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

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

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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)*

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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

Inactive LPL monomer

Free fatty acid

Milk fat globule

Active LPL dimer

activators

inhibitors
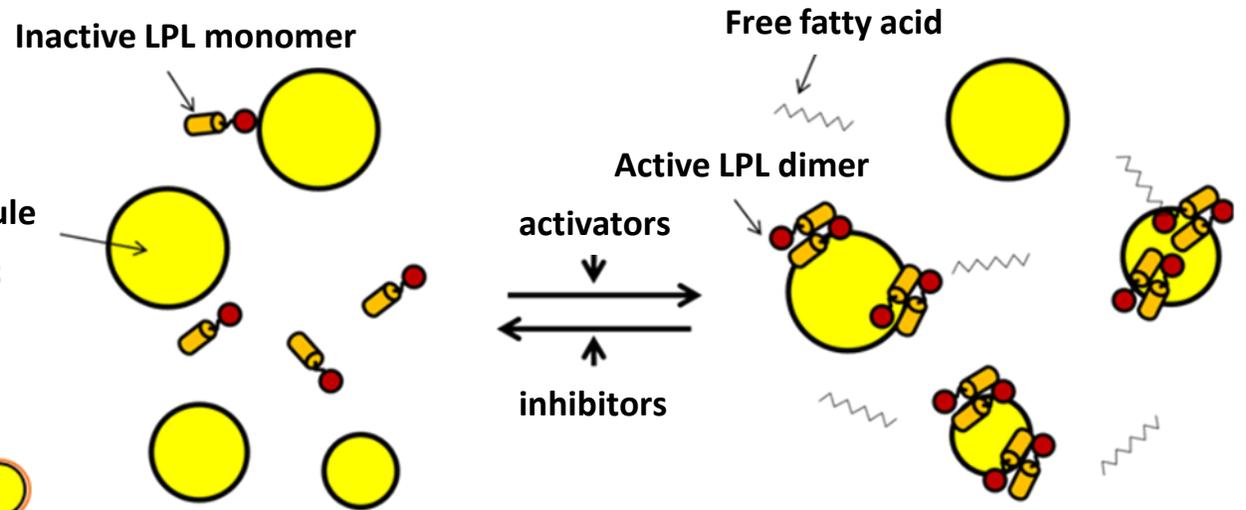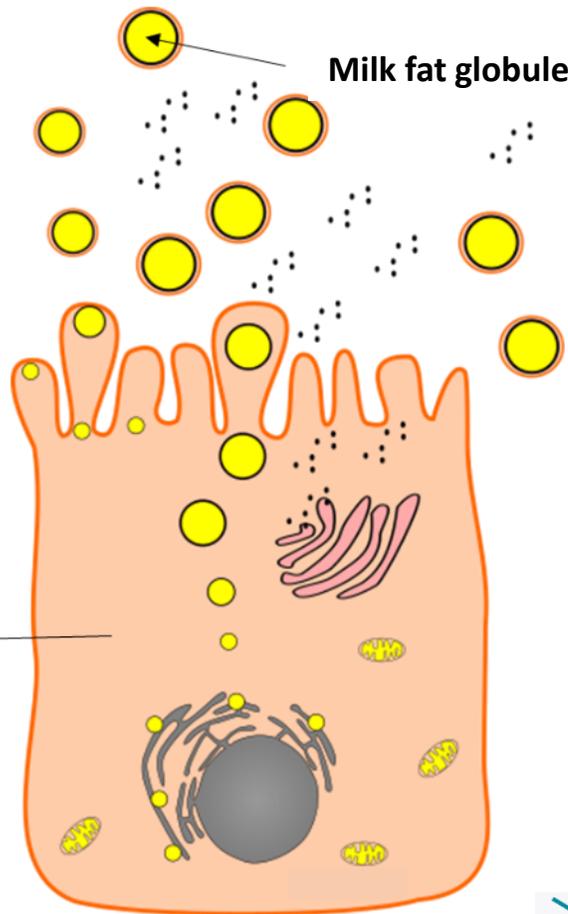
Hydrolysis of triglycerides of milk fat by LPL =>
release of FFA and mono- and diglycerides

