Breeding factors of dairy cows and milk lipolysis and consequences of milk lipolysis on semi-hard cheese and fresh cream

Catherine Hurtaud, Laurence Bernard, Anne Thierry, Gilles Garric, Marielle Harel-Oger, Christelle Cebo

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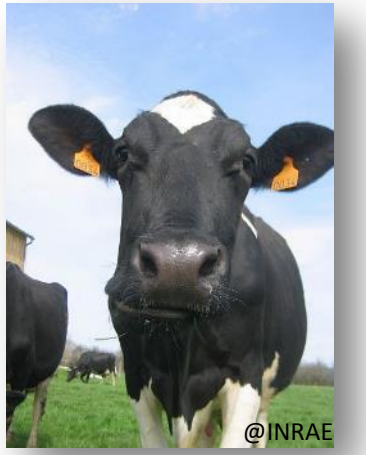

Breeding factors of dairy cows and milk lipolysis and consequences of milk lipolysis on semi-hard cheese and fresh cream

C. Hurtaud[a], L. Bernard[b], A Thierry[c], G. Garric[c], M. Harel-Oger[c] and C. Cebo[d]

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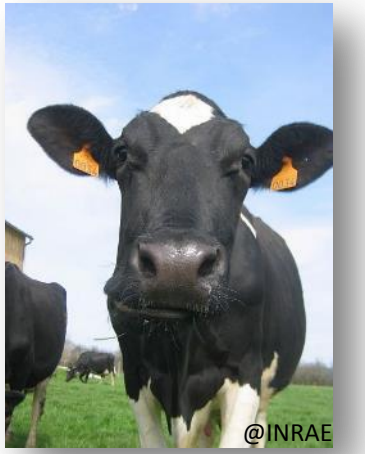
Presentation plan

- State of art of spontaneous lipolysis in 2023



- 3 experimentations concerning spontaneous lipolysis in dairy cows:
  - ✓ Feeding restriction
  - ✓ Milking interval
  - ✓ Milking frequency

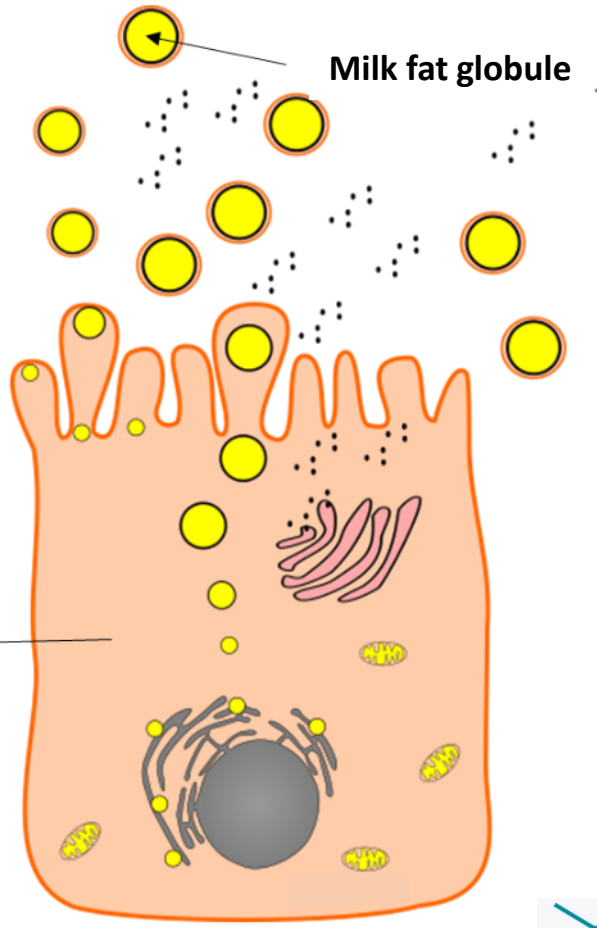
- Impact of the extent of milk spontaneous lipolysis on the lipolysis induced during transformation

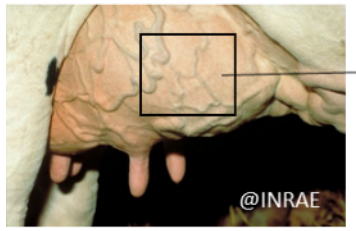
- ANR LIPOMEC project *(funding of the experiments)*

State of art of spontaneous lipolysis in 2023

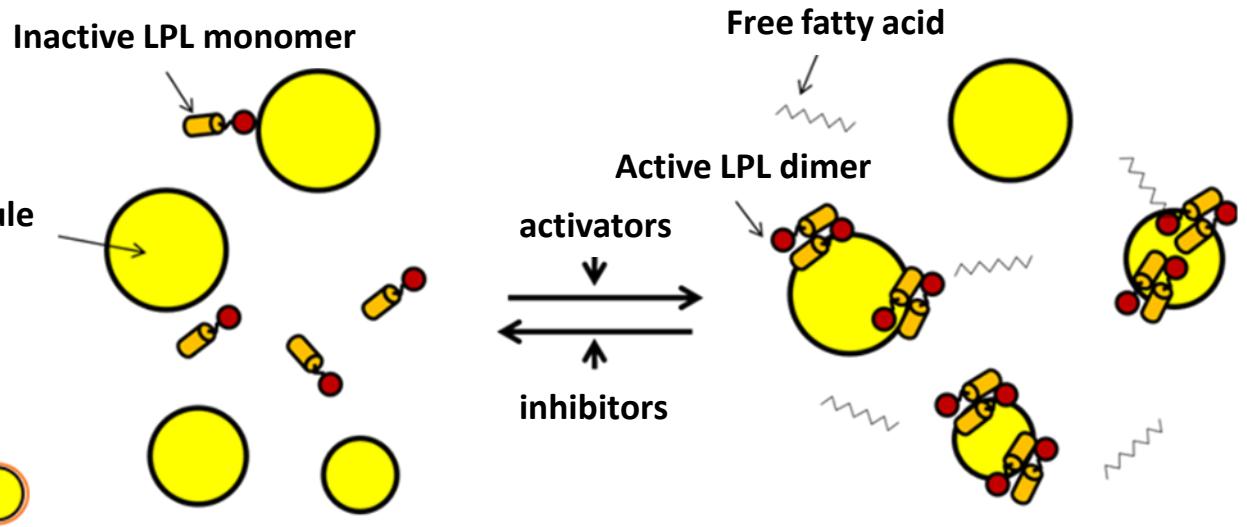
A definition: spontaneous lipolysis is initiated by the simple act of cooling raw milk to <10°C after it is taken from the cow (Deeth, 2006)

Milk lipolysis ?





