

# Authentication of ruminant meat, milk and cheese produced in grassland based production systems

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# An increased interest in authenticating ruminant products from grassland based production systems

- Consumer demand for assurance about mode of production of ruminant because of food crises
- Healthier products (*FA profile, antioxidants*)
- Interest in production practices which are *natural, environmentally friendly, respectful towards animal welfare, ...*
- Interest for producers to obtain market recognition and premium and to avoid piracy of their brands



# Development of specifications via quality labels



*Label Rouge*

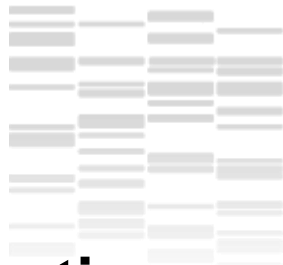


*Protected designation  
of origin*



*Organic  
Farming*

Analytical tools to be able to guarantee that the specification commitments have been fully met and to help constructing them



# PDO lamb meat



## Specification commitments

Raise lambs on the salt-marsh pastures for a minimum duration

Keep the concentrate supplementation below a predefined ceiling

Not to finish the lambs on concentrate indoors for longer than 30 days

## *Research questions*

*Identifying markers of pasture-feeding and of specific pastures*

*Investigating their latency of appearance and persistence in the animal's tissues*

# PLAN



- ❖ **Analytical approaches**
- ❖ **The case of less contrasted diets**
- ❖ **Examples of validation in the field**
- ❖ **Conclusions**

# PLAN



## ❖ Analytical approaches

### ❖ The case of less contrasted diets

### ❖ Examples of validation in the field

### ❖ Conclusions

## Diet fed to the animals



## Composition of meat and milk

### *Specific compounds*

Directly transferred from the feed to the end product

Transformed or produced by ruminal microorganisms or the animal's metabolism

### *Fingerprints*

Differences in the meat and milk composition induce differences in their optical properties



# Potential biomarkers

## Carotenoids

*(Prache & Thériez 1999, Serrano et al 2006; Röhrle et al 2011)*

## Terpens and other volatil compounds

*(Cornu et al 2001 & 2002; Bugaud et al 2001; Priolo et al 2004; Vasta & Priolo 2006; Priolo et al 2009)*

## Phenolic compounds

*(Besle et al 2010)*

## Fatty acid composition

*(Aurousseau et al 2004, Osorio et al 2013)*

## Isotopic composition of the tissues and products

*(Renou et al 2004; Schmidt et al 2005; Moreno-Rojas 2008; Osorio et al 2011)*

## Stereoisomer profile of $\alpha$ -tocopherol

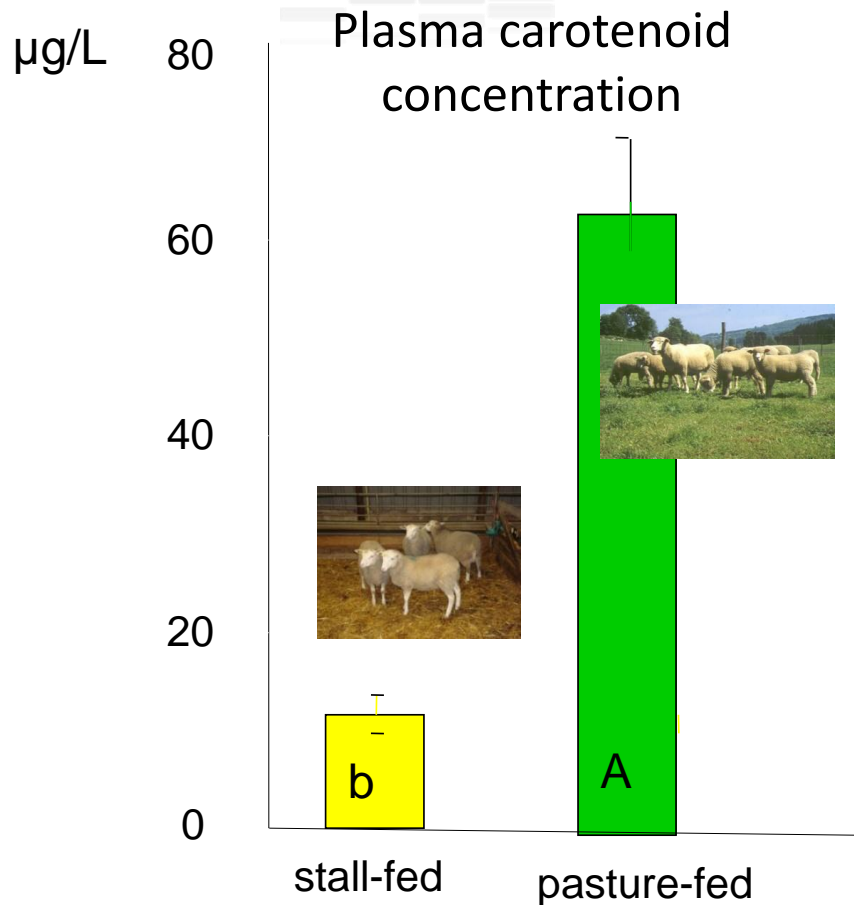
*(Röhrle et al 2011)*

## **Fingerprint approaches** based on optical properties (VIS, NIR, MIR)

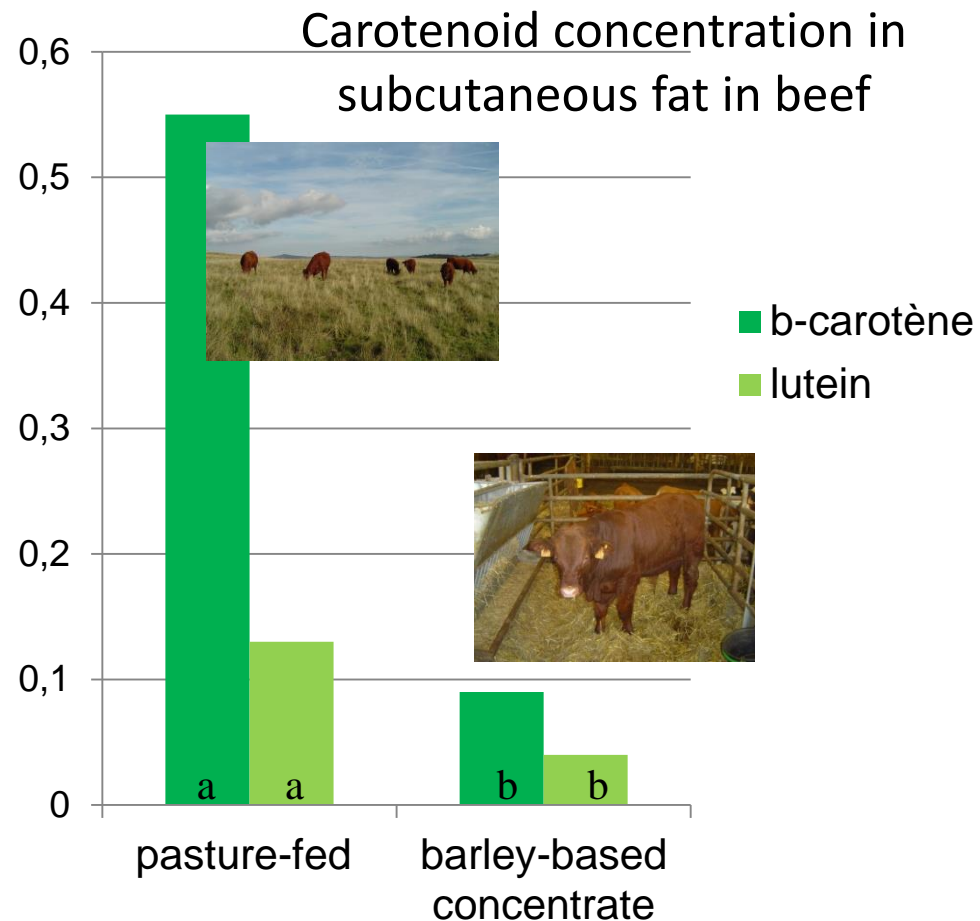
*Prache & Thériez 1999, Cozzolino et al 2002; Dian et al 2007 & 2008, Karoui R & De Baerdemaeker J., 2007 ; Röhrle et al 2011*