↓organoleptic properties
(rancid taste)

↓technological properties
(foaming capacity)

Decreased QUALITY of milk and dairy products

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« 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*

Milking time

**Genetics,
breed**

Once day milking
2 x/d
4 x/d

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

**Milking
frequency**

2023

**Rank of
lactation**

Mid lact. : Multi Primi

Beginning lact. : Multi Primi

*Connolly 1978 ; Chilliard et Lamberet, 1984 ;
Vanbergue 2017*

Feeding

**Physiological
stage**

Feeding restriction

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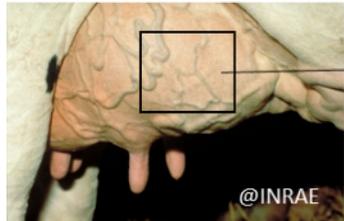Deeth et Fitzgerald,
1976; Chazal et al.,
1987; O'Brien et al.,
1996; Ferlay et al.,
2006 ; Vanbergue, 2017*

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*

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low lipolysis **high lipolysis**

3 experimentations concerning spontaneous lipolysis in dairy cows

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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

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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

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Material and methods: Analyses

❑ <u>Measurements and laboratory analysis</u>

✓ Milk yield, fat and protein contents
✓ Lipolysis
✓ Plasma metabolites

✓ Milk fat globules and casein micelles size
✓ Casein and minerals contents

❑ <u>Statistical analysis</u>

   Effects of feeding levels on parameters : <u>SAS MIXED procedure</u>

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

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)

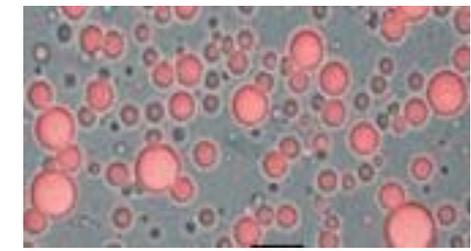- no effect on fat and lactose contents: surprising for lactose content considered as

biomarker of energy deficit
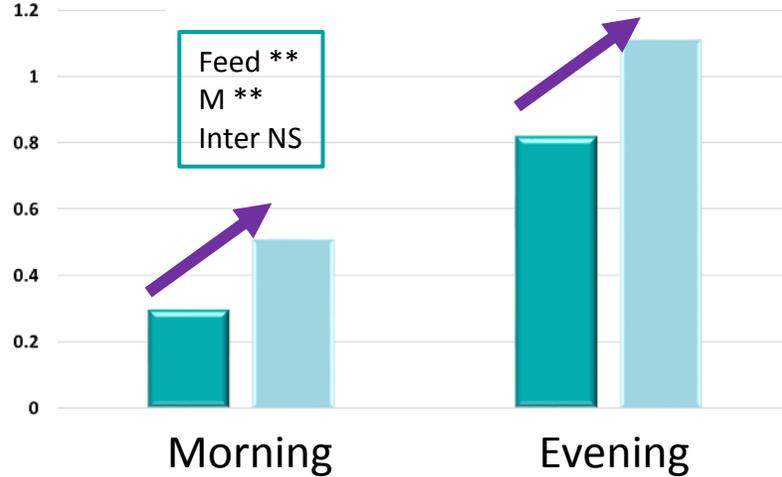
Vanbergue et al, 2018