LPL = lipoprotein lipase



Hydrolysis of triglycerides of milk fat by LPL =>

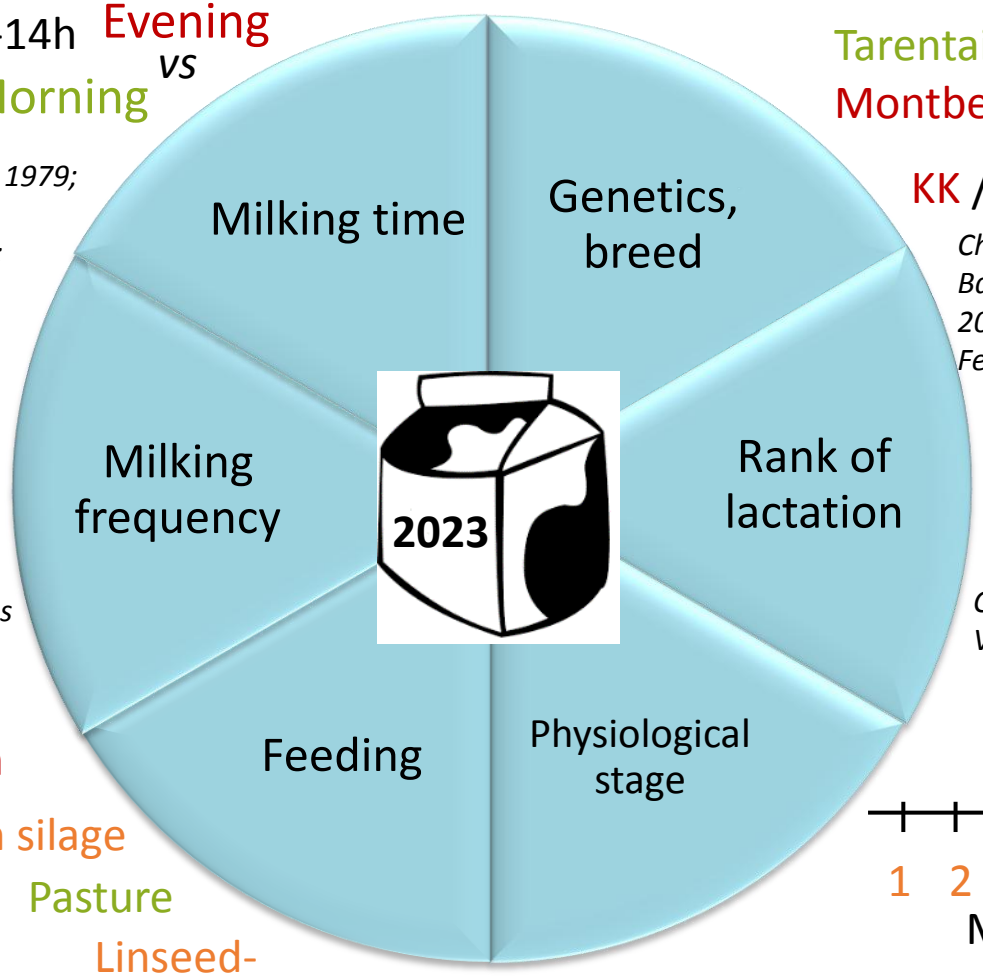
release of FFA and mono- and diglycerides

↓ organoleptic properties (rancid taste)

↓ technological properties (foaming capacity)

Decreased QUALITY of milk and dairy products

State of art of spontaneous lipolysis



« susceptible milk » « non susceptible milk» *Vanbergue, 2017*

10h-14h Evening
vs
Morning

Jensen, 1959; Murphy et al., 1979; Jellema 1975; Arhné et Björck, 1985 ; Vanbergue , 2017

Tarentaise, Jersiaise, Normande
Montbeliarde, Holstein, Salers

KK / KA et AA *DGAT-1*

Chilliard, 1982 ; Chazal et al., 1987 ; Bachman et al., 1988; Vanbergue et al, 2016 and 2017
Ferlay et al., 2006; Cozma et al., 2013

Once day milking
2 x/d
4 x/d

Wiking et al., 2006 ; Pomiès et al., 2007
Vanbergue et al, 2016

Mid lact. : Multi Primi

Beginning lact. : Multi Primi

Connolly 1978 ; Chilliard et Lamberet, 1984 ; Vanbergue 2017

Feeding restriction

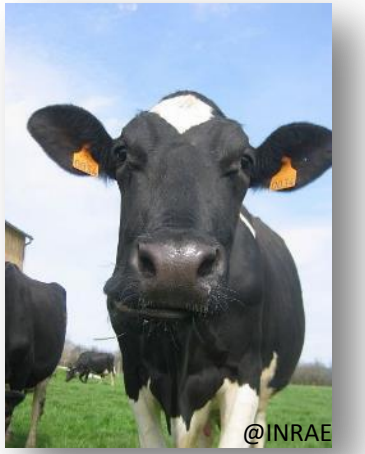
Stobbs et al., 1973 ; Deeth et Fitzgerald, 1976; Chazal et al., 1987; O'Brien et al., 1996; Ferlay et al., 2006 ; Vanbergue, 2017

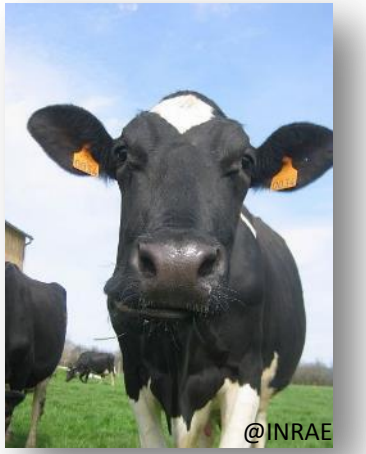
Corn silage
Pasture
Linseed-microalgae

1 2 3 4 5 6 7 8 9 10 11
Month of lactation

Arhné et Björck, 1985; Chazal et Chilliard, 1986; Bachman et al., 1988 ; Vanbergue, 2017

