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

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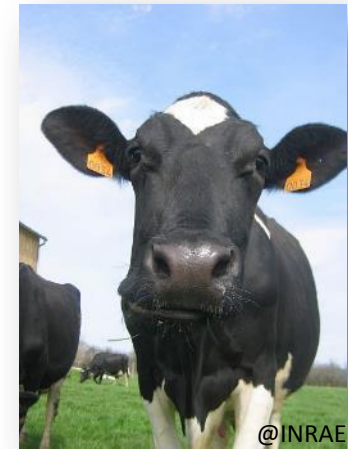
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➤ Presentation plan

- ❑ 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*)



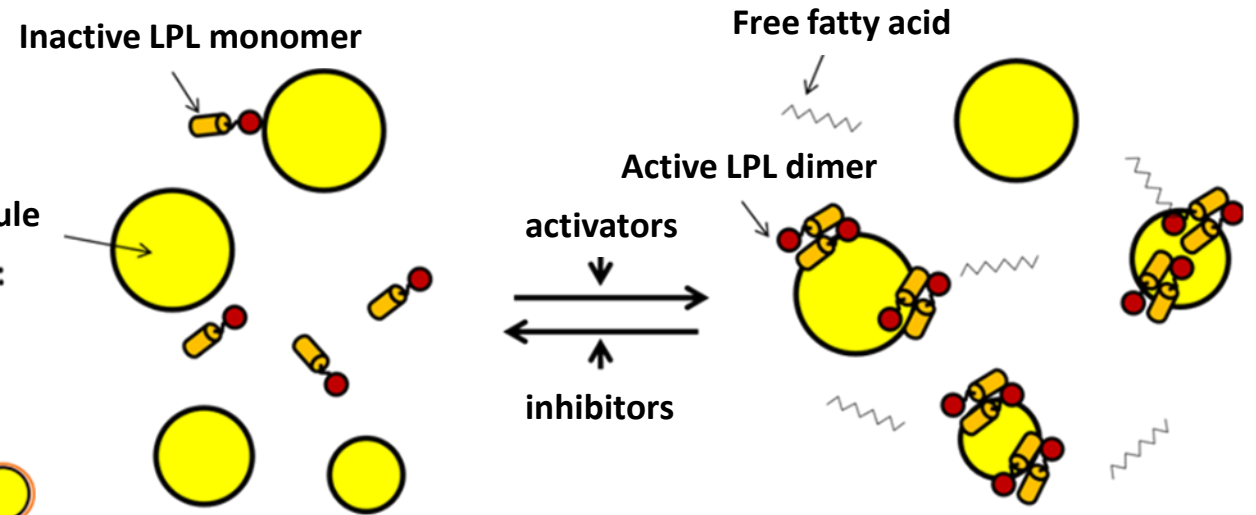
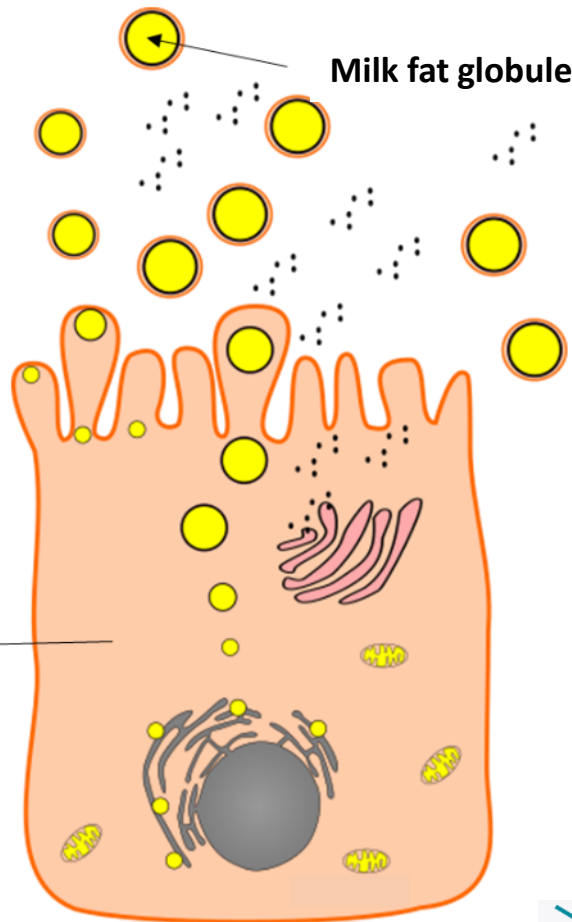
➤ State of art of spontaneous lipolysis in 2023

A definition: spontaneous lipolysis is initiated by the simple act of cooling raw milk to $<10^{\circ}\text{C}$ after it is taken from the cow (Deeth, 2006)



> Milk lipolysis ?