Milk lipolysis

Lipolysis, meq/100 g fat

Feed **
M **
Inter NS

Morning　　Evening

MFG diameter, µm

Feed **
M **
Inter **

Morning　　Evening

MFG area, m^2

Feed NS
M **
Inter **

Morning　　Evening
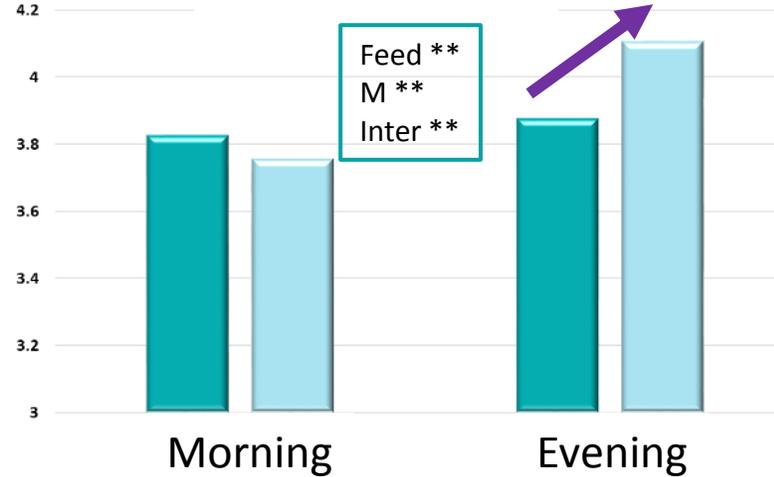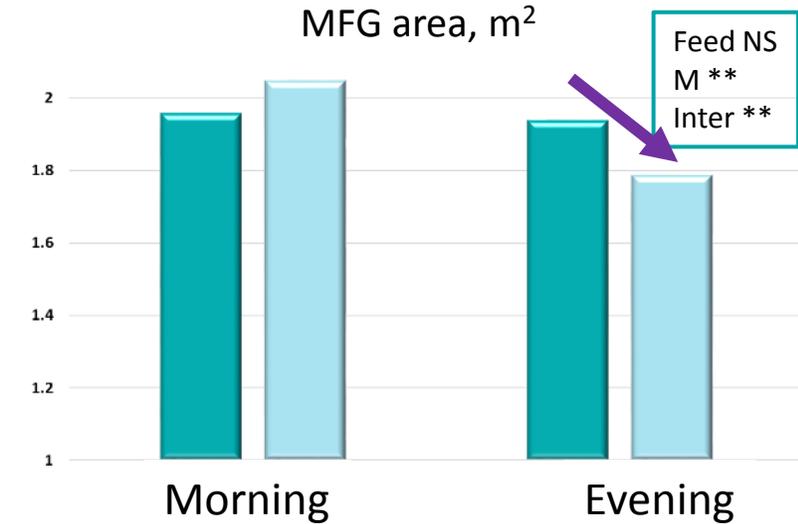
■ 100% ad lib　　■ 65% ad lib

❑ 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

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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
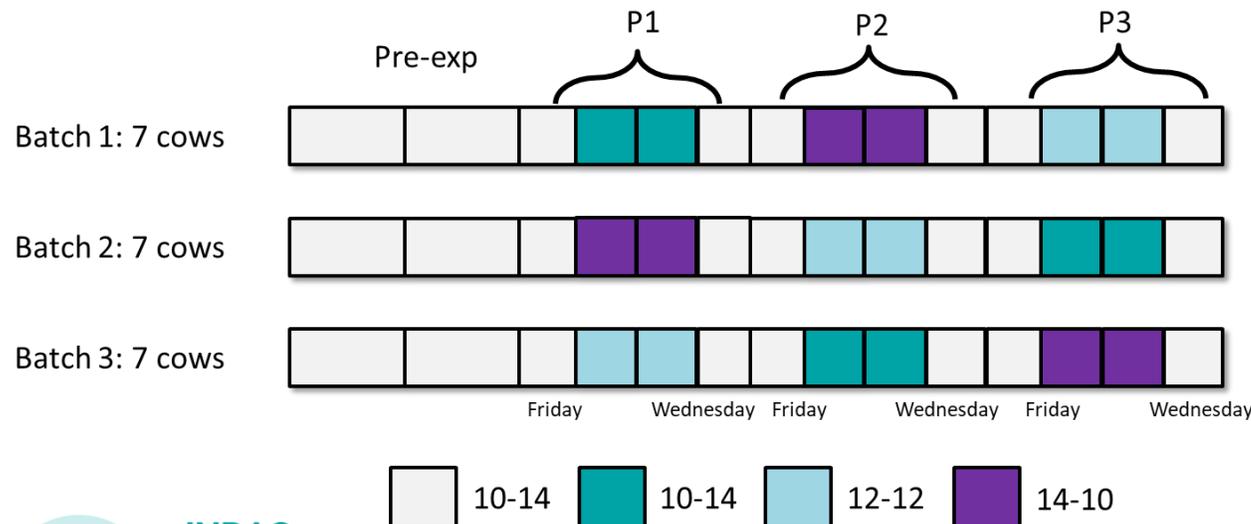
Material and methods: Analyses