3 experimentations concerning spontaneous lipolysis in dairy cows





Feed restriction as a tool to describe and understand the mechanisms underlying lipolysis in milk in dairy cows

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Objectives of the present study

STATEMENT: Lipolysis is higher in milks from feed restricted cows

ASSUMPTION:

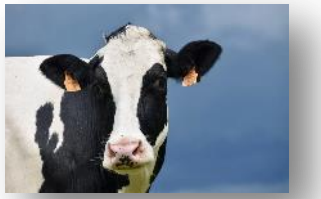
Changes in MFG composition could explain greater sensitivity of milk fat to lipolysis

Objectives =

- to induce spontaneous lipolysis in milk by **reducing the amount of feed** supplied to dairy cows
- to study the mechanisms of milk lipolysis

→ a better understanding of the molecular mechanisms controlling the degradation of milk fat through integrative biology approaches

Material and methods: experimental design



44 dairy cows (2 × 2 × 11)
165 ± 16 days in milk

2 weeks	1 week	2 weeks
Corn silage + conc. 100 % ad lib		Corn silage + conc. 65 % ad lib
Corn silage + conc. 65 % ad lib		Corn silage + conc. 100 % ad lib

Once at the end of each period:

- ✓ Milk sampling, morning and evening milking
- ✓ Blood sampling after morning milking
- ✓ Mammary biopsies

Material and methods: Analyses

Measurements and laboratory analysis

- ✓ Milk yield, fat and protein contents
- ✓ Lipolysis
- ✓ Plasma metabolites
- ✓ Milk fat globules and casein micelles size
- ✓ Casein and minerals contents

Statistical analysis

Effects of feeding levels on parameters : SAS MIXED procedure

$$Y_{ijkl} = \mu + Group_i + Milking\ Time_j + Period_k + Feeding\ level_l + Milking\ Time_j \times Feeding\ level_l + CovY_{ijkl} + e_{ijkl}$$

Milk production and composition

	100% *ad lib*	65% *ad lib*
Milk, kg/d	30.6	**25.5**
Milk protein content, g/kg	31.4	**30.1**
Mik fat content, g/kg	40.0	40.1
Milk lactose content, g/kg	49.3	49.5

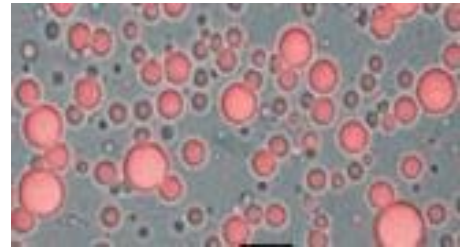
- Feed restriction was accompanied by

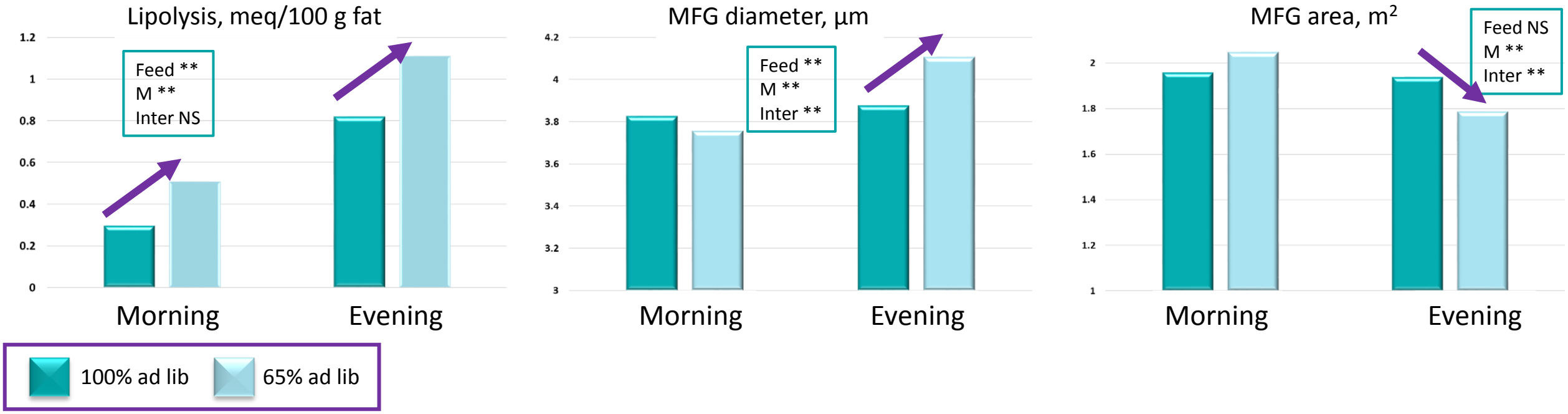
  - a decrease in milk yield (- 5.1 kg) and in protein content (-1.3 g/kg)

  - no effect on fat and lactose contents: surprising for lactose content considered as biomarker of energy deficit

Vanbergue et al, 2018

Milk lipolysis





- Increase of lipolysis with feeding restriction *(Vanbergue et al, 2017 and 2018)*
- Larger fat globules associated with higher degree of lipolysis, higher fat/protein ratio *(Couvreur and Hurtaud, 2017)* without variation of milk fat content

Assumptions & Interpretation

- Change in protein composition and polar lipids of MFG membrane (biomarkers of high lipolysis)
  With an effect on the size of the MFG and on the solidity of its membrane => accessibility of the LPL to its substrate.