# Carotenoid pigments



*Prache et al., 2003*



*Rörhle et al., 2011*



# Terpens

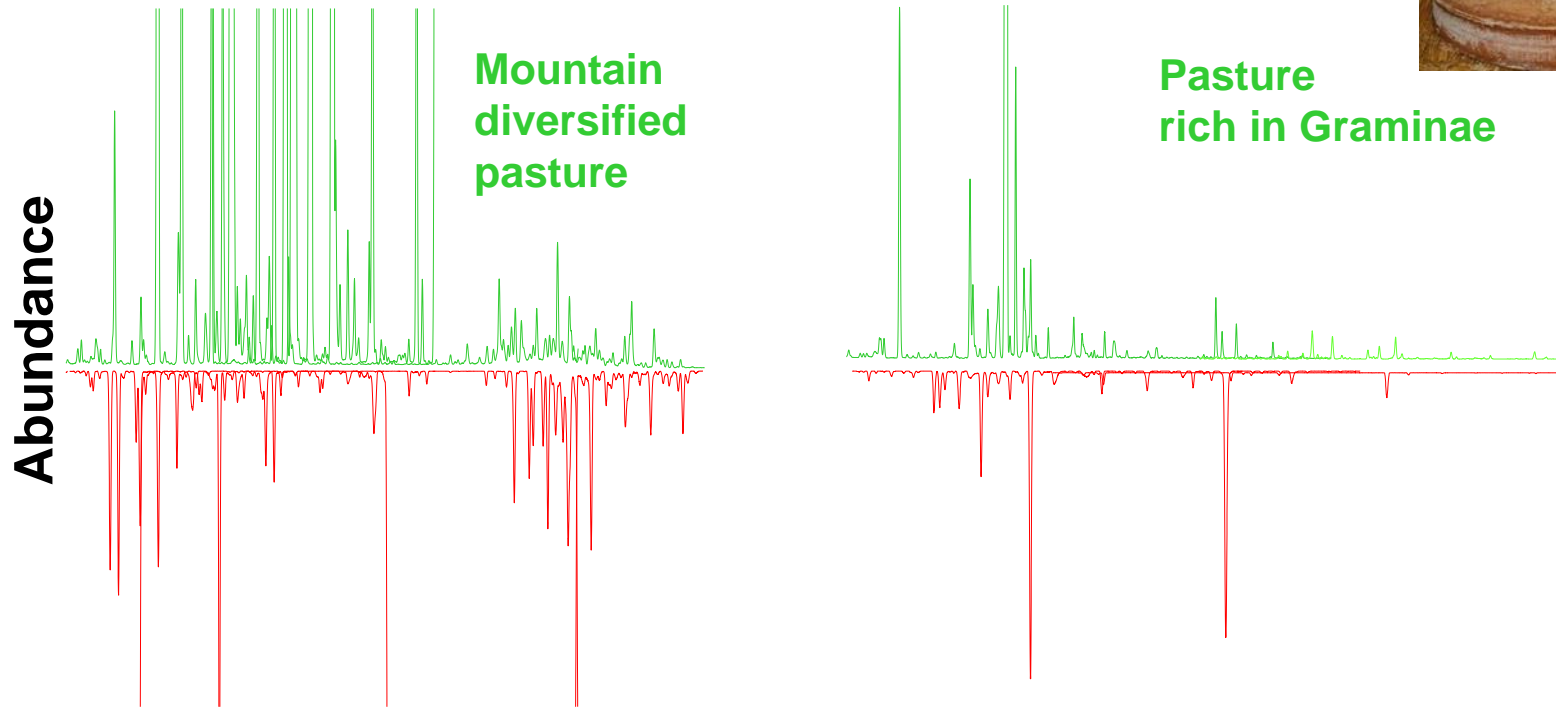
- ✓ Exclusively synthesized by plants
- ✓ Found in meat and dairy products
- ✓ In grassland plants, quantities and nature = f (botanical family)

Higher amounts in permanent highland pasture than in lowland (*Mariaca et al 1997*)

*Cornu et al. (2002)*

# Terpens in pasture and Abondance cheese

## Terpenes composition in grass



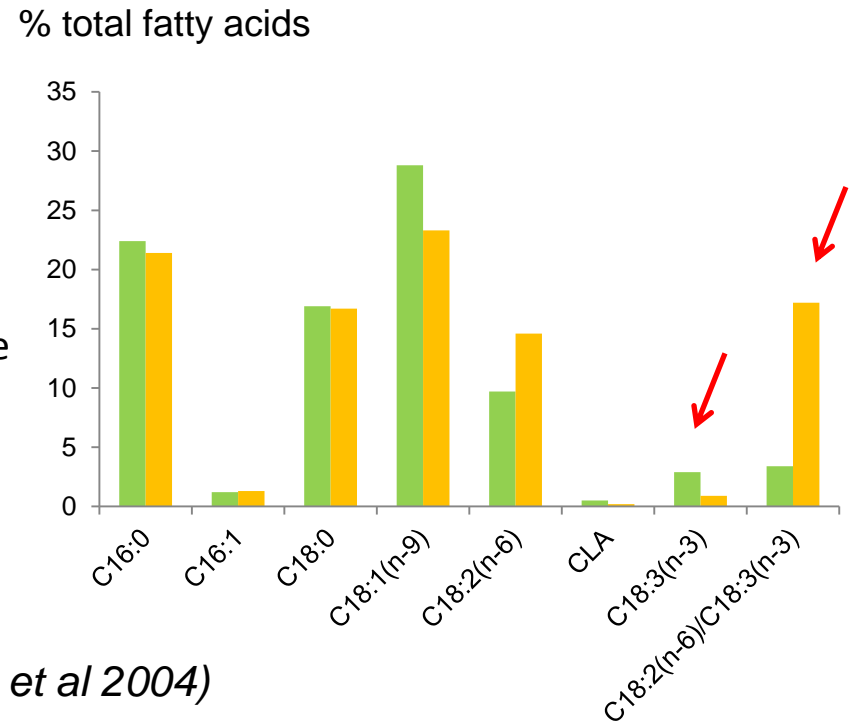
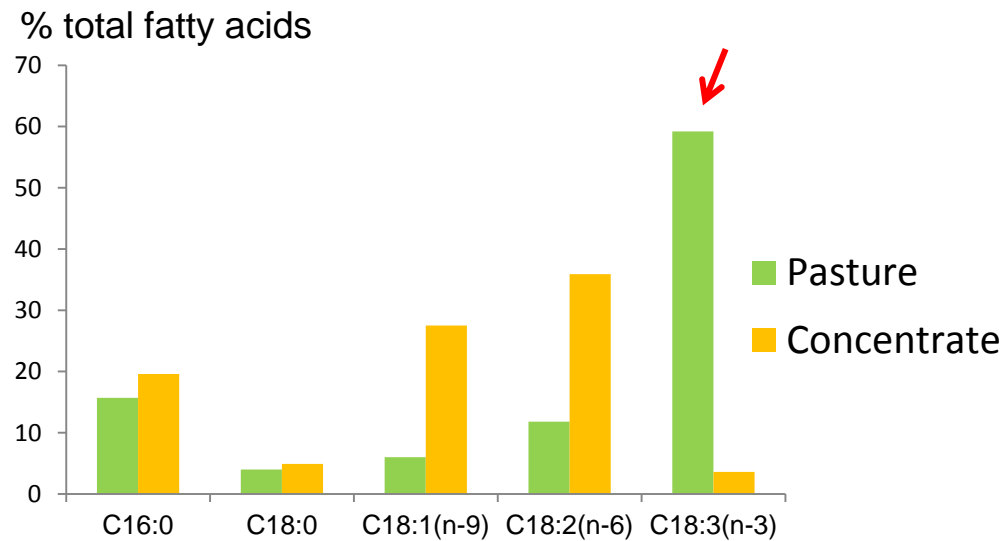
## Terpenes composition in cheese

