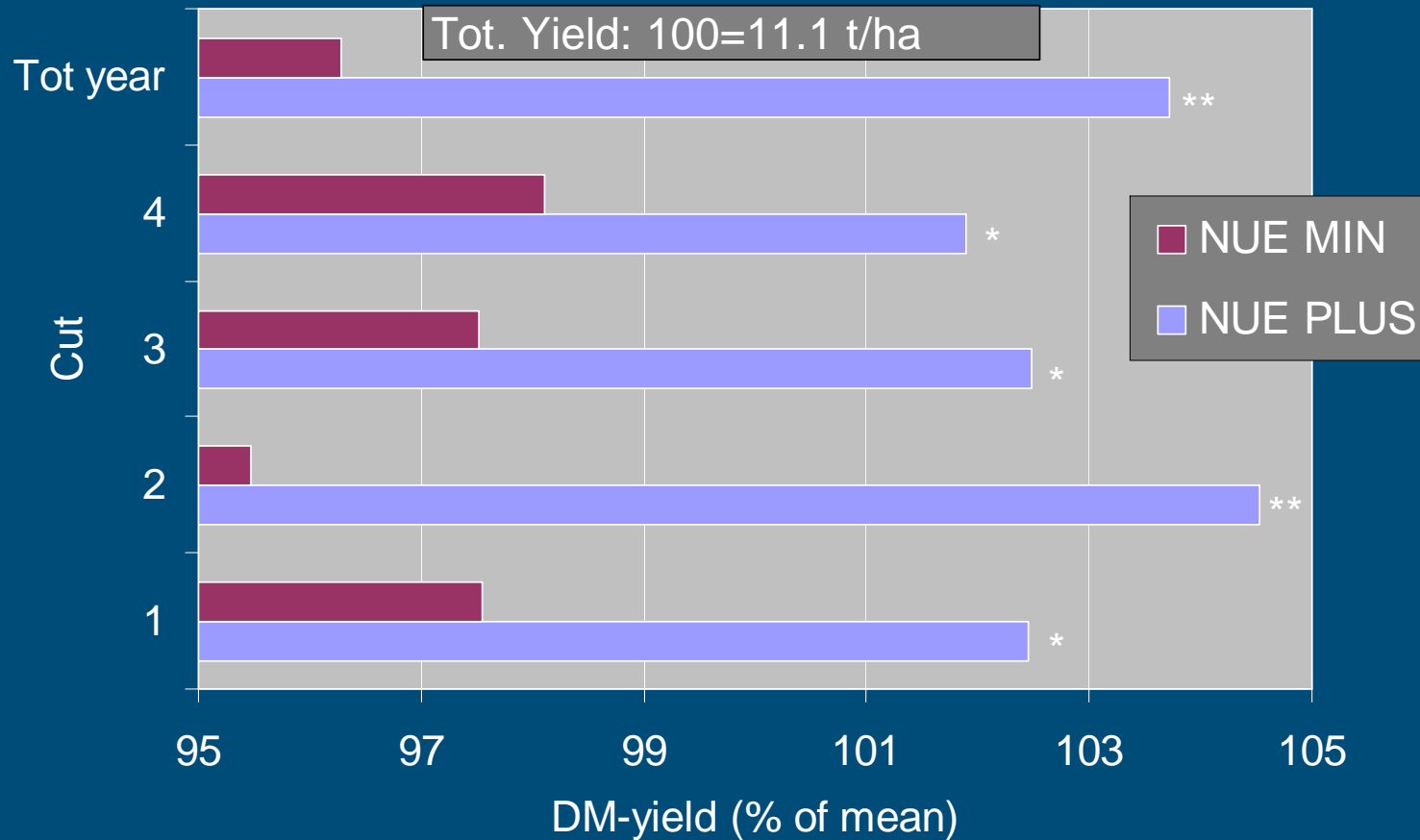
DM-yield at low N-supply (250 kg N/ha)

Field trial !!