- ↗LPL activity measured in morning milk with feeding restriction (708.8 vs. 501.7 ηmol/min/mL)

- ↗ LPL expression (mRNA measured on mammary tissue (biopsies) and on epithelial cells isolated from milk)? No result for the moment

- Action on inhibitors (PP5,…) or activators (apolipoproteins, Glycam1,…) of LPL

Conclusion

- Objective of the experiment achieved: induce lipolysis by restricting feed to dairy cows

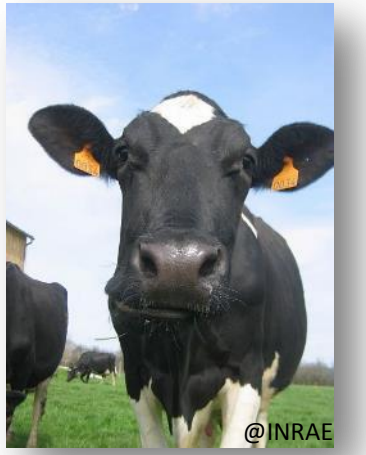
- Milk samples and biopsies performed on cows with extreme lipolysis: genomic, proteomic, lipidomic analyzes in progress.

  → Integrative biology approach, linking milk proteomics to mammary gland transcriptomics

- What's next?

  → 2 experiments with the same feeding strategy on ewes and on goats in 2021 for a better understanding of the response and mechanisms regulating the lipolytic system among species (results published soon).

  Ultimately, a comparative analysis between the 3 species will be also done.



Impact of milking interval on milk spontaneous lipolysis in dairy cows

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Objectives of the present study

STATEMENT: Lipolysis is higher in milks from the evening milking than in those from the morning milking with a 10-14 h interval

ASSUMPTION: levels of lipolysis in milk depends on:

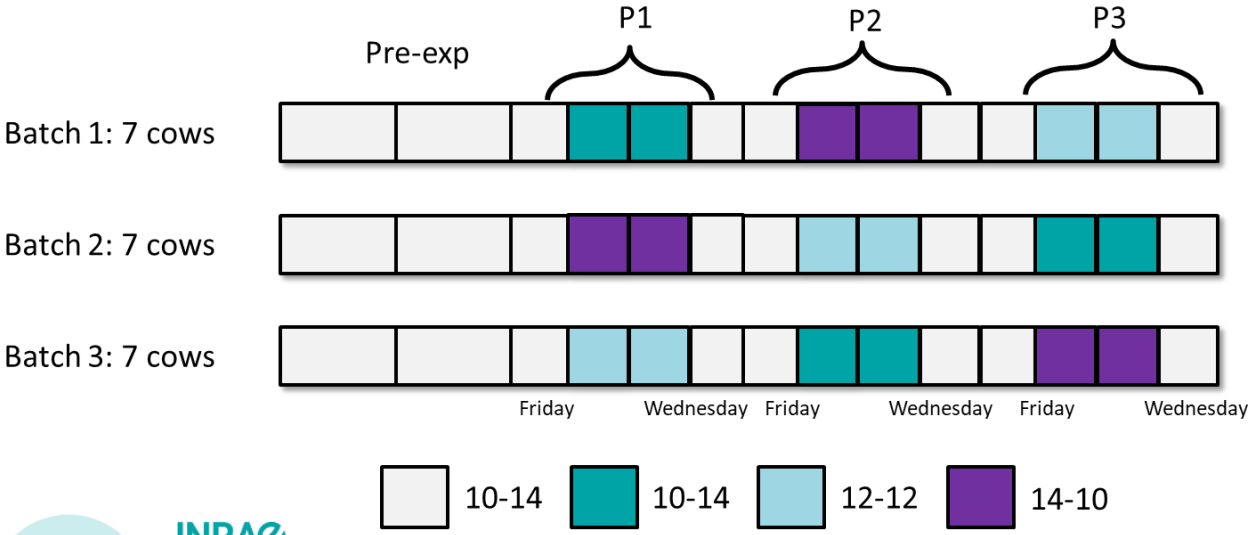
- the length of the intervals between milkings ?
- the nychthemeron ?

Objectives = to study the impact of the interval duration between 2 consecutive milkings on the spontaneous lipolysis of milk

Material and methods: Experimental design

- 21 primiparous and multiparous dairy cows chosen according to their susceptibility to lipolysis (measured by MIR)

- 3 treatments :
  - ✓ 10–14 (milking 6:30 am, 4:30 pm)
  - ✓ 12–12 (milking 6:30 am, 6:30 pm)
  - ✓ 14–10 (milking 6:30 am, 8:30 pm)



Latin Square Design

- 3 periods of 2 weeks:
  - ✓ 5 days of adaptation to treatment
  - ✓ 1 sampling day
  - ✓ 8 return days to 10 h – 14 h

Material and methods: Analyses

Measurements and laboratory analysis

- ✓ Milk yield, fat and protein contents
- ✓ Lipolysis
- ✓ Milk fat globules and casein micelles size
- ✓ Casein and minerals contents

Statistical analysis

Effects of duration of milking intervals on parameters : SAS MIXED procedure

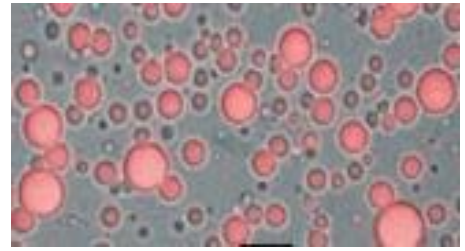
$$Y_{ijk} = \mu + Cow_i + Period_j + MilkingInterval_k + \varepsilon_{ijk}$$