*Bugaud et al., 2001*

# Fatty acid composition

## Feed fatty acids

## Lamb meat fatty acids

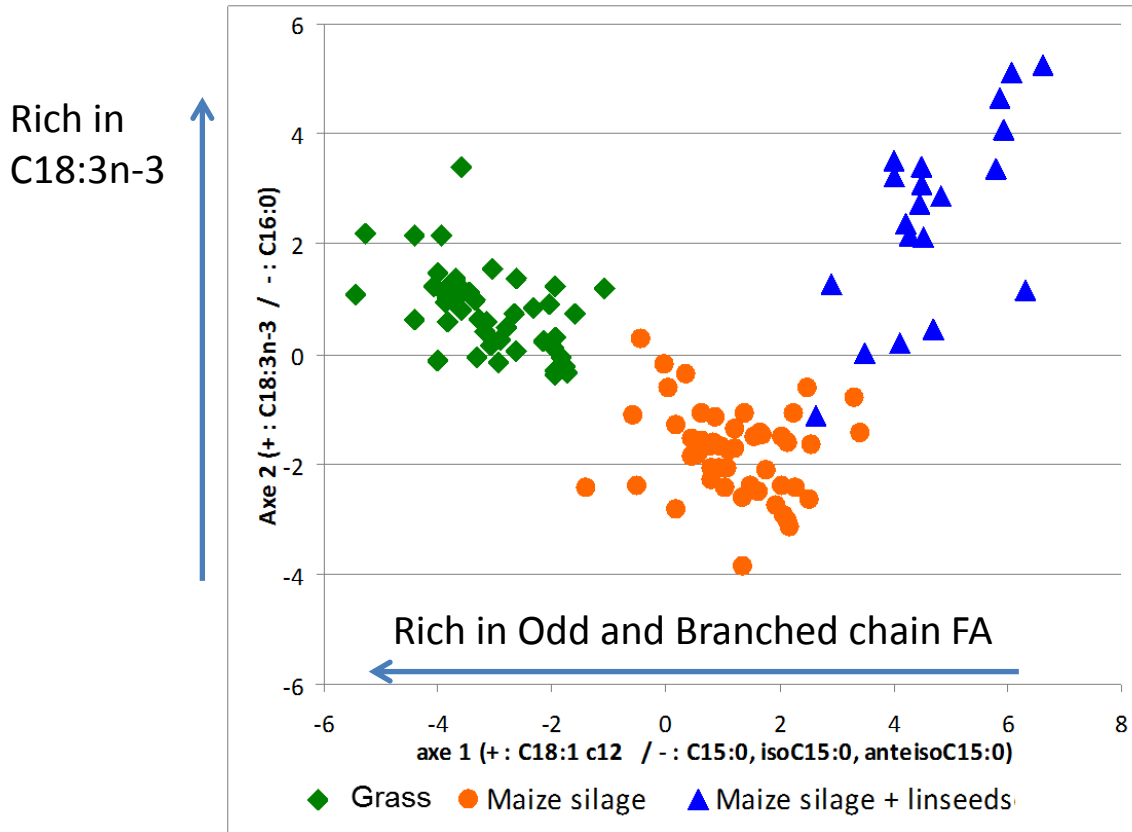


(Aurousseau et al 2004)

**Inclusion of oil seeds in animal diet can change the fatty acid profile**

# Misclassification of milk when linseed added?

Bulk milks from French farms sampled 5 times a year (summer and winter)



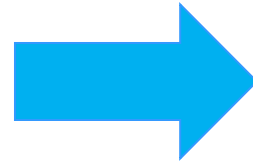
The supplementation of maize diet with linseed increases C18:3-n-3 but the discrimination is reliable when using Odd and Branched chain FA

Hurtaud et al, under revision



# Stable isotopes

Ratio of  
heavy isotope/light isotope  
in the feed and water  
animals consume



Ratio of  
heavy isotope/light isotope  
in meat and milk

$^2\text{H}/^1\text{H}$ ,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ ,  $^{18}\text{O}/^{16}\text{O}$ ,  $^{34}\text{S}/^{32}\text{S}$

$\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ ,  $\delta^{34}\text{S}$  (per mil)





# C4 plants have a higher $^{13}\text{C}$ proportion than C3 plants

**High**  $^{13}\text{C}/^{12}\text{C}$  ratio

**Low**  $^{13}\text{C}/^{12}\text{C}$  ratio

Maize

Grassland plants (temperate areas)

Tropical grasses

Barley

Soya bean

Sugar beet

*(Beef meat, de Smet et al 2004)*

*(Lamb meat, Moreno-Rojas 2008; Norman et al 2009)*

# $^{15}\text{N}/^{14}\text{N}$ ratio in pastures is influenced by the proportion of legume and the fertilizer

**High**  $^{15}\text{N}/^{14}\text{N}$  ratio

Low proportion of legumes in the pastures

Organic fertilizer

**Low**  $^{15}\text{N}/^{14}\text{N}$  ratio

High proportion of legumes in the pastures

Mineral fertilizer



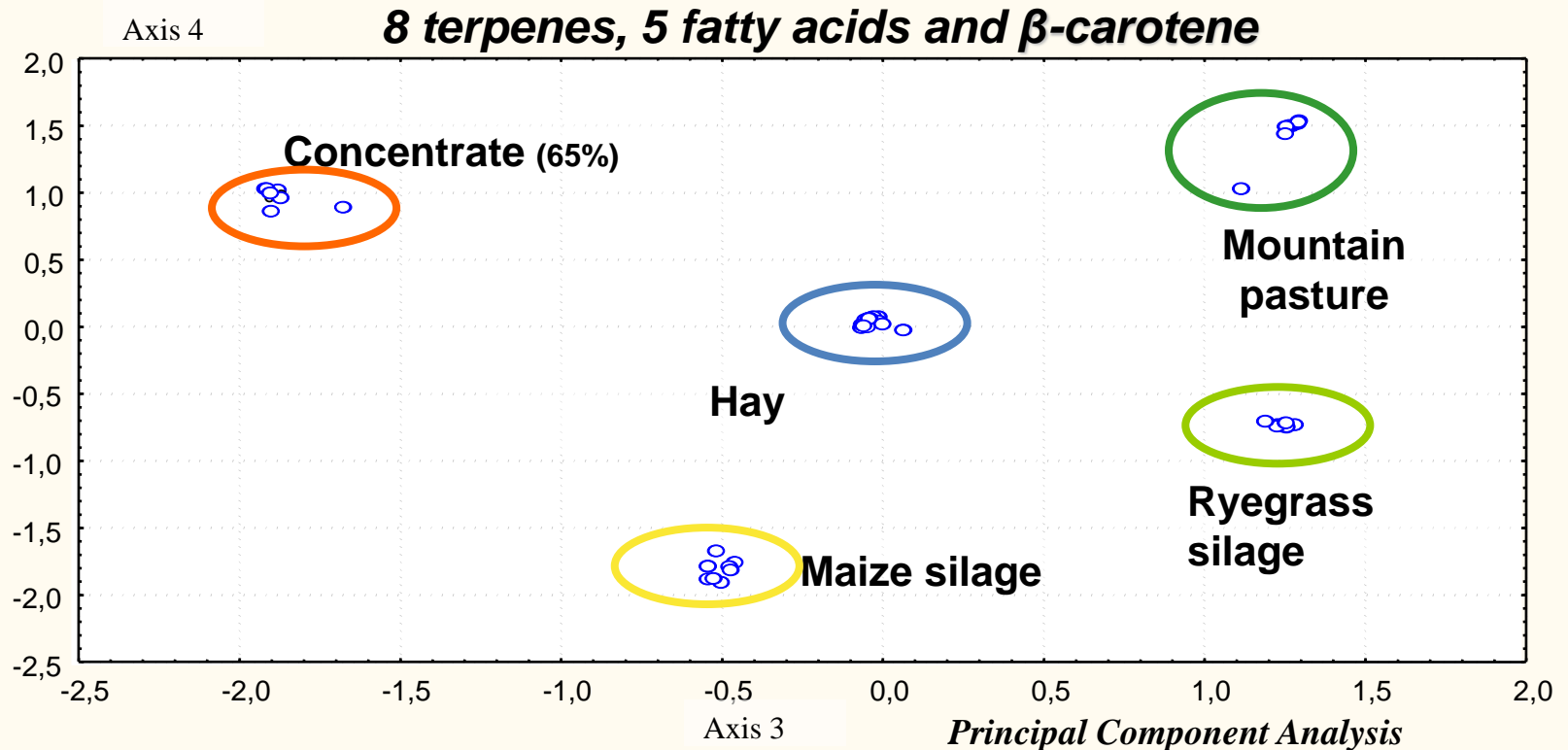
*Discrimination between **organic** and **conventional** beef (Boner & Forstel 2004; Schmidt et al 2005; Bahar et al 2008)*