LPL = lipoprotein lipase

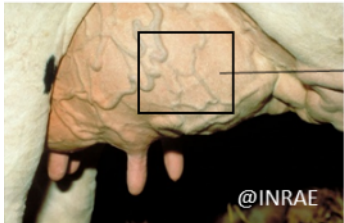


Hydrolysis of triglycerides of milk fat by LPL => release of FFA and mono- and diglycerides

↓ organoleptic properties (rancid taste)

↓ technological properties (foaming capacity)

Decreased QUALITY of milk and dairy products

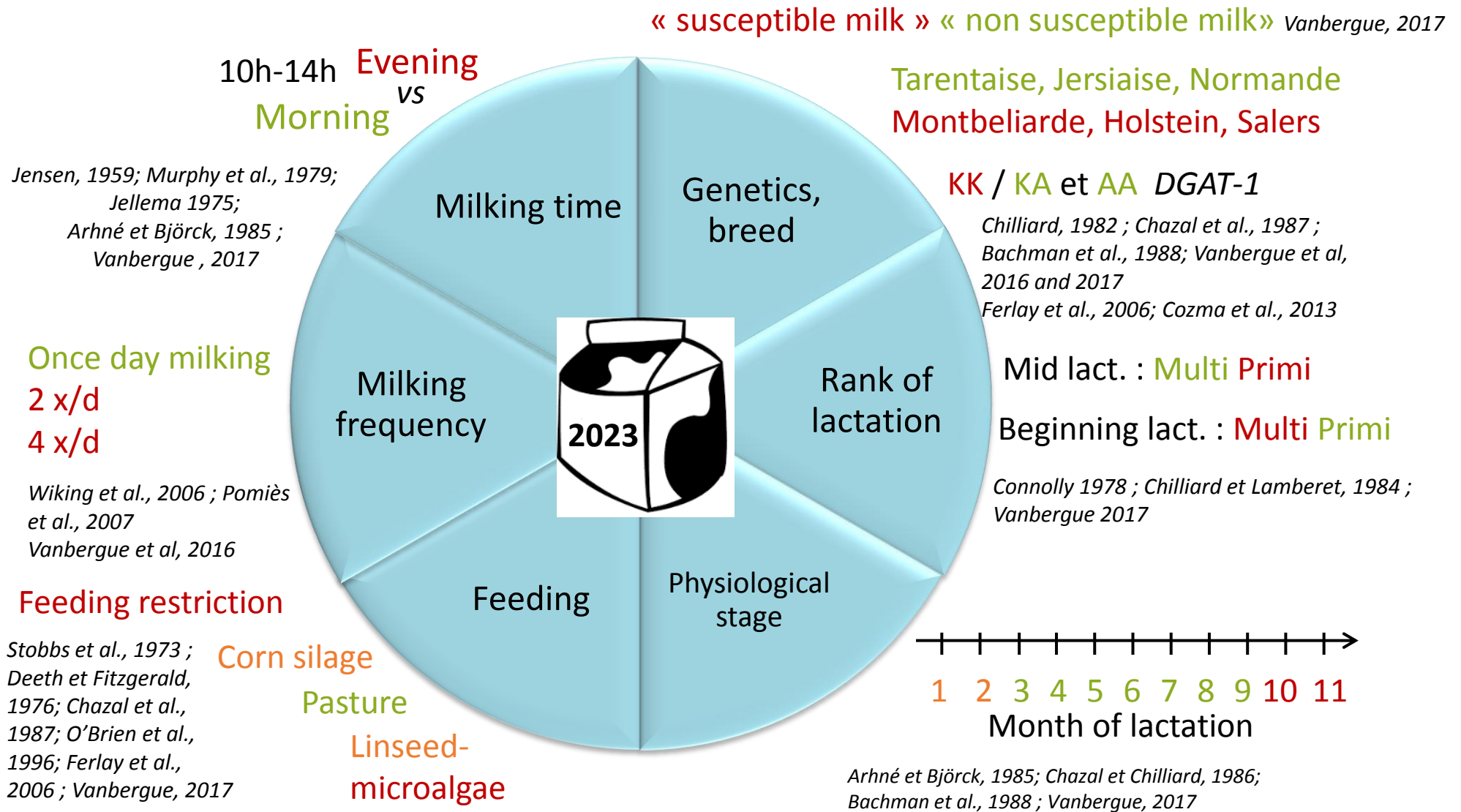


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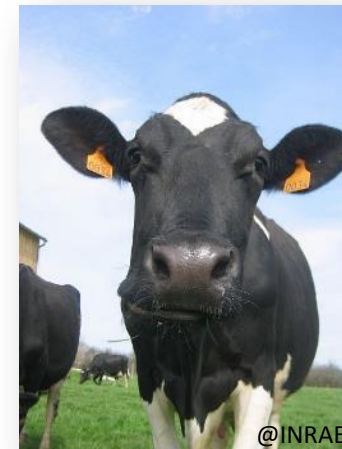