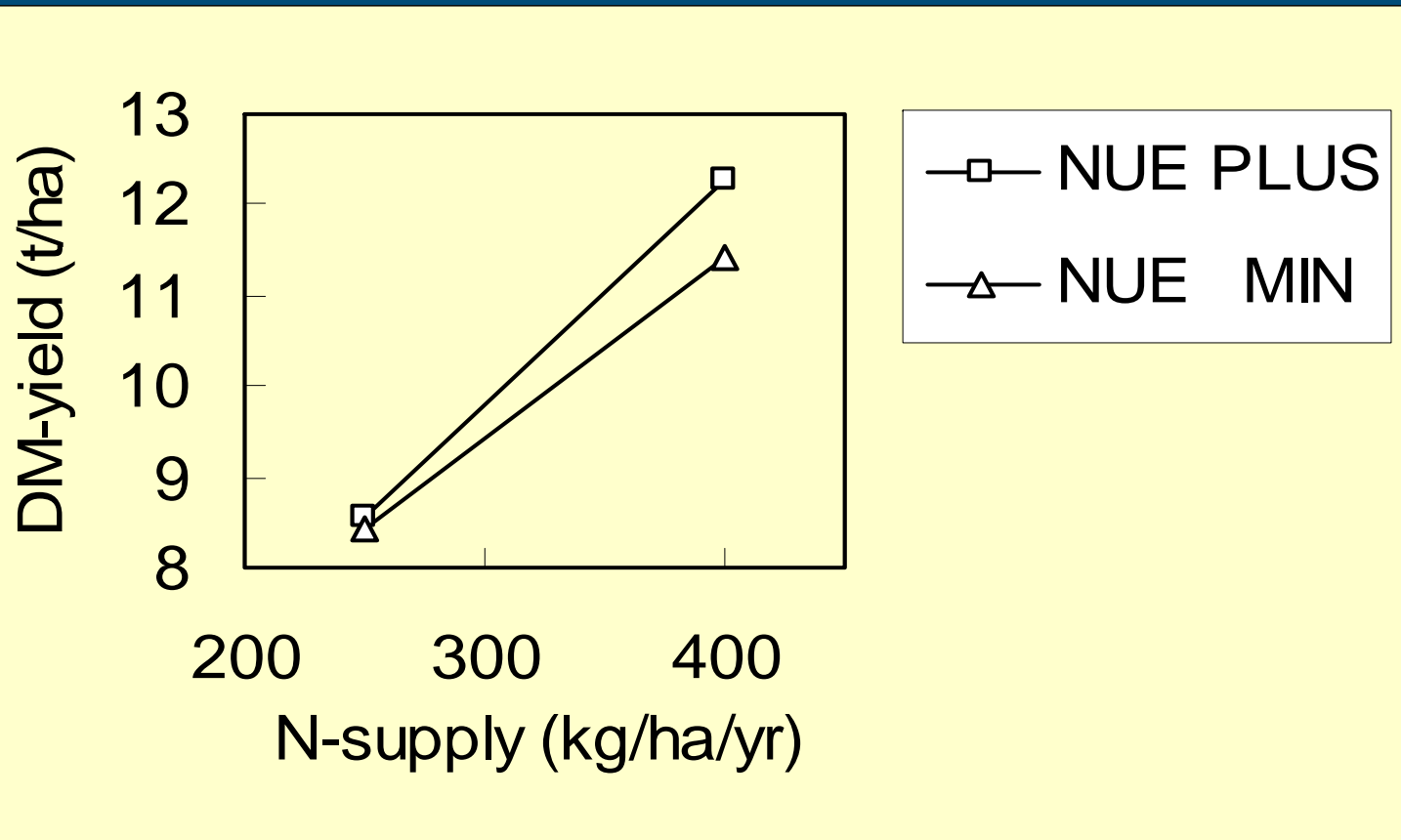
DM-yield at high N-supply (400 kg N/ha)

Field trial !!