❑ <u>Measurements and laboratory analysis</u>

- ✓ Milk yield, fat and protein contents
- ✓ Lipolysis

- ✓ Milk fat globules and casein micelles size
- ✓ Casein and minerals contents

❑ <u>Statistical analysis</u>

  Effects of duration of milking intervals on parameters : <u>SAS MIXED procedure</u>

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

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

SUMMARY

✓ 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!

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Results-Discussion: Lipolysis and milk fat globules

Lipolysis, meq/100 g fat

Legend:
- 10-14
- 12-12
- 14-10

(Morning, Evening bar chart with T and *** annotations)
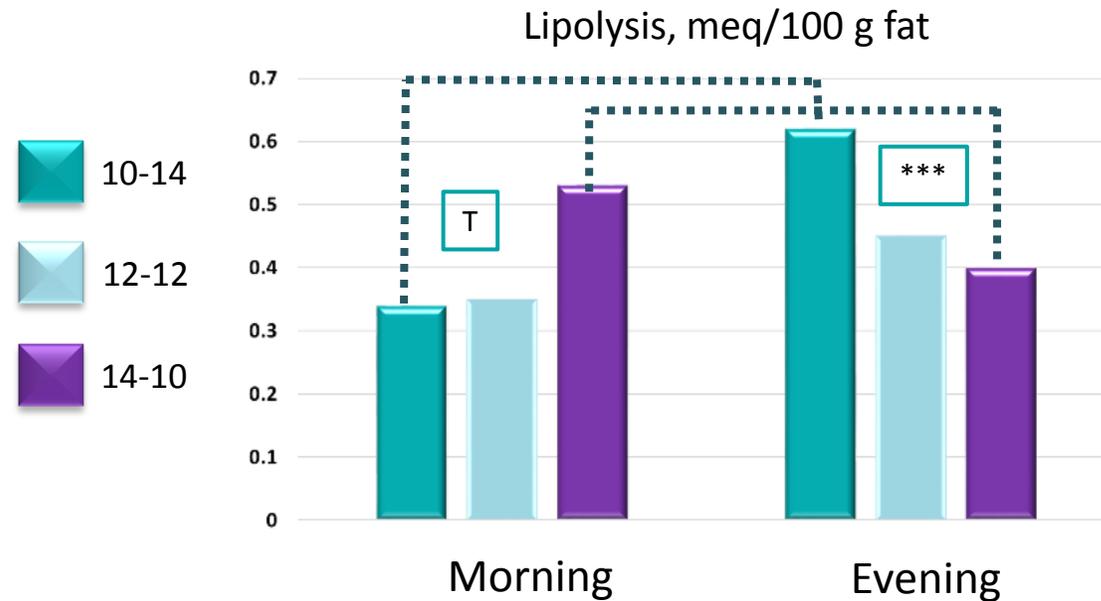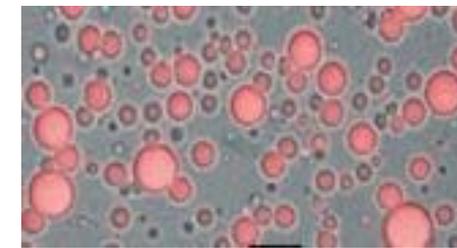
No significant effect of milking intervals on MFG diameter and area

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)*

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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

C, Hurtaud[1], L. Bernard[2], D. Taillebosq[1], et C. Cebo[3]

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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