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➤ State of art of spontaneous lipolysis



➤ 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

$$Y_{ijkl} = \mu + \text{Group}_i + \text{Milking Time}_j + \text{Period}_k + \text{Feeding level}_l + \text{Milking Time}_j \times \text{Feeding level}_l + \text{Cov}Y_{ijkl} + e_{ijkl}$$

➤ Milk production and composition

| | 100% <i>ad lib</i> | 65% <i>ad lib</i> |
|----------------------------|--------------------|-------------------|
| Milk, kg/d | 30.6 | 25.5 |
| Milk protein content, g/kg | 31.4 | 30.1 |
| Milk fat content, g/kg | 40.0 | 40.1 |
| Milk lactose content, g/kg | 49.3 | 49.5 |

❑ Feed restriction was accompanied by

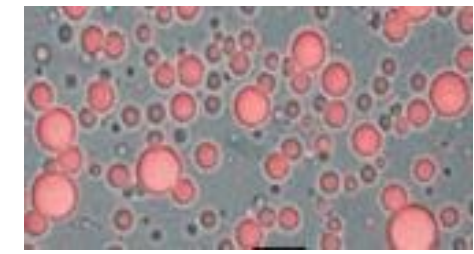
- a decrease in milk yield (- 5.1 kg) and in protein content (-1.3 g/kg)