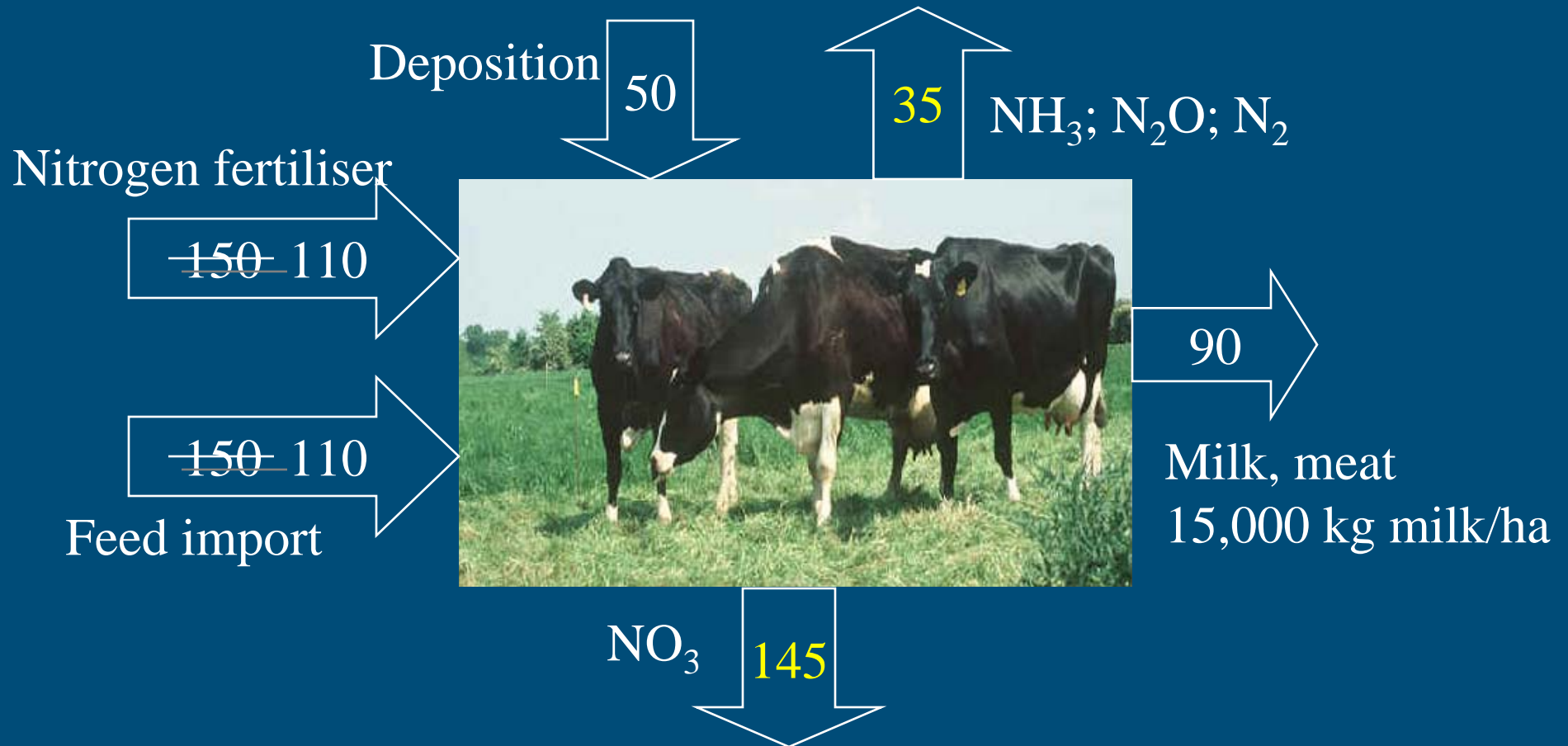


Marker selection changed N-response

Field trial !!