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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
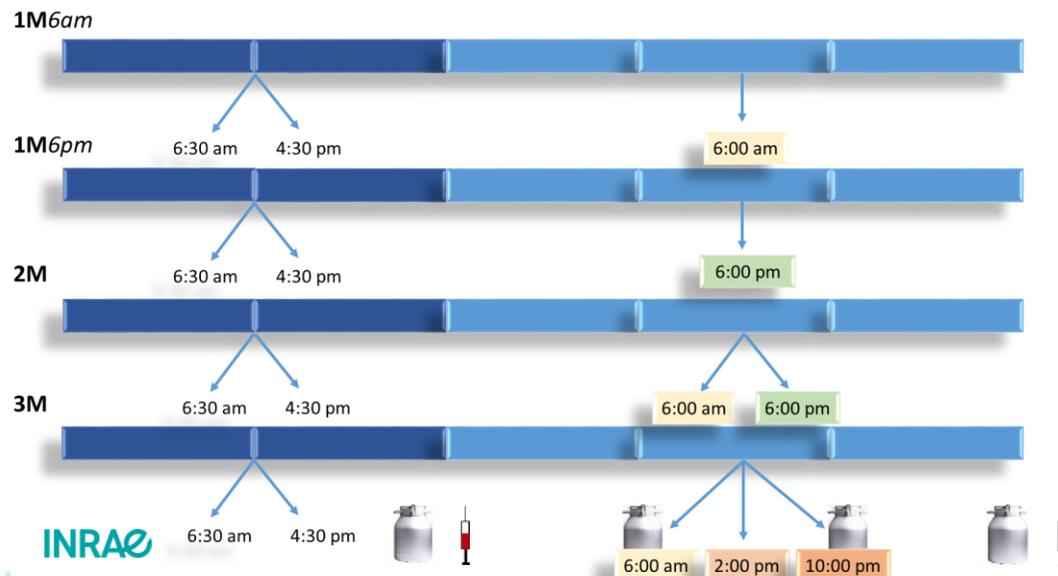
At the end of the pre-experiment and week 3

- ✓ Milk samples at each milking
- ✓ Blood samples

Material and methods: Analyses

❑ <u>Measurements and laboratory analysis</u>

➢ Milk yield, fat and protein contents
➢ Lipolysis

➢ Milk fat globules and casein micelles size
➢ Casein and minerals contents

❑ <u>Statistical analysis</u>

   Effects of milking frequencies on parameters in wk 3 : <u>SAS MIXED procedure</u>

$$Y_{ij} = \mu + \text{Milking}_i + \text{LipoSens}_j + \text{LipoSens}_j \times \text{Milking}_i + \text{Cov } Y_{ij} + \varepsilon_{ij}$$

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Results-Discussion: Milk production

		1M*6am*	1M*6pm*	2M	3M	Effect SEN	Effect M	Effect 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			

SUMMARY
- ✓ 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

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Results-Discussion: Lipolysis and milk fat globules

NSus **Sus**

Lipolysis, meq/100 g fat

Sus **
M **
Inter **

1M*6am* 1M*6pm* 2M 3M

MFG diameter, µm

Sus NS
M *
Inter ns

1M*6am* 1M*6pm* 2M 3M

MFG area, m^2

Sus NS
M ***
Inter ns

1M*6am* 1M*6pm* 2M 3M
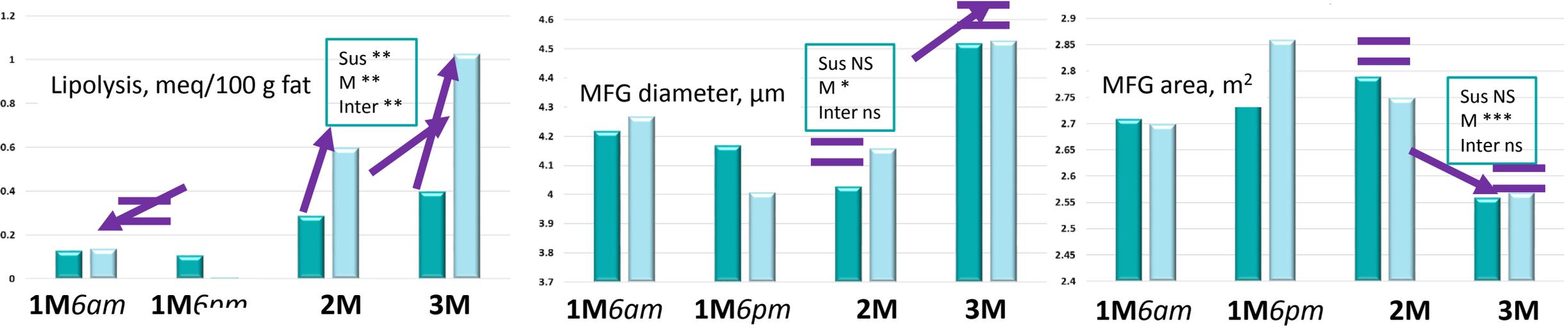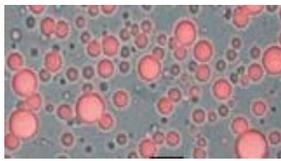
☐ Compared to **2M**, decrease in lipolysis with **1M** and increase with **3M**