*To what extent may the **nature of the fertilization** modulate (mineral/manure) ?*



# The simultaneous analysis of milk fatty acids, terpenes and $\beta$ -carotene enabled the reliable discrimination of 5 diets in dairy cows



Martin et al., 2005

# Potential biomarkers

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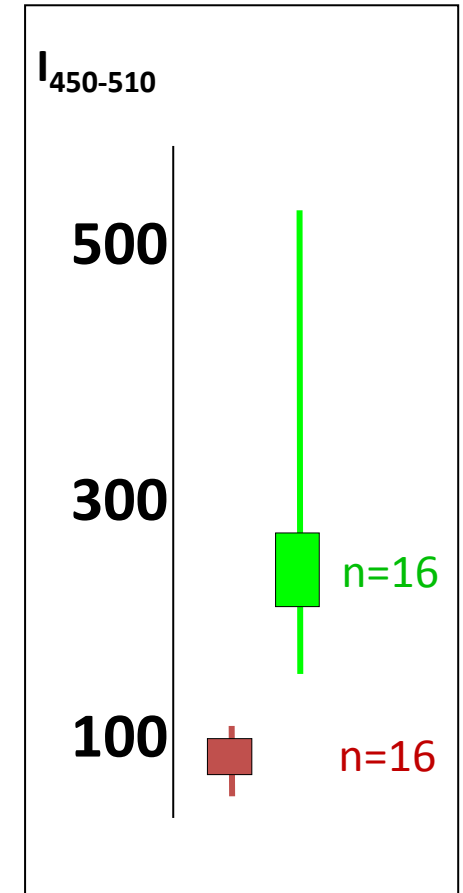
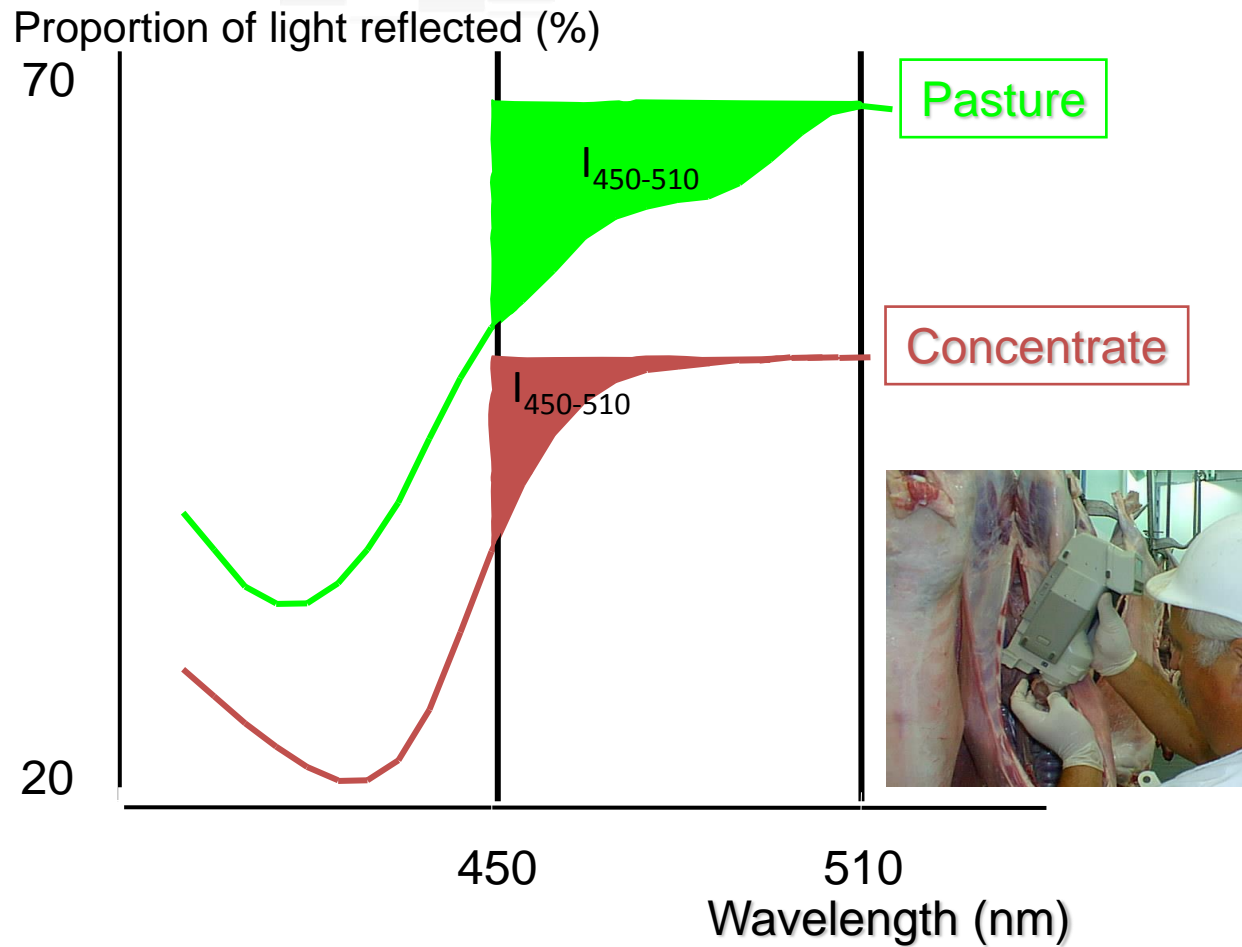
## Stereoisomer profile of $\alpha$ -tocopherol

*(Röhrle et al 2011)*

## **Fingerprint approaches** based on optical properties (VIS, NIR, MIR)

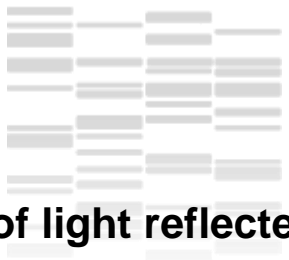
*Prache & Thériez 1999, Cozzolino et al 2002; Dian et al 2007 & 2008, Karoui R & De Baerdemaeker J., 2007 ; Röhrle et al 2011*

# Reflectance spectrum of perirenal fat in lamb

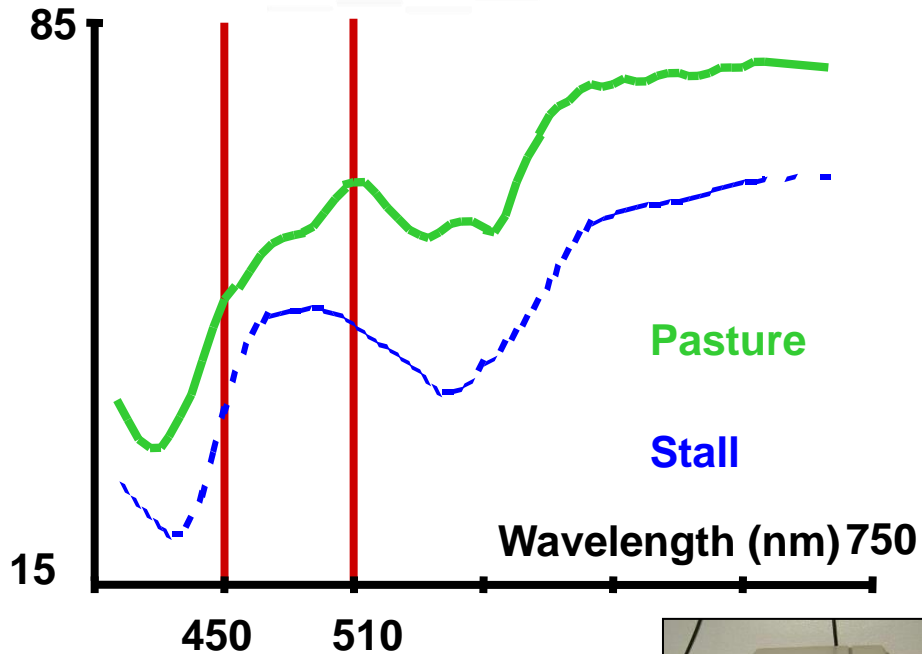


(Prache & Thériez 1999)