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

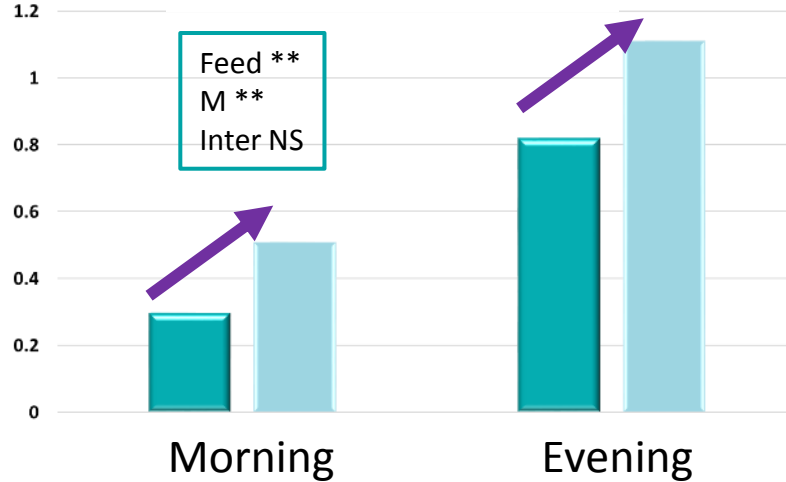
biomarker of energy deficit

Vanbergue et al, 2018

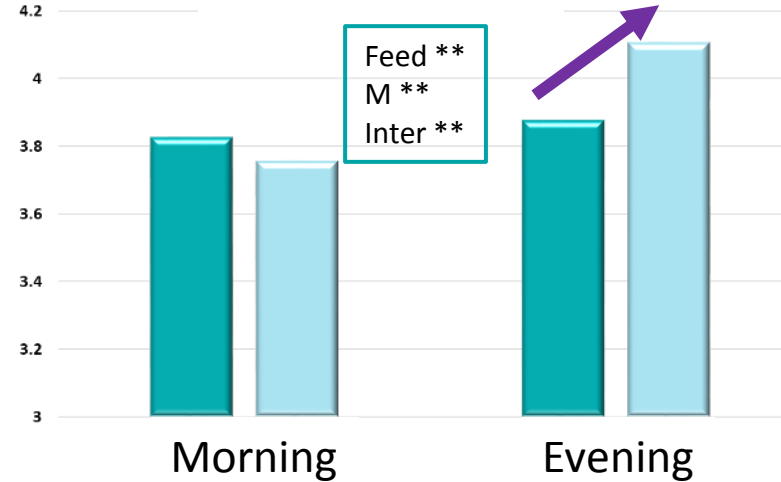
➤ Milk lipolysis



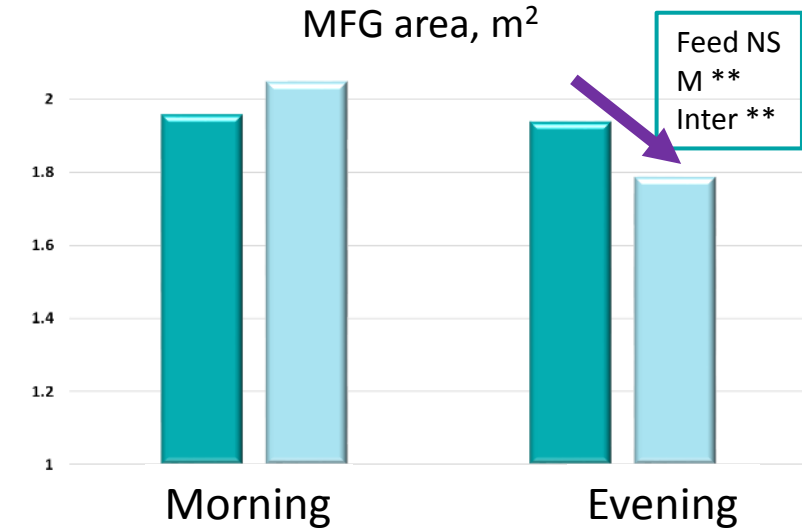
Lipolysis, meq/100 g fat



MFG diameter, μm



MFG area, m^2



 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 η mol/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 nycthemeron ?

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

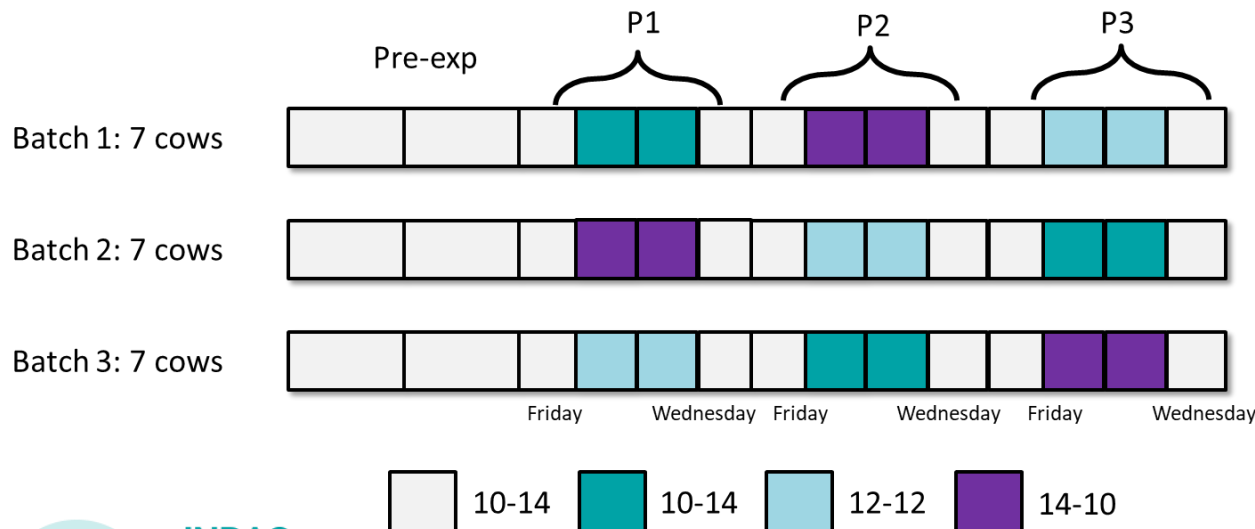


➤ Material and methods: Experimental design

- ❑ 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

$$Y_{ijk} = \mu + \text{Cow}_i + \text{Period}_j + \text{MilkingInterval}_k + \varepsilon_{ijk}$$