☐ With **3M**, MFG diameter ↗ and MFG specific 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)*

☐ 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.

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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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https://www6.rennes.inrae.fr/plateforme_lait

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

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Main results: example of semi-hard cheese

Semi-hard Raclette-type cheese process


| Day before: milk preparation | D day: cheese processing | D+1 | D+2 | D+3 | D+4 | 7 weeks |

Milking — Collect — Pumping — Pasto — **Skimming** — Standard — **Cold maturation** — Pumping — Reheating — Cheese processing — Moulding — Pressing — Demoulding — Brining — Wipening/(film)Ripening

Centrifugal pump / 75°C/20 s / 50°C / Fat/Prot / Innoculation / Centrifugal pump / 32°C / Coag / Draining / removal lactose / 50g/cm² /4 h / 24h / 12°C/92%HR

Milk sampling

Closed tank

Cheese sampling

✓ 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)

Overall lipolysis increase

meq/100g fat

0.3
0.2
0.1
0.0

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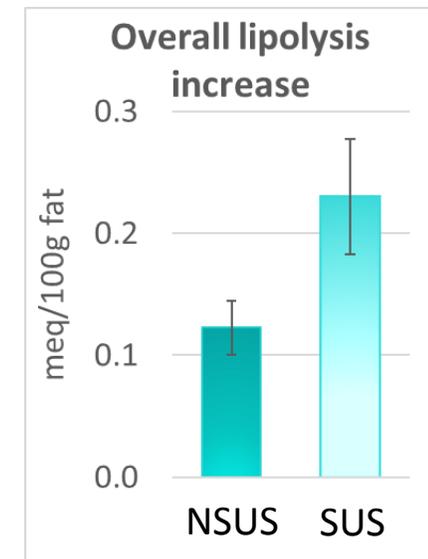
Main results: sensory evaluation for semi hard cheese and sour cream

✓ 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

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> And the full story of the adventure INRAE "Lipolysis"…