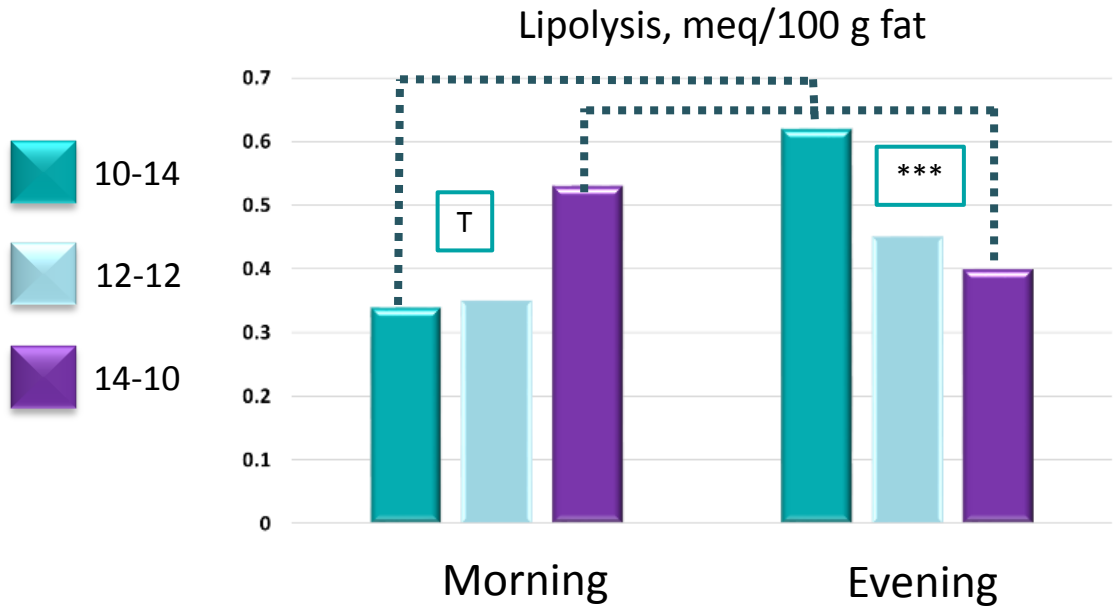
Results-Discussion: Milk production

		10-14	12-12	14-10	Interval effect
Milk, kg	Morning	**19.2**	**17.5**	**15.5**	***
	Evening	**13.9**	**16.0**	**17.8**	***
	Day	33.1	33.6	33.2	NS
Fat content, g/kg	Morning	39.2	40.4	**43.4**	***
	Evening	**42.9**	40.5	39.1	***
	Day	40.7	40.2	41.1	NS
Protein content, g/kg	Morning	**30.9**	**30.2**	**29.1**	***
	Evening	30.2	**31.4**	**31.6**	***
	Day	30.5	30.7	30.4	NS

- ✓ No effect of milking intervals on milk yield, fat and protein contents
- ✓ Opposite effects between 10-14 and 14-10 on morning and evening milks
- ✓ 12-12 : slight differences in milk yield and protein content between morning and evening
- ✓ For 12-12 and 14-10, increase of synthesis of milk constituents during the night, not for 10-14!

Results-Discussion: Lipolysis and milk fat globules





No significant effect of milking intervals on MFG diameter and area

SUMMARY

Fluctuation of lipolysis between morning and evening milking ⟺ interval between milkings *(Wiking et al, 2006)*, not to nychthemeron

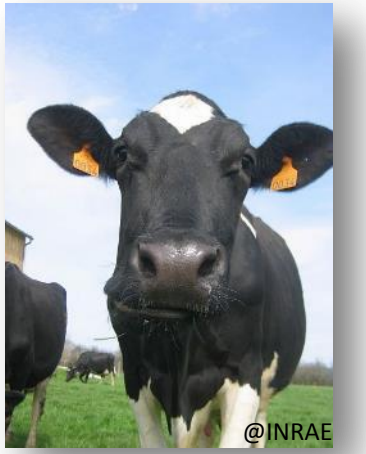
Penalization of the reconstitution of the membrane which surrounds the triglycerides *(Connolly, 1978)*

LPL activity with 10-14 especially in evening milk (737 vs 642 vs 572 ηmol/min per mL)
12-12 14-10

No correlation between LPL activity and lipolysis *(Cartier and Chilliard, 1990)*

Conclusion

- This original study
  - ✓ shows that milking interval prevails over nychthemeron in affecting milk lipolysis
  - ✓ still raises a number of questions about the mechanisms of lipolysis



Impact of milking frequency on milk spontaneous lipolysis in dairy cows

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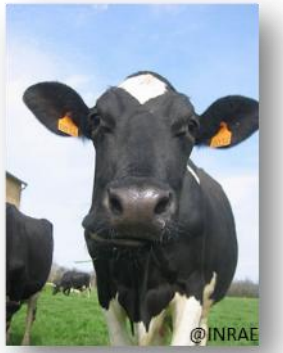


Objective of the present study

Statement: Lipolysis is higher in milks from automatic milking systems (AMS) in relation to milking frequency and lower in once day milking

HYPOTHESIS: levels of lipolysis in milk depend on:

- ✓ the length of the intervals between milkings?
- ✓ the milk yield?

Objectives = to study the impact of **milking frequency** on the spontaneous lipolysis of milk

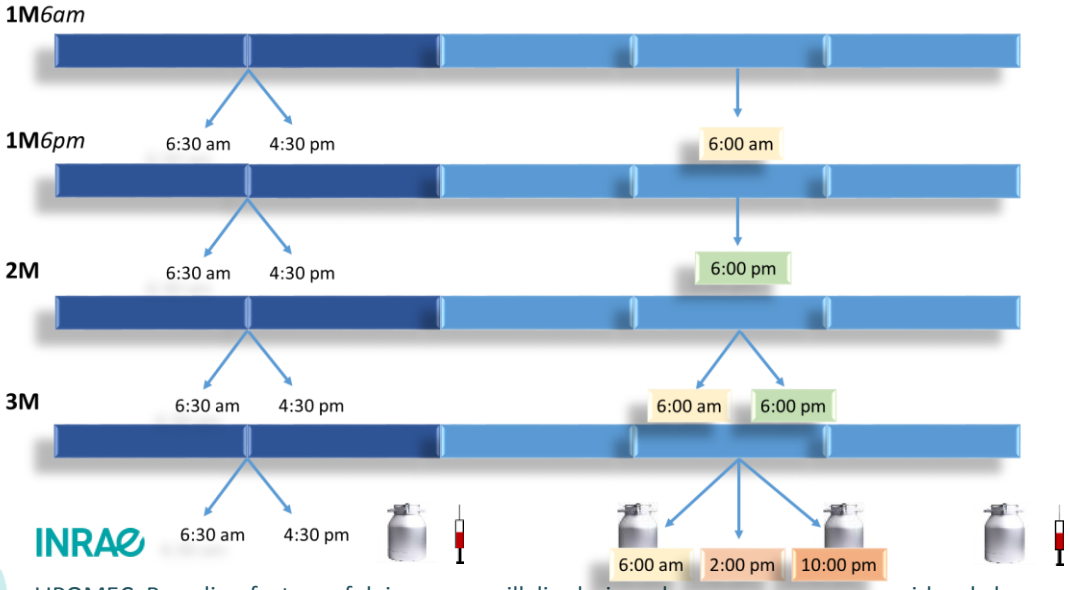
Material and methods: Experimental design