The solution: lower N-input, same output



Farm monitoring in the Netherlands, ~~2001~~ 2010

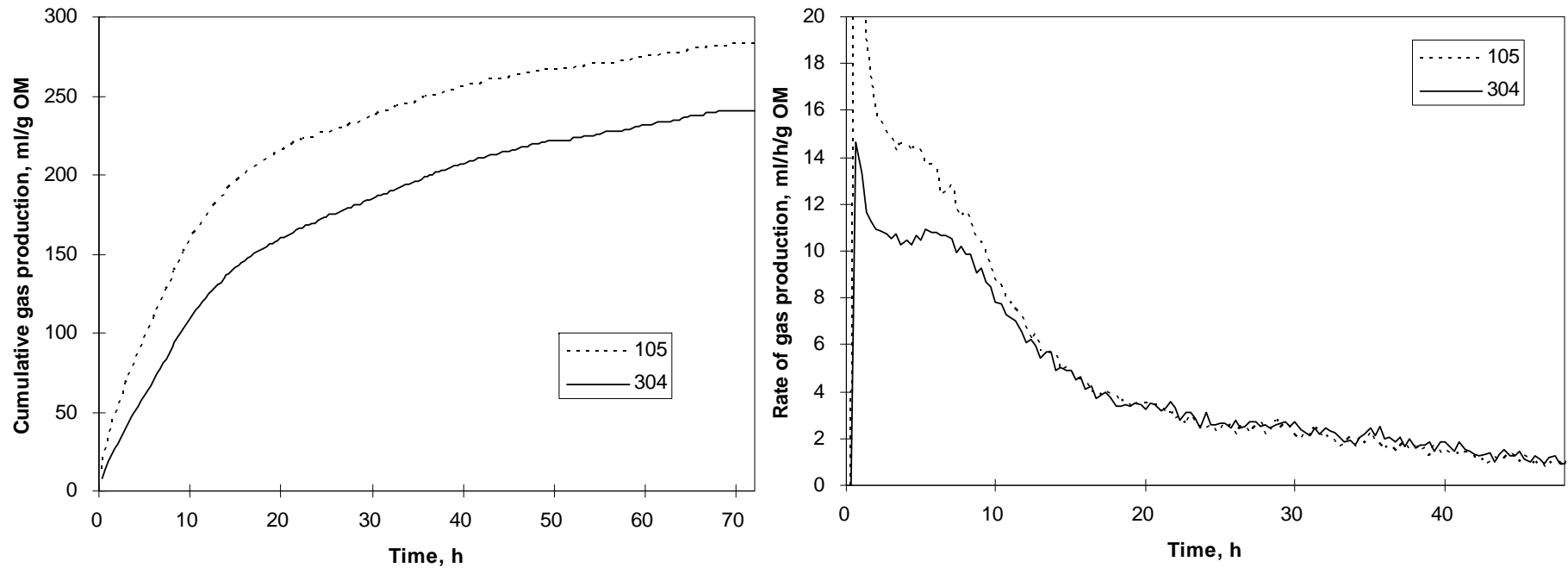


Conclusions marker selection NUE Lolium

- Substantial improvement of NUE through marker-aided selection achievable
- The yield response in the field to marker selection proves the presence of relevant genes near the QTL-loci
- NUE-related traits in hydroponics system relevant!



Ex. 2: Rate of cell wall fermentation of two grass genotypes



Cone, Van Loo & Deinum, 2002

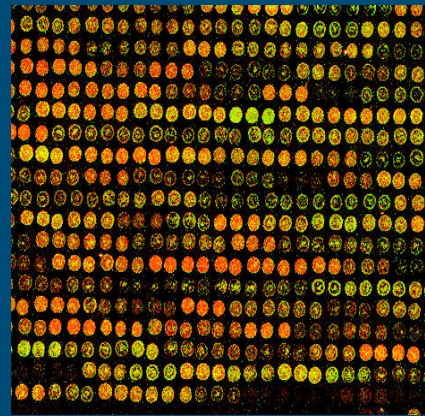
Bannink, Dijkstra & Van Loo, 2004

Next steps in Lolium

- Implementing molecular marker selection in practical breeding: how?
- Find more allelic variation for established QTLs (followed up in EU-project GRASP)
- Use of new tools (like pyrosequencing, 454-sequencing) to find allelic frequency shifts in breeding programmes



Example 3: eQTLs for cell wall digestibility maize



Koning-Boucoiran, Dolstra,
Giuliani, Tuberosa, Vorst,
Jansen, Maliepaard & Van Loo,
2005



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Most influential genes

Genes possibly contributing to genotypic variation in cell-wall digestibility among maize RILs.

Clone number	Putative function	E value
4217	87 EcoR1-XhoI: 0.8kb KIN cDNA	
4348	UDP-glucuronic acid decarboxylase, <i>O. sativa</i>	2e-04
4876	MADS-domain transcription factor, <i>Z. mays</i>	2e-108
4922	Transport inhibitor response 1, <i>A. thaliana</i>	6e-37
5604	Multi-drug resistance protein EST 23	

Expression study combined with QTL-analysis of traits can narrow down number of candidate genes: validation needed!