# Spectral methods



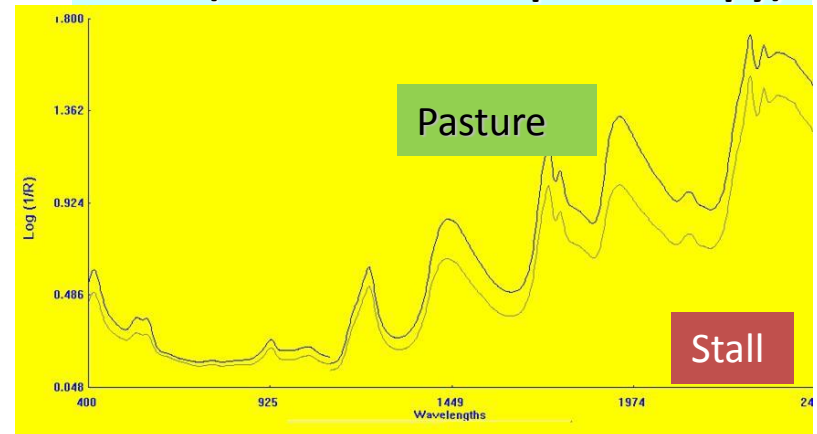
Proportion of light reflected (%)



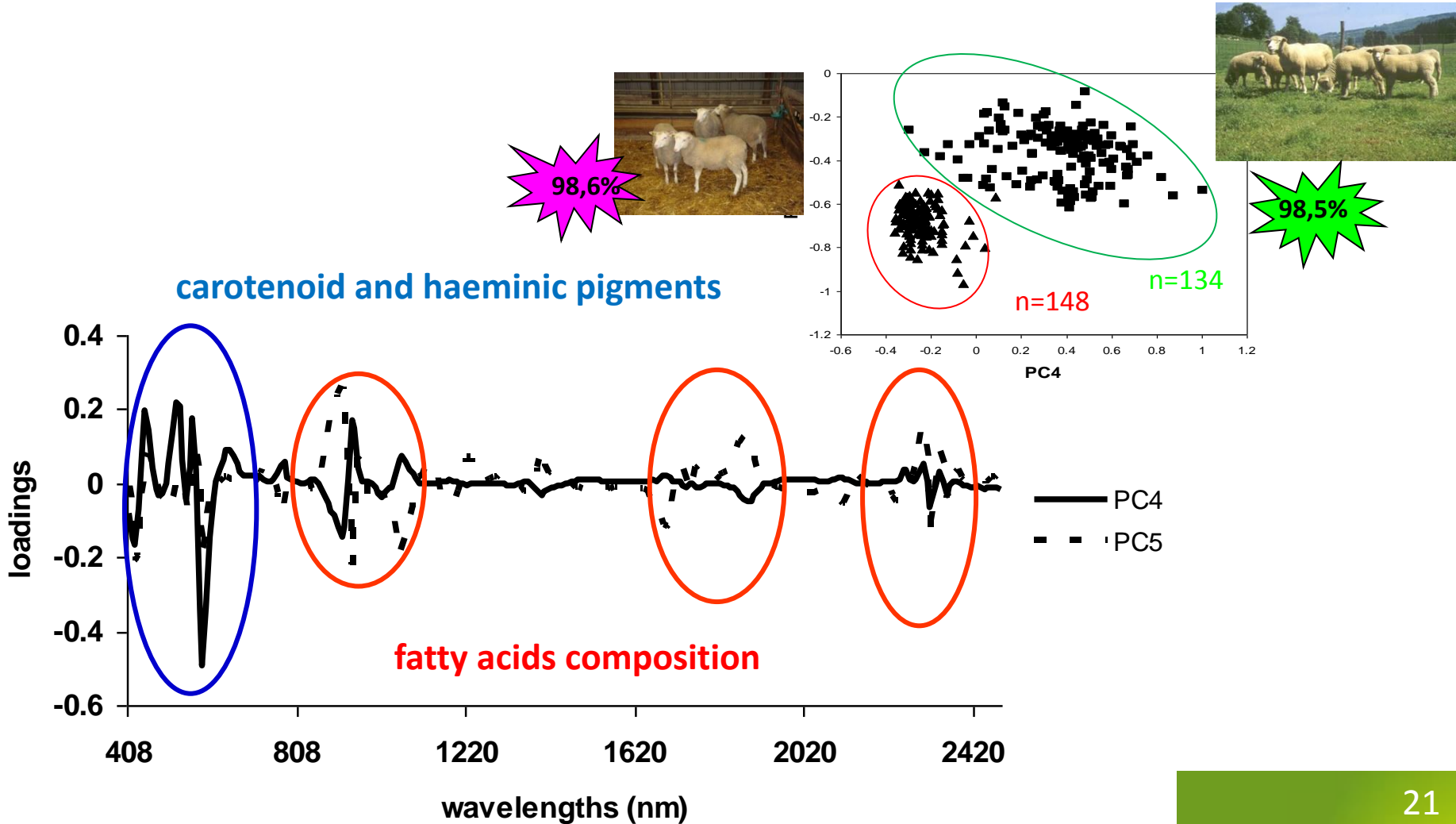
Spectroradiometry  
400-700 nm



NIRS (Near infra-red spectroscopy)



# VIS-NIRS reflectance spectrum of perirenal fat : reliable discrimination between pasture-fed and concentrate-fed lambs



# PLAN



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# A number of factors may make the authentication more complex

Some variations in... The plants' concentration in the potential markers  
The animals' herbage intake level  
Animals' dietary choices at pasture

May depend on ... Seasonal variations (*Prache et al 2003*)  
Phenological stage (*Mariaca et al 1997; Tornambé et al 2006*)  
Grazing management (*Tornambé et al 2006*)  
Herbage allowance  
Concentrate supplementation (*Zawadzki et al 2013*)

Dose-dependent response studies when possible ...

Dietary switches

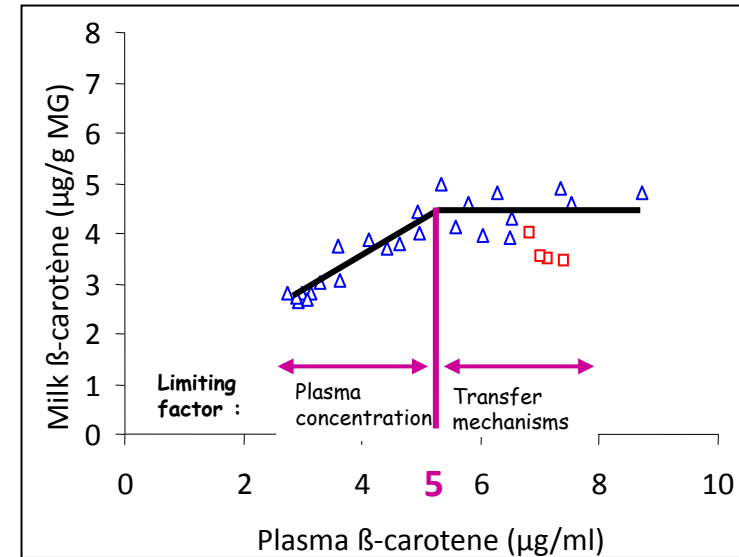
# Dose-dependent responses

## Carotenoid pigments

Plasma carotenoid concentration linearly related to carotenoid intake level

(dairy cows : Calderon et al 2007; lamb : Dian et al 2007)

Milk carotenoid content : curvilinearly related to plasma carotenoid content (Calderon et al 2007)



## Stable isotopes ratios



Linear relationships :

% of C4 plants in the diet/ $\delta^{13}\text{C}$  in skim milk (Crittenden et al 2007, Camin et al 2008)