- 32 primiparous and multiparous dairy cows chosen according to their susceptibility to lipolysis (measured by MIR and copper soap method) (4 susceptible dairy cows (**Sus** > 0.6 mEq/100 g fat**)** and 4 non-susceptible dairy cows (**Nsus** <0.6 mEq/100 g fat**)** by batch)

- 4 treatments :
  - ✓ **1M***6am*: one milking/ day at 6:00 am
  - ✓ **1M***6pm*: one milking/ day at 6:00 pm
  - ✓ **2M**: 2 milkings / day at 6:00 am and 6:00 pm
  - ✓ **3M**: 3 milkings / day at 6:00 am, 2:00 pm and 10:00 pm

Continuous design

- 2 weeks of pre-experiment
- 3 experimental weeks



At the end of the pre-experiment and week 3

- ✓ Milk samples at each milking
- ✓ Blood samples

Material and methods: Analyses

Measurements and laboratory analysis

- Milk yield, fat and protein contents
- Lipolysis
- Milk fat globules and casein micelles size
- Casein and minerals contents

Statistical analysis

Effects of milking frequencies on parameters in wk 3 : SAS MIXED procedure

$$Y_{ij} = \mu + Milking_i + LipoSens_j + LipoSens_j \times Milking_i + Cov\ Y_{ij} + \varepsilon_{ij}$$

Results-Discussion: Milk production

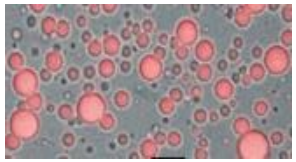
						Effect		
		1M*6am*	1M*6pm*	2M	3M	SEN	M	SEN*M
Milk, kg/d	NSus	27.3	18.3	**39.5**	**37.0**	NS	***	NS
	Sus	25.4	23.2	**42.3**	**38.2**			
Milk fat content, g/kg	NSus	45.1	40.2	35.7	40.4	NS	T	NS
	Sus	47.2	41.5	40.7	40.6			
Milk protein content, g/kg	NSus	33.3	31.8	31.3	32.5	NS	NS	NS
	Sus	32.7	31.9	32.3	31.4			

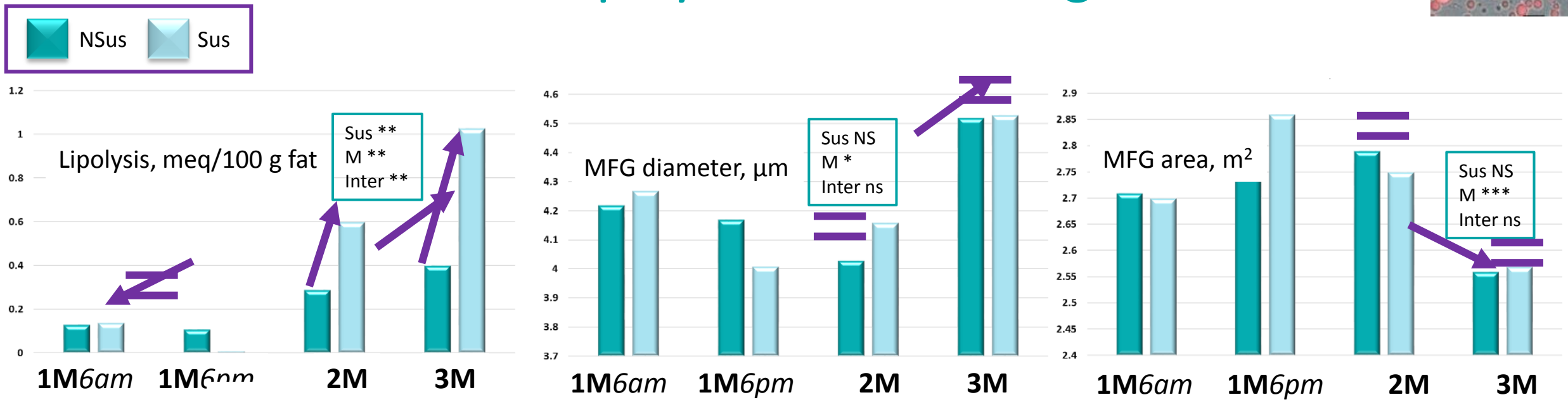
- ✓ No significant effect of milking frequencies on milk fat and protein contents
- ✓ Higher milk production for **2M** and **3M**, intermediate for **1M***6am* and lower for **1M***6pm*.

For **1M***6pm*, consequence of a large decrease for 2 dairy cows (more than 20 kg !)

- ✓ No effect of the individual susceptibility to lipolysis on these parameters

Results-Discussion: Lipolysis and milk fat globules





- Compared to **2M**, decrease in lipolysis with **1M** and increase with **3M**
- With **3M**, MFG diameter ↗ and MFG specific area ↘

- No effect of dairy cow susceptibility to lipolysis with **1M**, but increase of lipolysis with **2M** and **3M** for susceptible dairy cows, without any effect on MFG diameter and area.

Penalization of the reconstitution of the membrane which surrounds the triglycerides *(Connolly, 1978)*: not enough time between milkings to synthesize MFGM *(Wiking et al, 2019)*

↗ LPL activity with ↘ interval *(Sundheim, 1988)*

Conclusion

- We confirmed the effects of milking frequencies: less lipolysis in once day milking and more lipolysis with 3 milkings per day (automated milking systems)

- Original result:
  - ✓ Similar decrease in lipolysis in susceptible and non-susceptible cows with once day milking
  - ✓ On the other hand, marked increase in lipolysis in susceptible cows with 2 or 3 milkings per day compared to non- susceptible cows

Impact of the extent of milk spontaneous lipolysis on the lipolysis induced during transformation

Impact of the extent of milk spontaneous lipolysis on the lipolysis induced during transformation