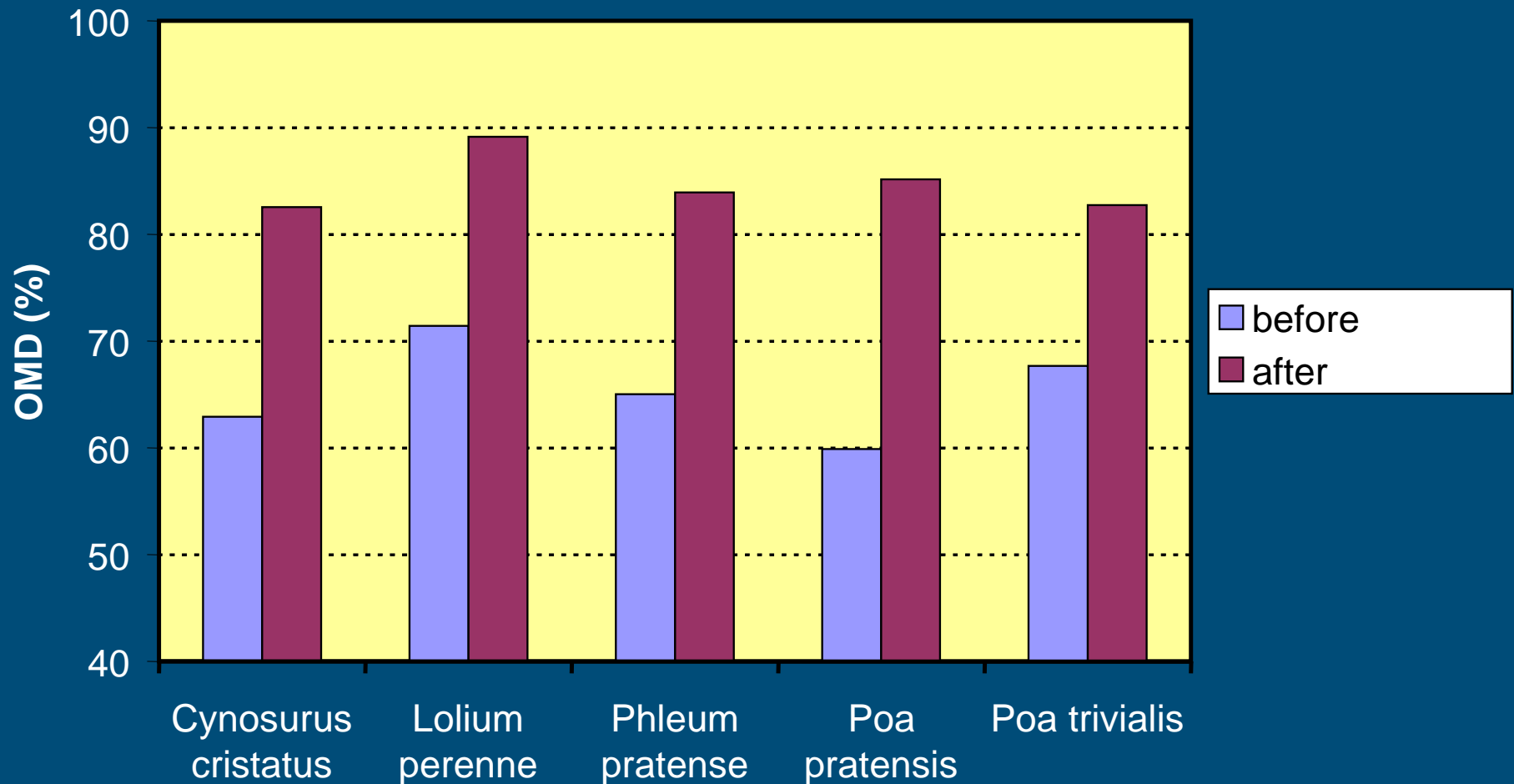
Opportunity: use genetic tools

- Marker aided selection
- Gene sequence based markers
- Allele mining
- Use allele frequency shift between selected and original population

- But for what traits: shifting use of forage



Treatment to upgrade 'nature' grass



Opportunity: new uses of forages

- Upgrade grass coming from nature conservation areas and farm areas with 'nature' function
- Fractionate grass into fibre and nutrient rich juice
 - E.g. grass biorefinery
- Different cultivars needed for new uses?