➤ 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

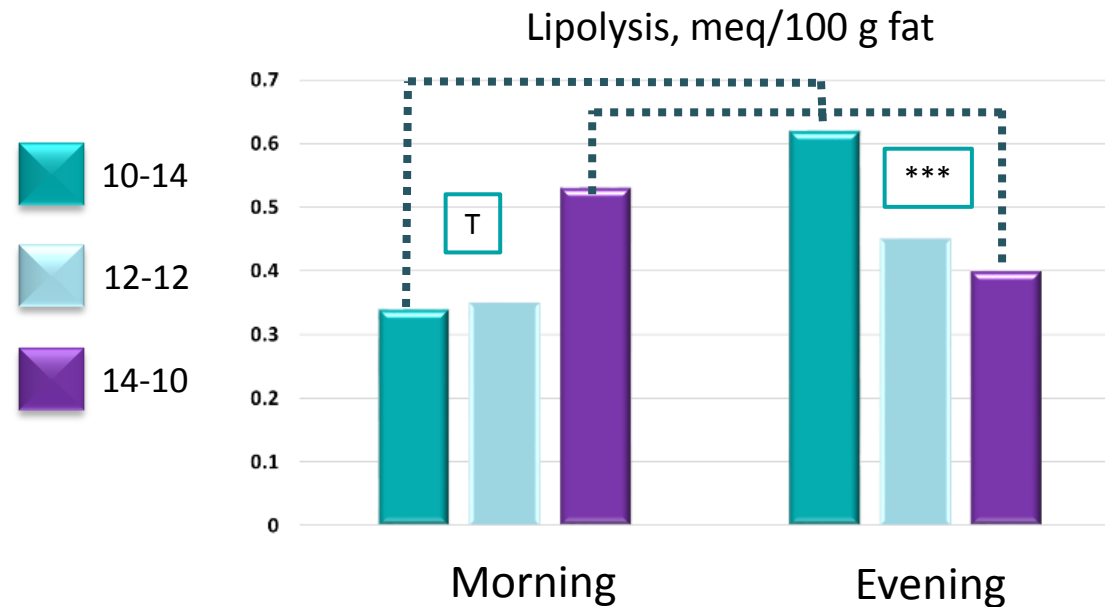
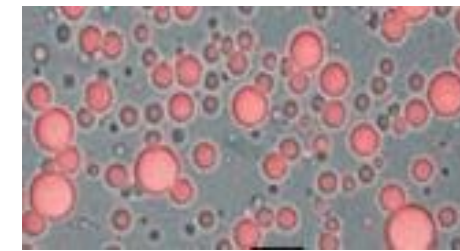
- ✓ No effect of milking intervals on milk yield, fat and protein contents
- ✓ Opposite effects between 10-14 and 14-10 on morning and evening milks
- ✓ 12-12 : slight differences in milk yield and protein content between morning and evening
- ✓ For 12-12 and 14-10, increase of synthesis of milk constituents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

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

20230628 / ADSA-INRAE INTERNATIONAL PARTNERSHIP SYMPOSIUM/ C. Hurtaud et al., INRAE

➤ 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 nycthemeron

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 nycthemeron 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?

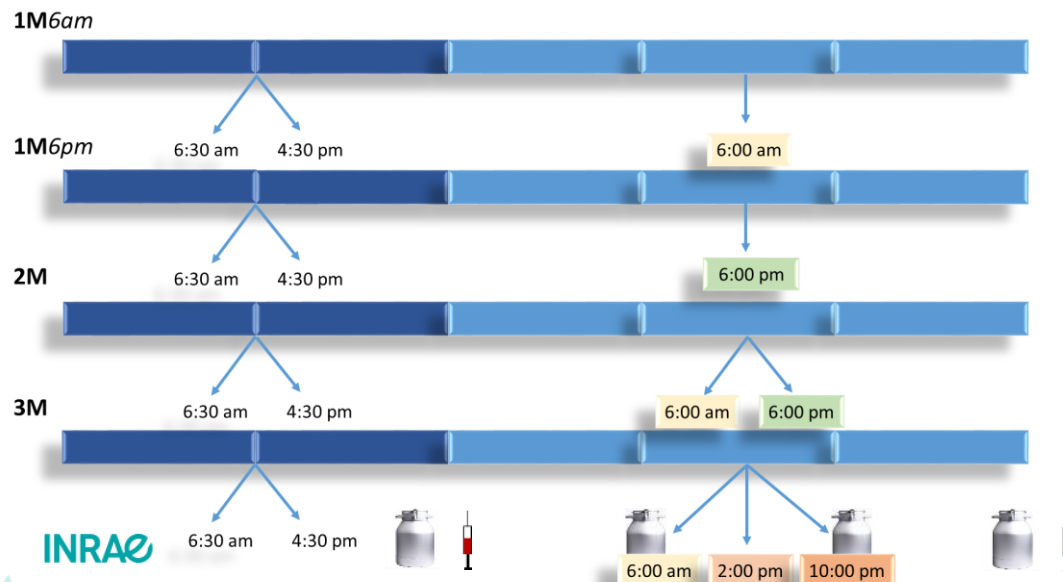
Objectives = to study the impact of **milking frequency** on the spontaneous lipolysis of milk

➤ 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 :
 - ✓ **1M6am**: one milking/ day at 6:00 am
 - ✓ **1M6pm**: 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