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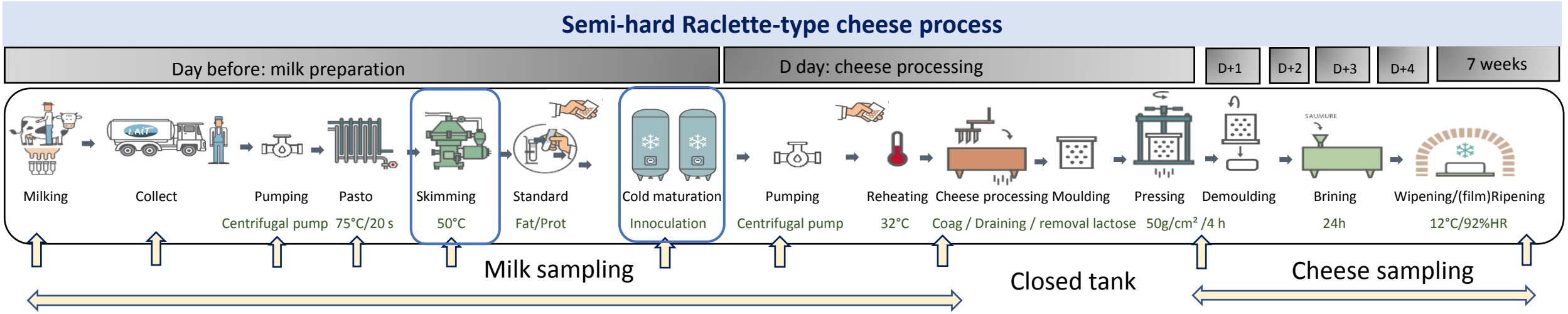
Objective of the present study

Statement: milk processing induce lipolysis due to fat globule damage

HYPOTHESIS: the induced lipolysis could be more pronounced in milk sensitive to spontaneous lipolysis

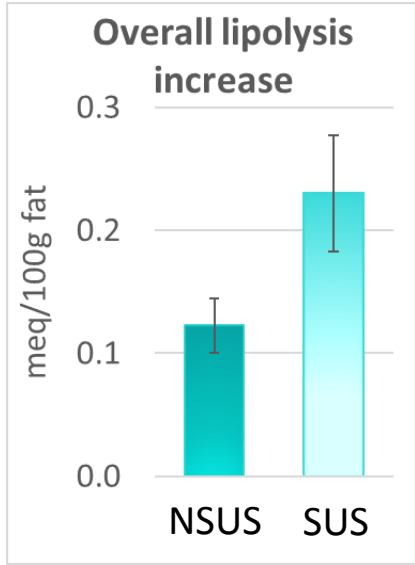
Objective: to compare the changes in lipolysis induced during milk processing operations between susceptible and non-susceptible milks

Main results: example of semi-hard cheese



- ✓ We measured lipolysis evolution all along the process from milking to renneting (by sampling)
- ✓ The most impactful steps were **skimming** and **cold maturation**

- ✓ We observed a significantly greater overall increase for **susceptible milks**, compared to **non-susceptible milks** (regardless the initial lipolysis degree)



Main results: sensory evaluation for semi hard cheese and sour cream

- ✓ We used Triangular tests to evaluate sensory differences
- ✓ No significant differences were perceived between products made from sensitive or non sensitive milks (regardless the product)

2 month-ripened cheeses

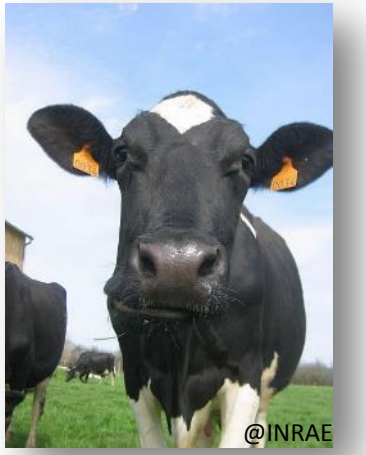
sour cream stored for 2 months at 4°C

Conclusion

- ✓ We measured a significantly greater lipolysis induced in sensitive milks during some key steps during milk transformation in cheese and sour cream
- ✓ However, these differences of induced lipolysis were not sensorially perceived in the two dairy products tested

- And the full story of the adventure INRAE “Lipolysis”…

  … the ANR LIPOMEC project



The ANR project LIPOMEC

Coordination C. Cebo (INRAE)

Reduction of lipolysis along the « milk way »

Genetic selection and milk spontaneous lipolysis in dairy ruminants

Knowledge transfer

WP7 DATA INTEGRATION

Modelling, data integration and dissemination

Milk lipolysis: going further into mechanisms

WP5 ANIMAL POPULATIONS
(building of MIR** equations & re-exploring the PhenoFinLait Programme)
Partners: GABI, ACTALIA, IDELE

Genomic region linked to lipolysis in cattle

WP6 TO THE MILKING PLANT and BEYOND
(modelling, « artificial udder », , cheese properties)
Partners: IDELE, STLO

Lipolysis biomarkers in milk and mammary gland in dairy ruminants

WP4 MAMMARY GLAND
(multi-omics (mRNA, miRNA), dissection of the casein genetic polymorphism in the goat species)
Partners: UMRH, GABI, PEGASE