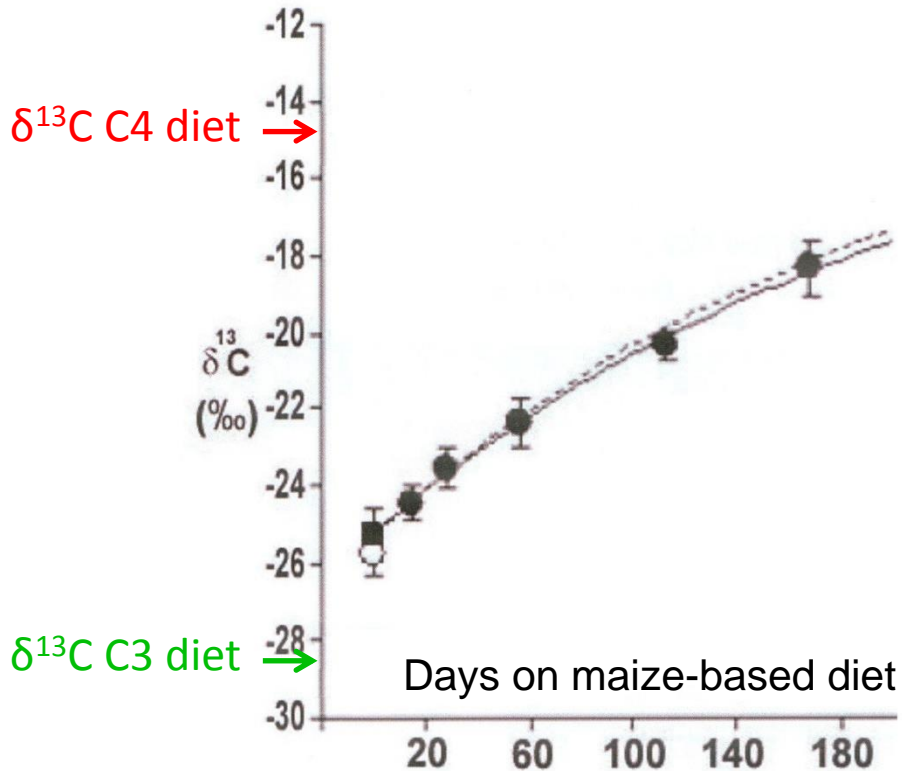


% of legumes in the diet/ $\delta^{15}\text{N}$  in meat (Devincenzi et al, in press)



# Dietary switch from C3 to C4 plants (maize) in beef

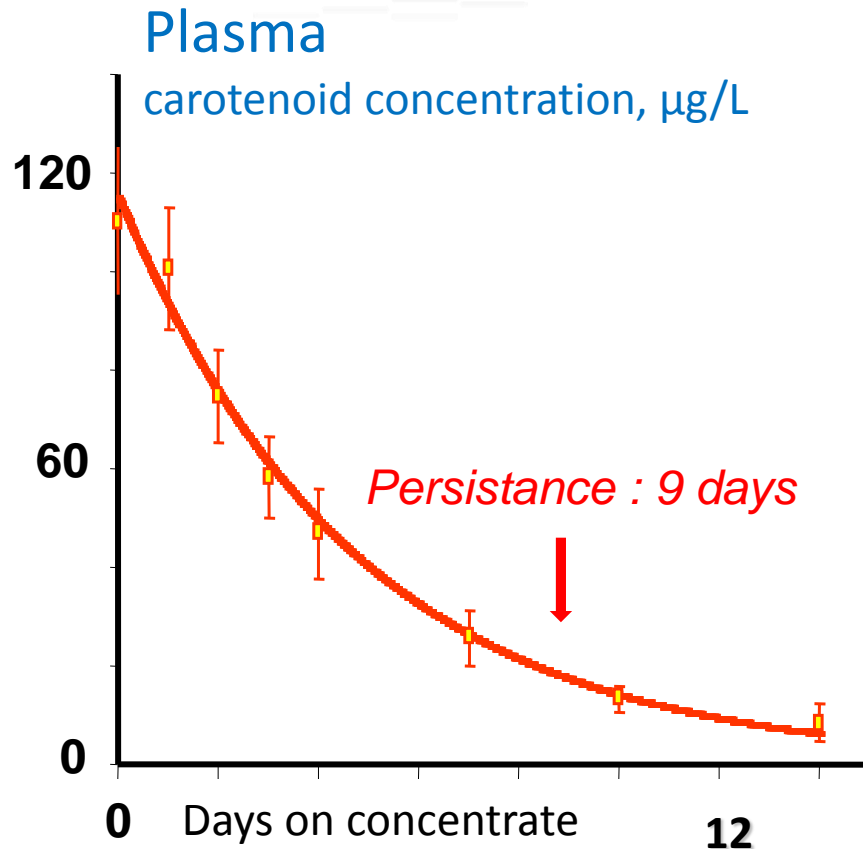
$\delta^{13}\text{C}$  in longissimus dorsi muscle



The feeding of the maize was reflected by the  $\delta^{13}\text{C}$  in muscle. Even though the isotopic equilibrium was not reached after 168 days of feeding maize, the isotopic ratios allowed to distinguish steers finished for different periods on maize

*Bahar et al (2009)*

# Dietary switch from pasture to concentrate in lambs



## Perirenal fat carotenoid concentration

Negatively related to liveweight gain during finishing period (kg)

*Persistence : 35 days*

	Plasma concentration ( $\mu\text{g/l}$ )	Spectrocolorimetric index perirenal fat
Pasture-fed	> 36.6	> 152
Stall-fed	< 20.6	< 152
Stall finished grazing lambs	< 20.6	> 152

*Prache et al 2003*

# PLAN



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# Spectral methods to authenticate cow's diet VIS/NIR spectroscopy applied to commercial cheeses



Cantal



Tomme de Savoie

Pasture



Preserved  
forages



Abondance

4.0% error, n=308

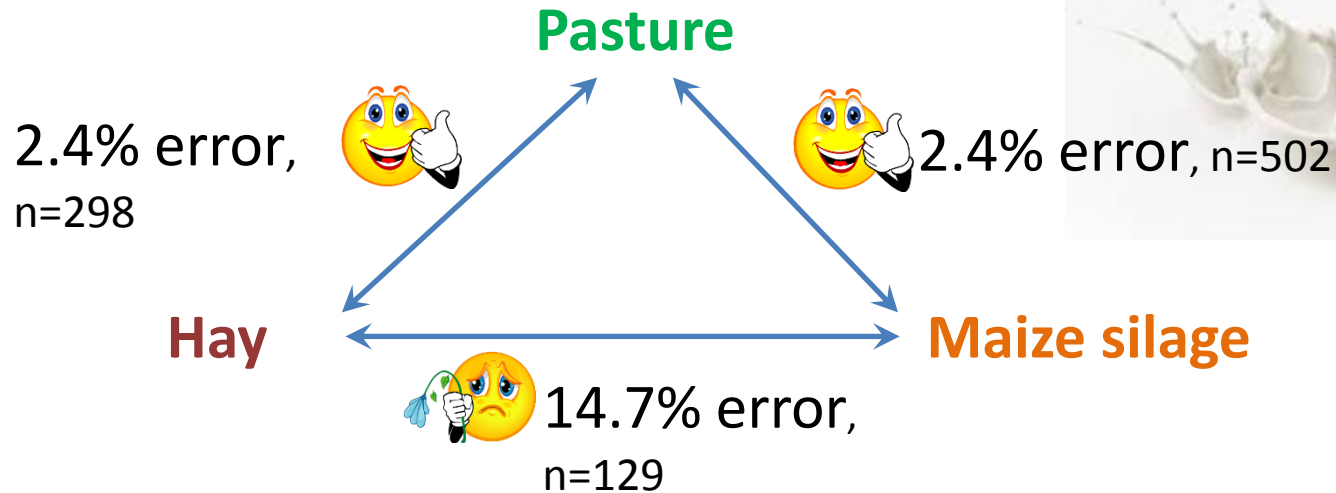


Salers

(Andueza et al., 2013)

Good discrimination due to  $\beta$ -carotene content and fatty acids profile of cheeses