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

➤ Results-Discussion: Milk production

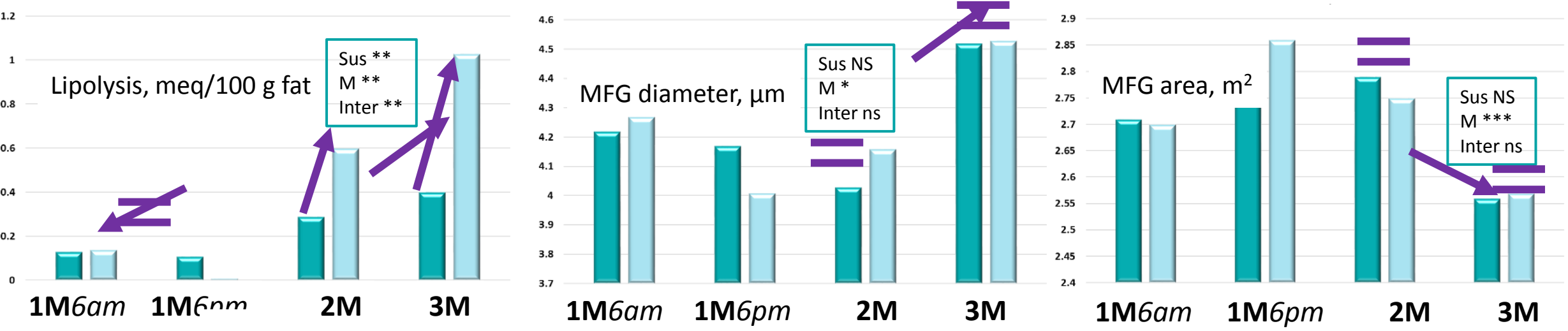
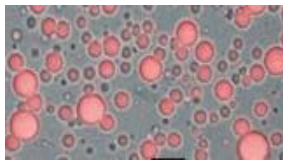


| | | | | | | Effect | | |
|----------------------------|------|------|------|-------------|-------------|--------|-------|----|
| | | | | | | 1M6am | 1M6pm | 2M |
| Milk, kg/d | NSus | 27.3 | 18.3 | 39.5 | 37.0 | NS | *** | NS |
| | Sus | 25.4 | 23.2 | 42.3 | 38.2 | | | |
| Milk fat content, g/kg | 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 **1M6am** and lower for **1M6pm**. For **1M6pm**, 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*)

SUMMARY



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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



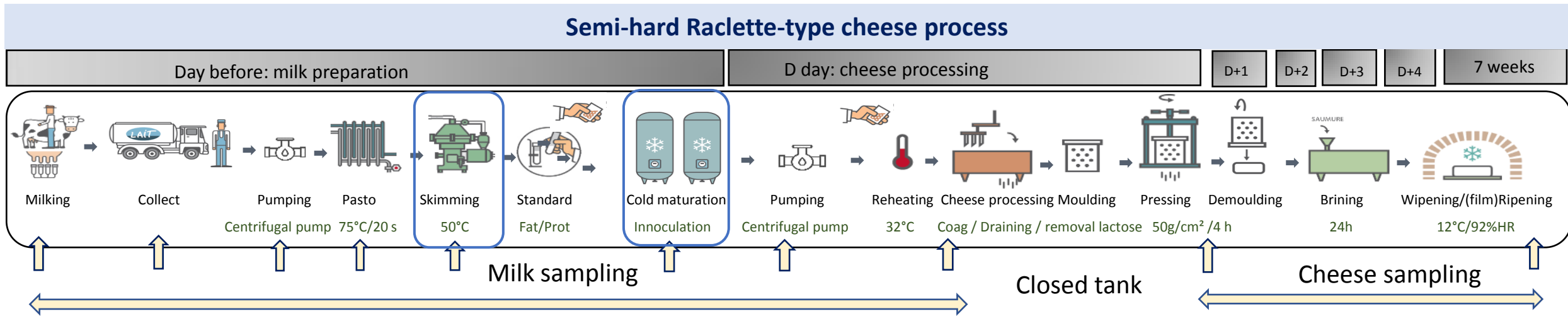
Objective of the present study

 Statement: milk processing induce lipolysis due to fat globule damage

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

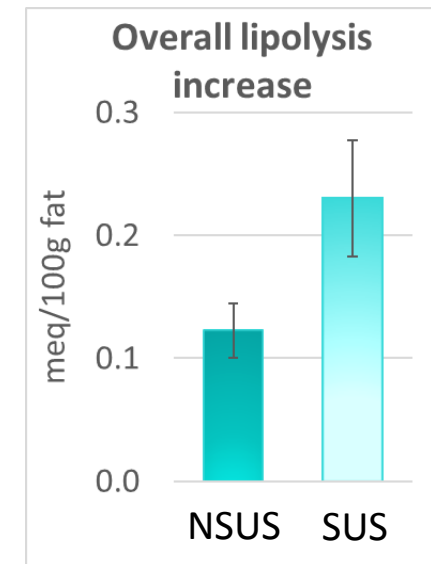
Objective: to compare the changes in lipolysis induced during milk processing operations between susceptible and non-susceptible milks