… the ANR LIPOMEC project

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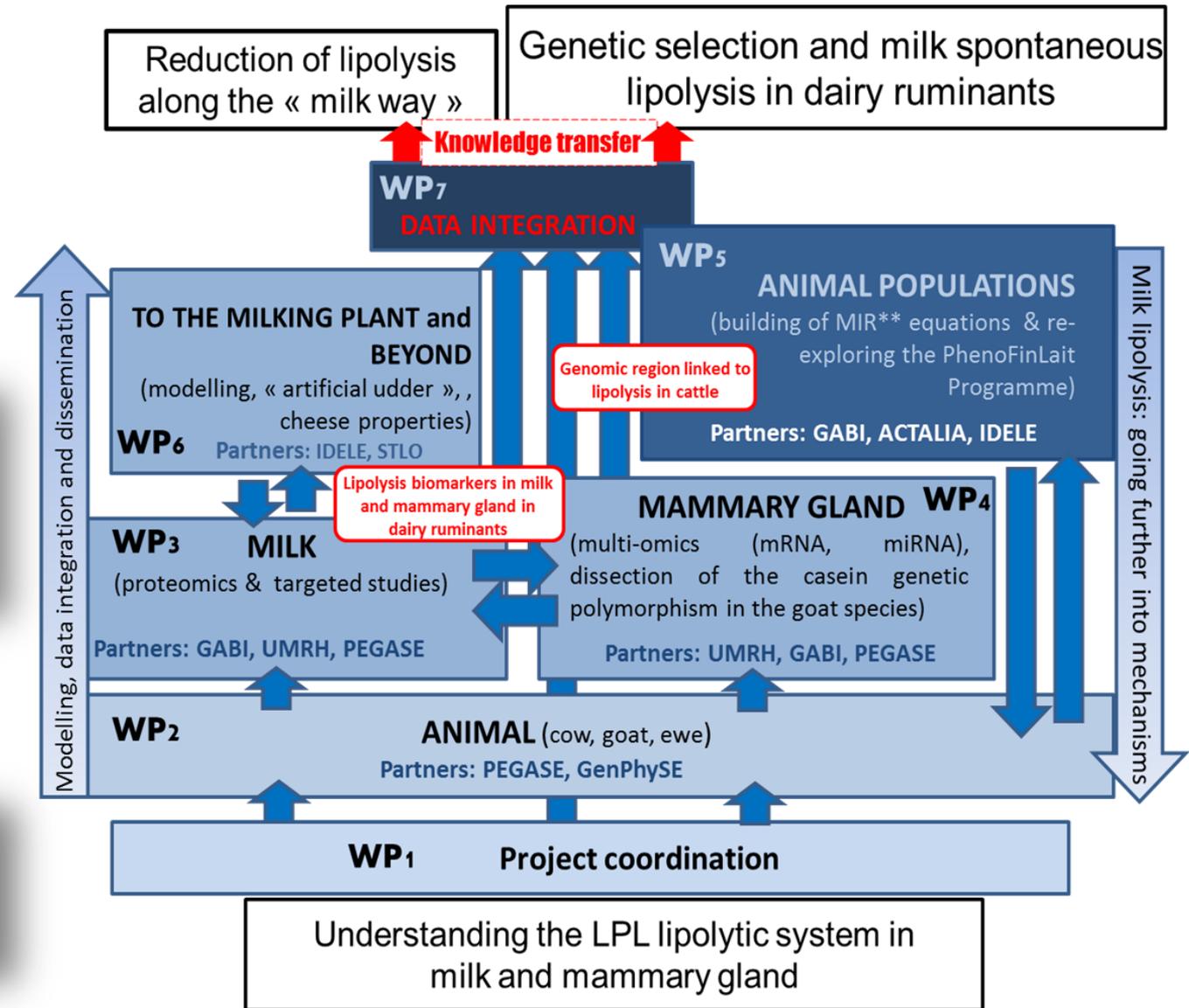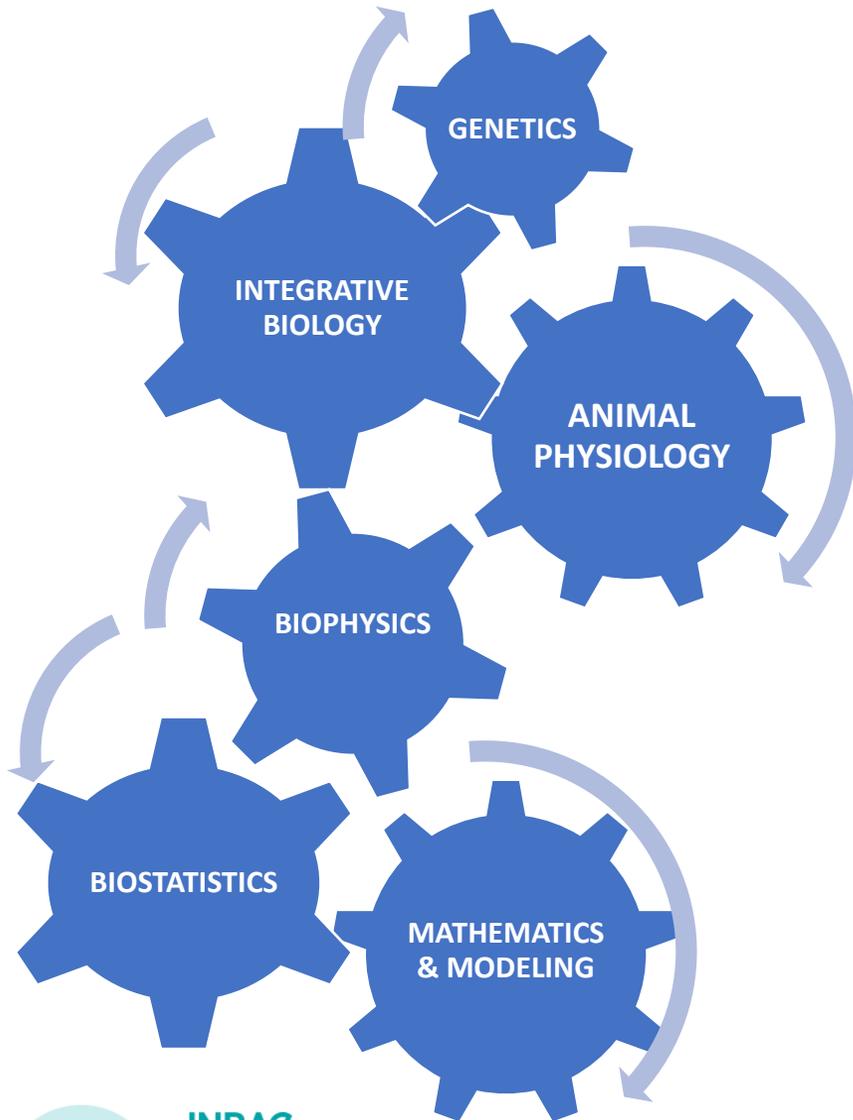
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The ANR project LIPOMEC