# Spectral methods to authenticate cow's diet mid infrared spectroscopy (MID) applied to milk



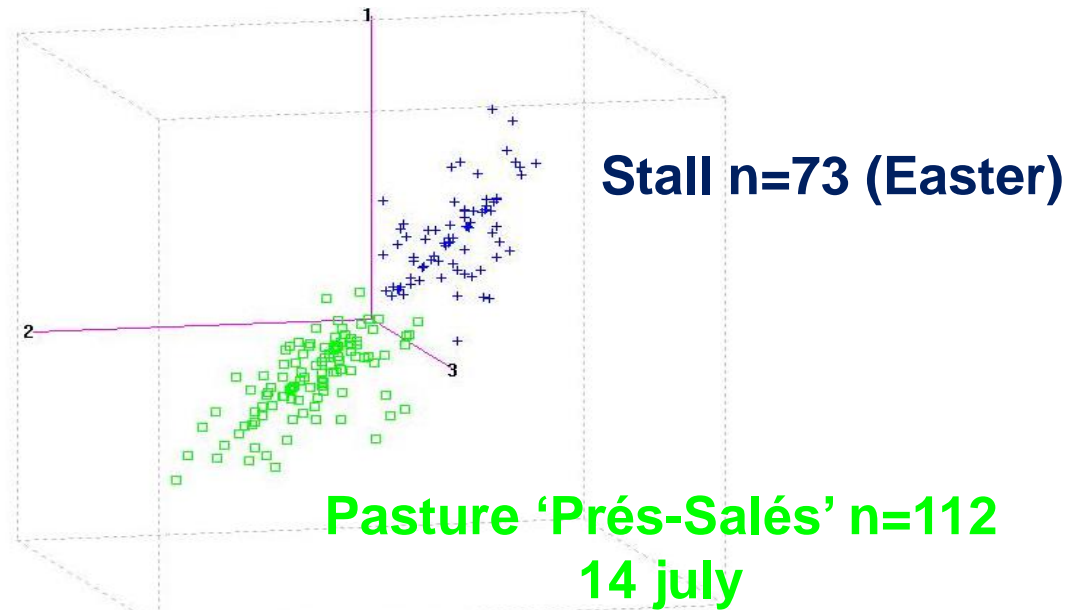
(Valenti et al., 2013)



5 farmers producing stall-fed lambs and salt marsh lambs 'Agneaux Prés-Salés'



VIS+NIRS perirenal fat



Prache et al, unpublished

# PLAN



- ❖ Analytical approaches
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- ❖ **Conclusions**



## Discrimination of contrasting diets

*A range of specific compounds or fingerprints*

The combined use of compounds and tissues may be necessary

## Huge potential of spectral methods

(provision of a global signature containing compositional information)

## Diet switches

*Latency and persistence of the markers*

## Sources of variation in the animal response

*Factors linked to the plants, animals and management*

## Validation procedure





## Questions that remain...

*Less contrasted feeding regimes*

*The cumulative effects of changes in feeding regimes*

*The sources of bias*

*The different methods may be  $\pm$  expensive and  $\pm$  difficult to implement*

Not systematic analyses, how to combine?

*Some commitments may be difficult to guarantee*

The proportion of pasture in the diet

Daily concentrate supplementation at pasture below ceiling

*In parallel with 'paper traceability'*

Controls using documents and inspection visits

# Acknowledgements



*Dr Yves Chilliard, INRA*

*Dr Anne Ferlay, INRA*

*Dr Donato Andueza, INRA*

*Dr Benoît Graulet, INRA*

*Dr Dominique Bauchart, INRA*

*Dr Pierre Nozière, INRA*

*Dr Agnès Cornu, INRA*

*Dr Didier Micol, INRA*

*Dr Jean-Michel Besle, INRA*

*Dr Bernard Aurousseau, INRA*

*Dr Catherine Hurtaud, INRA*

*Olivier Delfosse, INRA*

*Pr Claire Agabriel, VetAgro Sup*

*Dr JL Berdagué, INRA*

*Dr Erwan Engel, INRA*



*Dr Alessandro Priolo, Post doc*

*Cristina Barbosa, PhD student*

*E. Serrano, post-doc*

*Paulo Dian, PhD student*

*F. Calderon, PhD student*

*Dr Mauro Coppa, post-doc*

*A. Lucas, PhD student*

*Stefani Macari, PhD student*

*Lisandre de Oliveira, PhD student*

*F. Zawadzki, PhD student*

*Thais Devincenzi, PhD student*



*UE Monts d'Auvergne*

*UE Ruminants Theirx*





# Authentication

the procedure by which a food product is verified as complying with its label description, and conforming to what is established by regulations  
*(Dennis, 1998)*

Prove that a particular food product is as is stated on the product label

# French PDO lamb meat

## Specification commitments

to both preserve

*the specificities of lamb meat*

and

*the fragile equilibriums of the natural area*



## Specification commitments

Not to graze the salt-marsh pastures before beginning of March

Raise lambs having a minimum age on these salt-marsh pastures for a minimum duration

→ Seasonality of the production (mid-may to december)