➤ 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)



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➤ 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

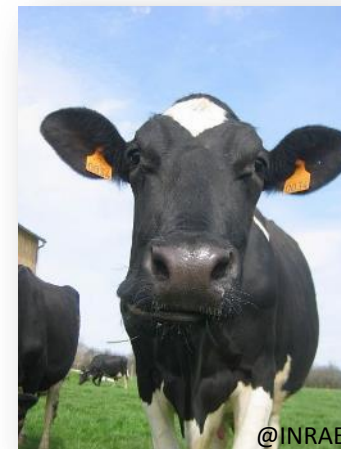


sour cream stored
for 2 months at 4°C

➤ 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 LIPOMECC project



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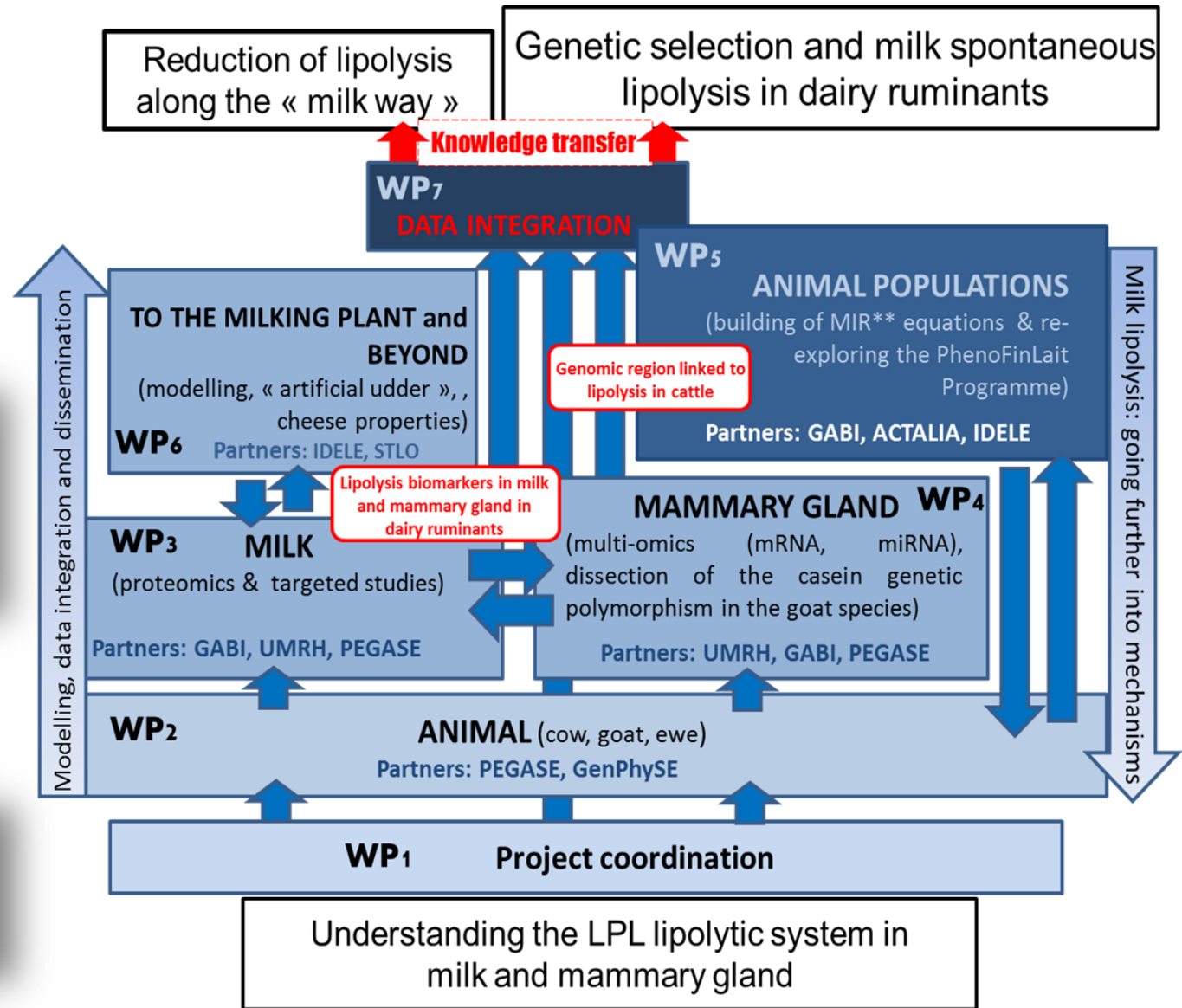
➤ The ANR project LIPOMECE