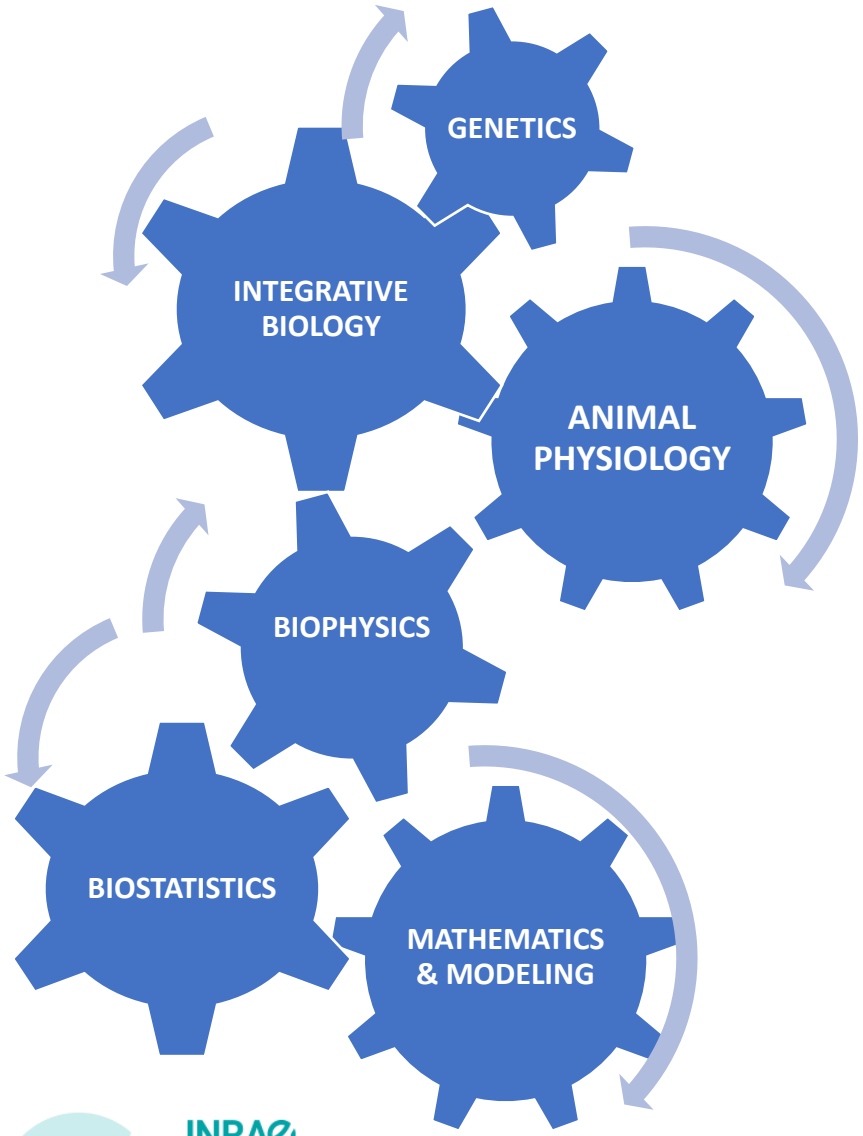
WP3 MILK
(proteomics & targeted studies)
Partners: GABI, UMRH, PEGASE

WP2 ANIMAL (cow, goat, ewe)
Partners: PEGASE, GenPhySE

WP1 Project coordination

Understanding the LPL lipolytic system in milk and mammary gland

LIPOMEC, a transdisciplinary, multi-actors and multi-scale program



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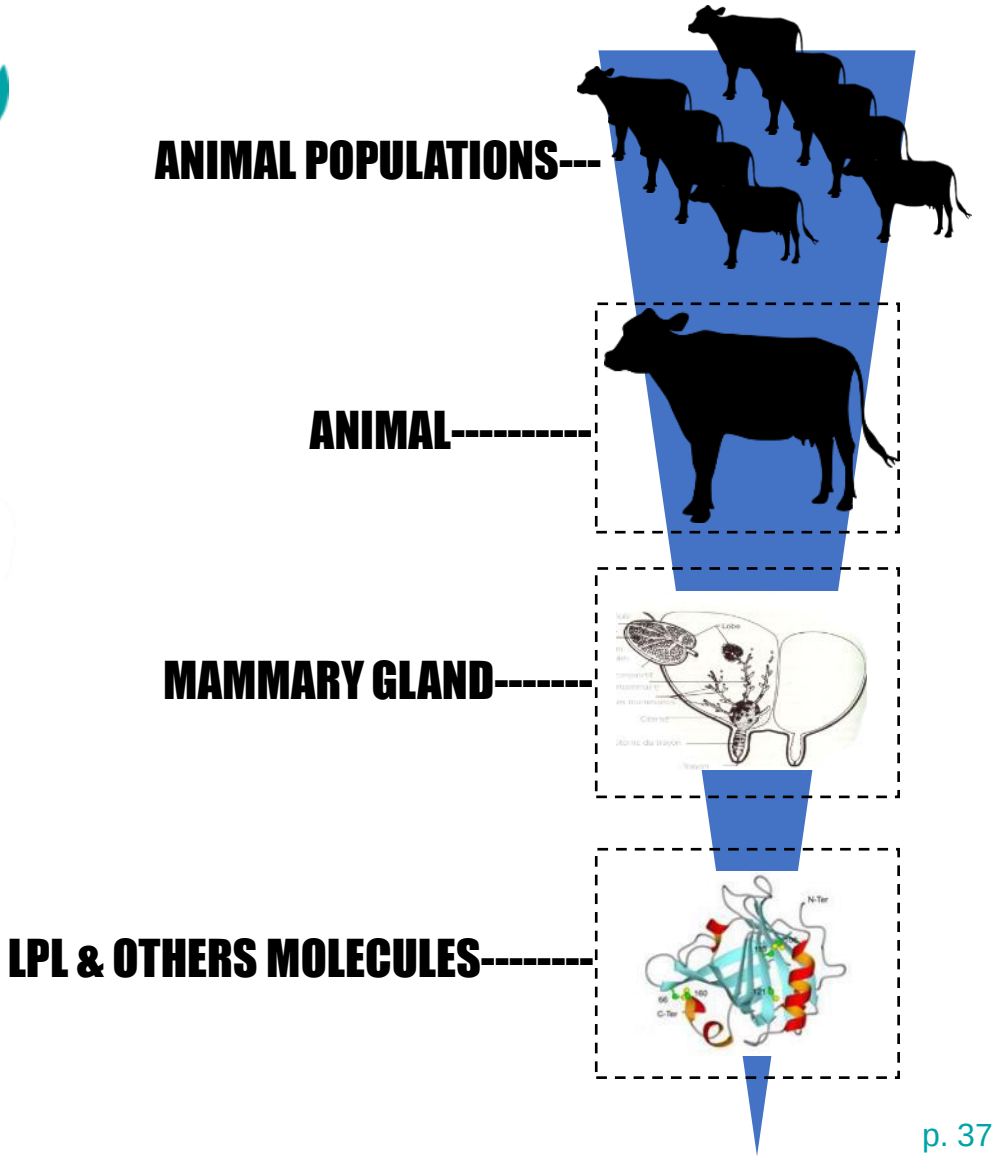

THANK YOU FOR YOUR ATTENTION !

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