Opportunities

- Maintain production at low N-input
- Understand better intake of forage by animal
- Increase degradability and decrease passage rate
- Increase healthy compounds (e.g. CLA)
- Improve quality of 'nature' grass
- Alternative uses ('biorefinery')
- Implement marker assisted selection in grass breeding
- Increase understanding of underlying genetics: o-mics toolbox for forage crops?
- Develop co-operation across forage production chain

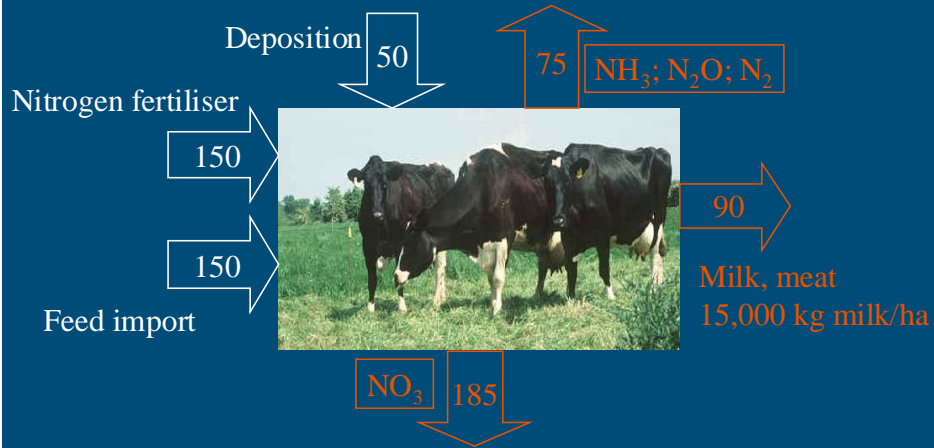


Thank you for your attention



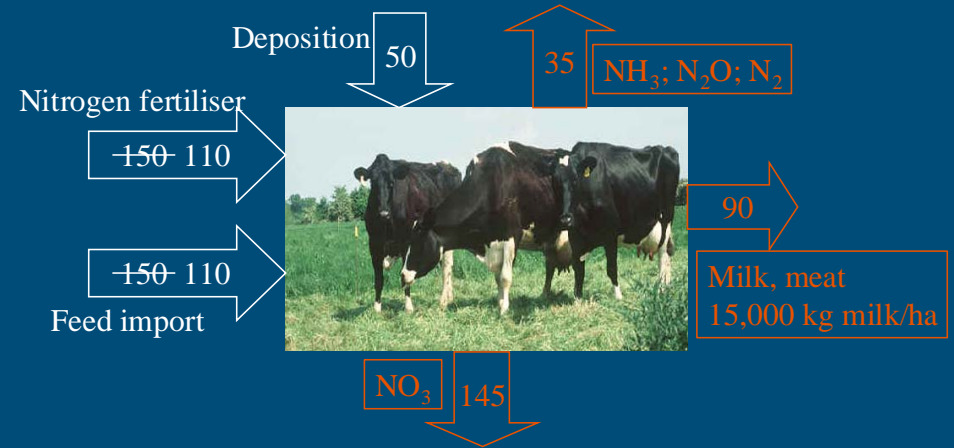
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The problem: Annual N-input >> N-output