Coordination C. Cebo (INRAE)

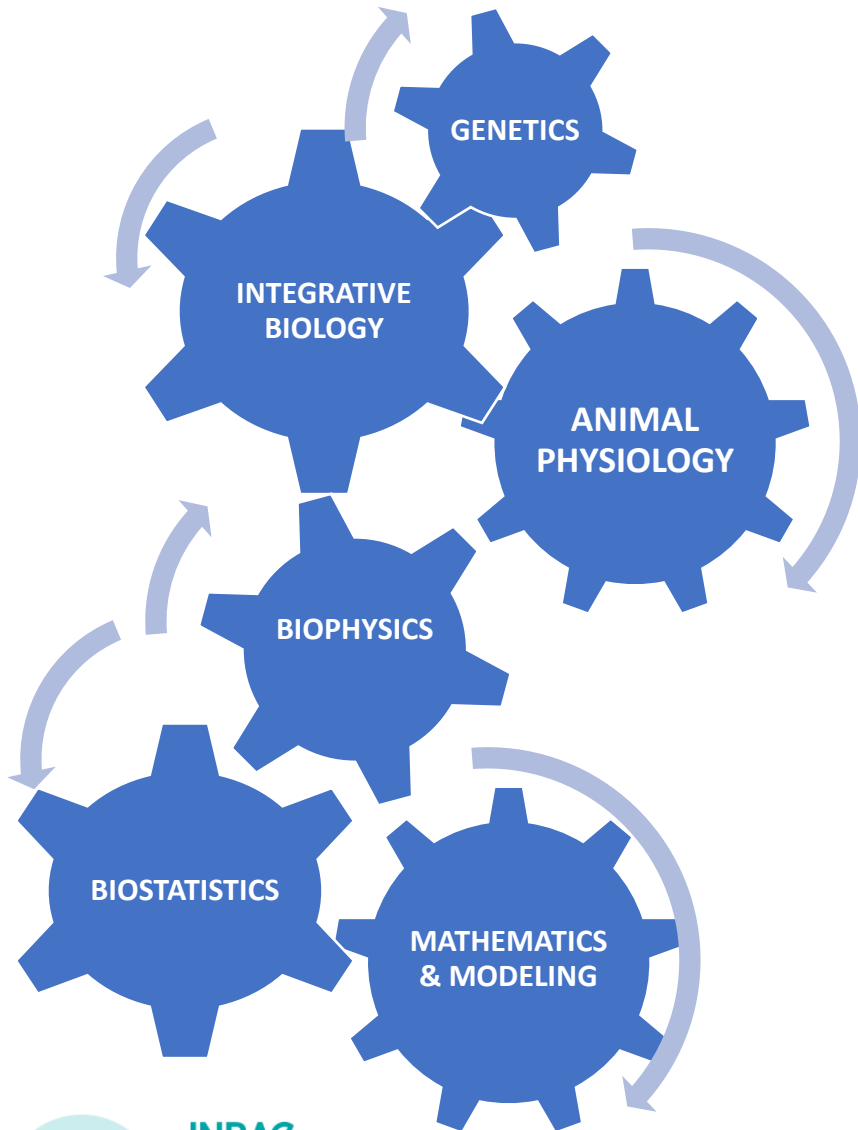


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➤ LIPOMECH, a transdisciplinary, multi-actors and multi-scale program



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APIS-GENE
Investir Innover Valoriser



INSTITUT DE L'ELEVAGE idele

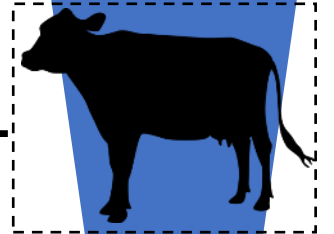


ACTALIA

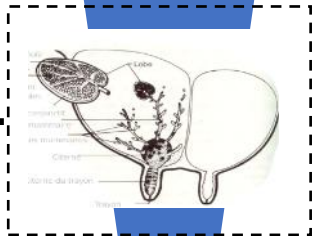
ANIMAL POPULATIONS



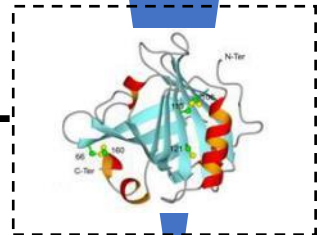
ANIMAL



MAMMARY GLAND



LPL & OTHERS MOLECULES



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