Coordination C. Cebo (INRAE)

ANR (690 k€)

WE ARE HERE

| 2020 | 2021 | 2022 | 2023 | 2024 |

Reduction of lipolysis along the « milk way »

Genetic selection and milk spontaneous lipolysis in dairy ruminants

Knowledge transfer

WP7 DATA INTEGRATION

WP5 ANIMAL POPULATIONS (building of MIR** equations & re-exploring the PhenoFinLait Programme)

Partners: GABI, ACTALIA, IDELE

Modelling, data integration and dissemination

TO THE MILKING PLANT and BEYOND (modelling, « artificial udder », , cheese properties)

WP6 Partners: IDELE, STLO

Genomic region linked to lipolysis in cattle

Lipolysis biomarkers in milk and mammary gland in dairy ruminants

WP3 MILK (proteomics & targeted studies)

Partners: GABI, UMRH, PEGASE

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

Partners: UMRH, GABI, PEGASE

Milk lipolysis: going further into mechanisms

WP2 ANIMAL (cow, goat, ewe)

Partners: PEGASE, GenPhySE

WP1 Project coordination

Understanding the LPL lipolytic system in milk and mammary gland

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

GENETICS

INTEGRATIVE BIOLOGY

ANIMAL PHYSIOLOGY

BIOPHYSICS

BIOSTATISTICS
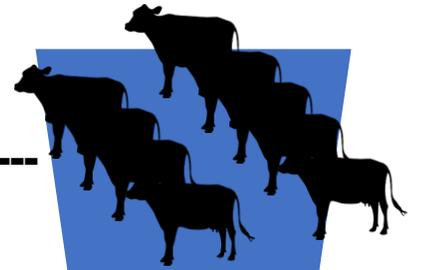
MATHEMATICS & MODELING

INRAe

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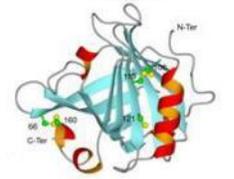INSTITUT DE L'ELEVAGE idele

ACTALIA

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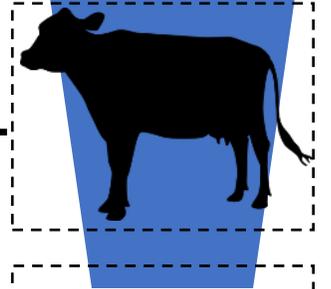
MAMMARY GLAND-------

LPL & OTHERS MOLECULES-------

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