Farm monitoring in the Netherlands, 2001

The solution: lower N-input, same output



Farm monitoring in the Netherlands, 2004-2010

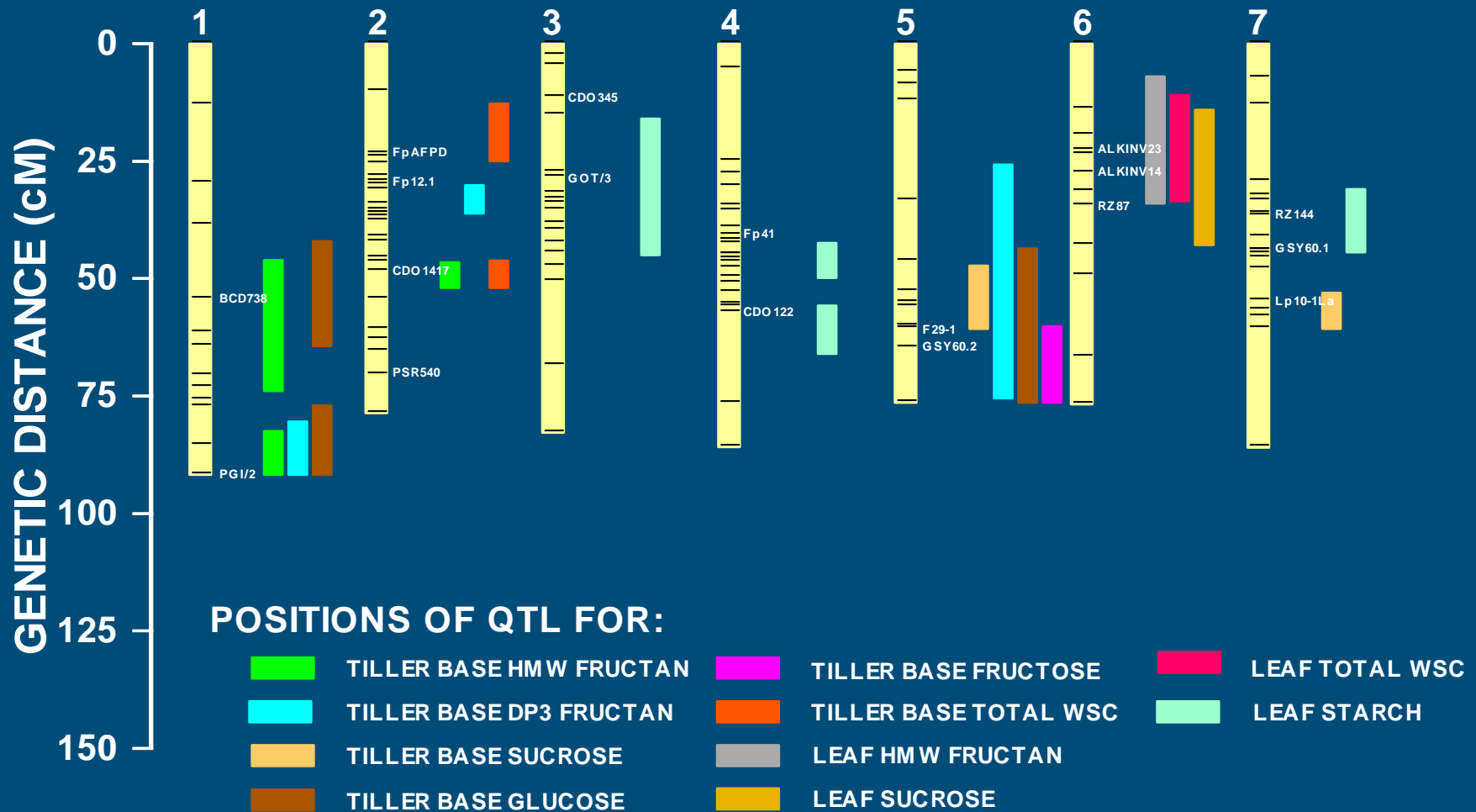
Less input in fertiliser and feed



Less output in soil and air
(same amount of milk, meat)

IGER CARBOHYDRATE QTLs

LINKAGE GROUP



Effect on N-recovery & N-utilisation

	% N-recovery	N-utilisation efficiency kg DM/kg N-uptake	
		250 kg N/ha	400 kg N/ha
NUEminus	62.3	49.4	42.8
NUEplus	67.5	51.4	46.1
<i>F2-pop</i>	63.7	50.3	43.2
Difference %	8.2	4.0	7.5

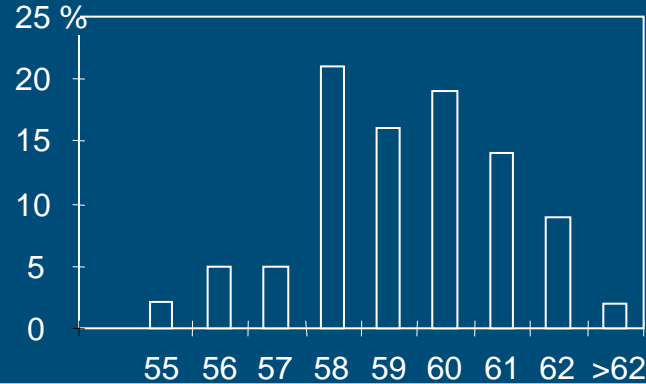


Conventional molecular selection: find QTLs

Segregating population



Cell wall (%)



N-use efficiency