Keep the concentrate supplementation below a predefined ceiling

Not to finish the lambs on concentrate indoors for longer than 30 days



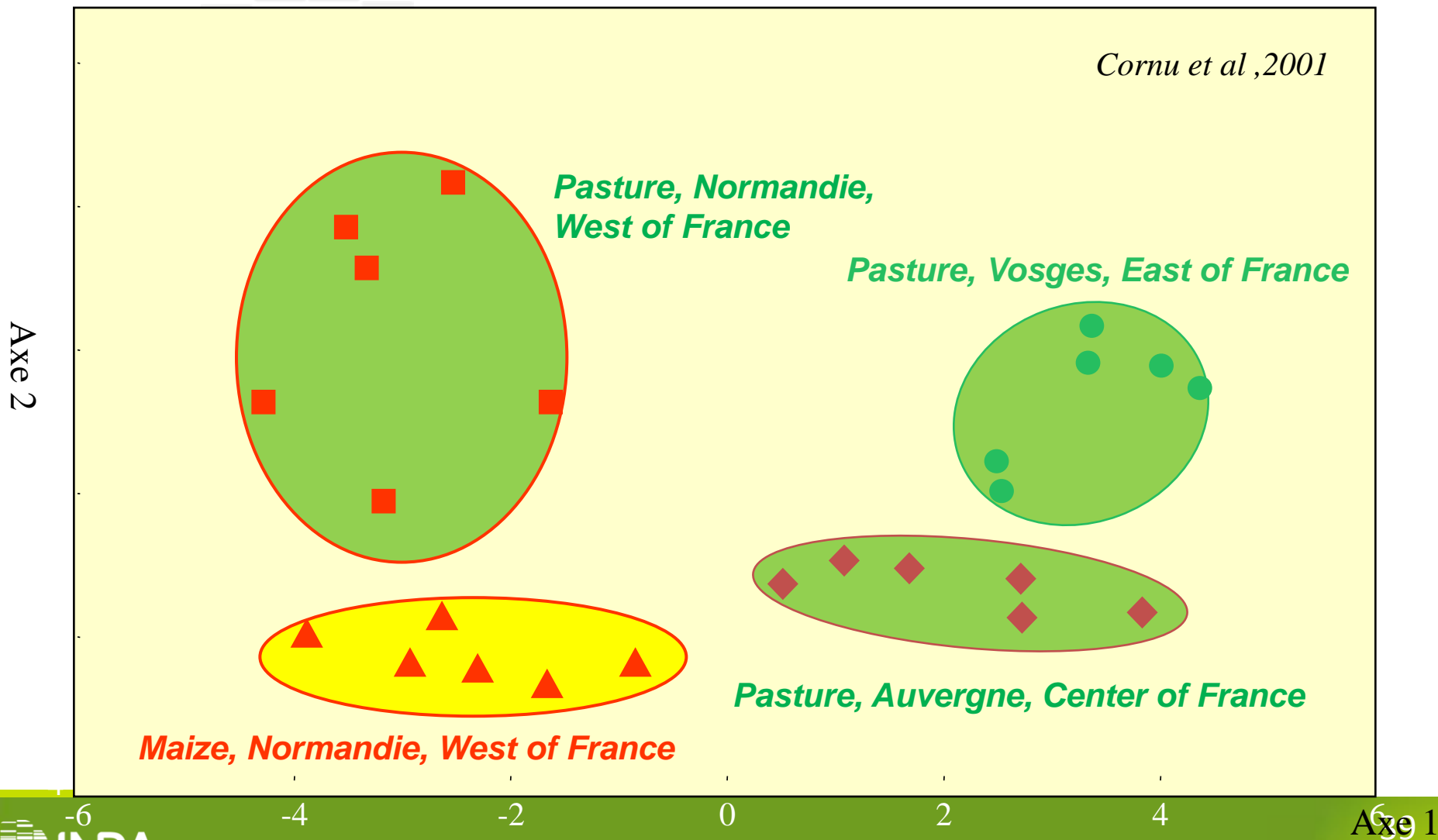
## Research questions

*Identifying markers of pasture-feeding and markers of specific grassland flora*

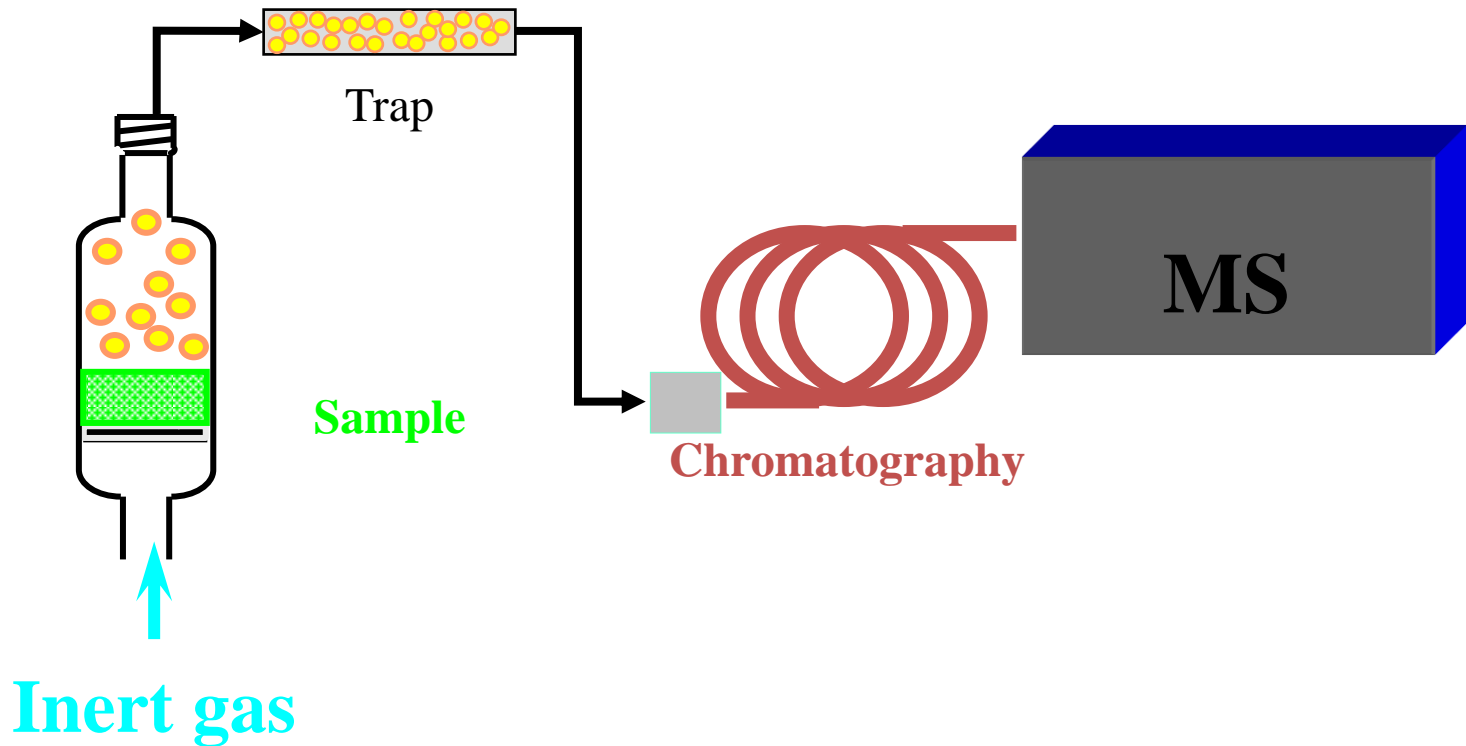
*Investigating their latency of appearance and persistence*

*Dose/response relating markers' concentration in animal tissues with intake levels*

# Discrimination from terpenes desorbed from beef fat of different diets on a same site and of grasslands from different geographic areas



# Dynamic headspace-gas chromatography-mass spectrometry





# Phenolic compounds in milk

mountain pastures

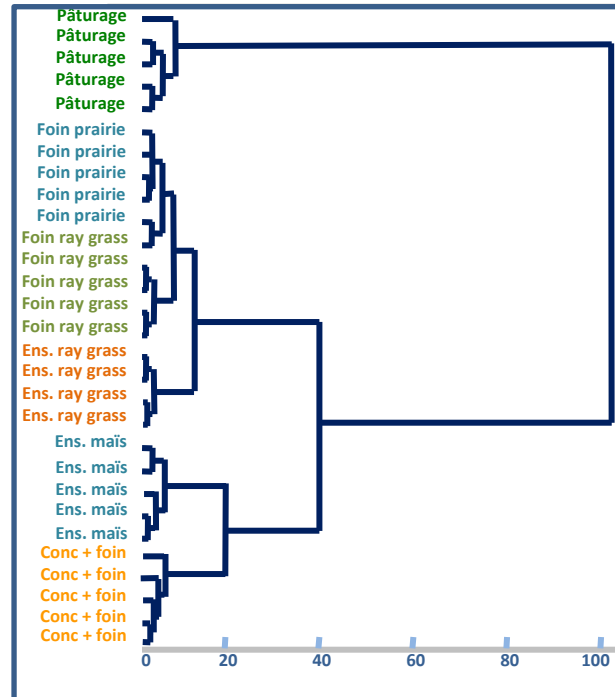
hay from mountain pastures

ryegrass hay

ryegrass silage

maize silage

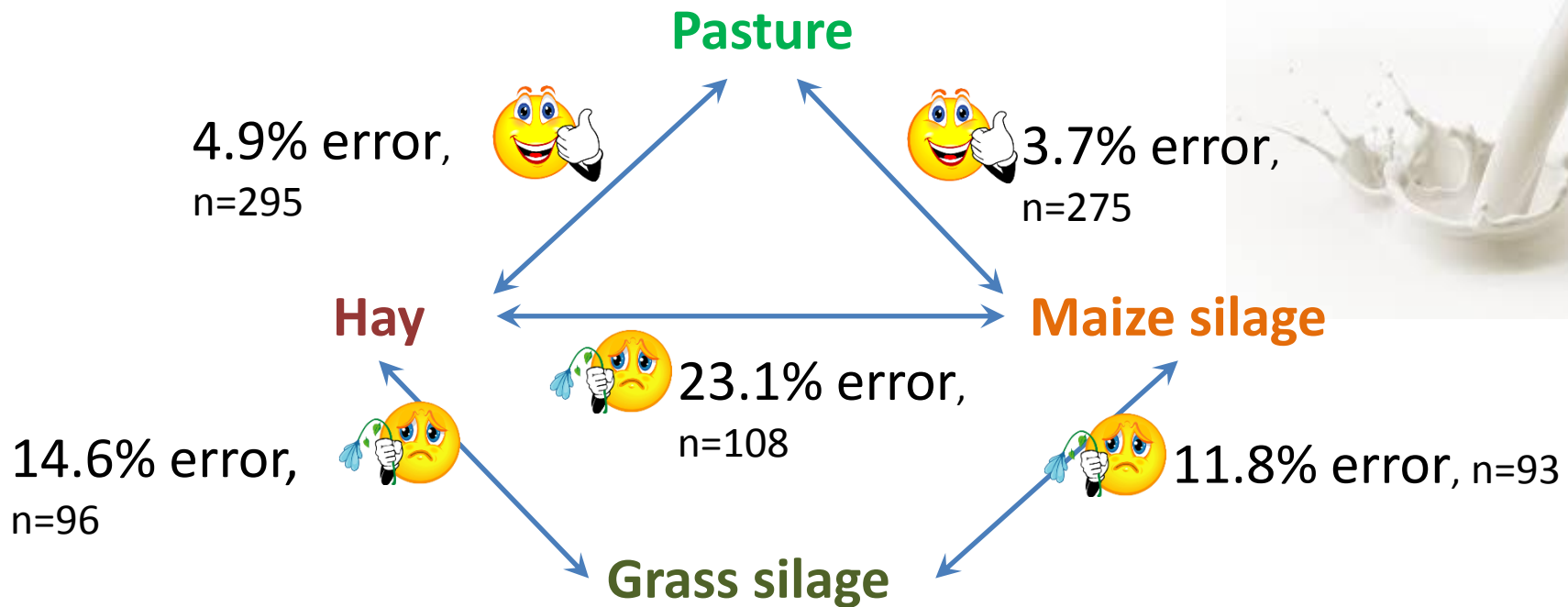
concentrate + hay



Hierarchical classification of milks according to their phenolic composition (Besle *et al* 2010)

# Spectral methods to authenticate cow's diet

## VIS/NIR spectroscopy applied to milk



(Coppa et al., 2012)