



Feed restriction in dairy ewes decreases milk lipolysis and remodels the milk proteome and lipidome

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➤ Milk lipolysis implications

Milk fat lipolysis = hydrolysis of fat globule triglycerides into free fatty acids.

High level of lipolysis in milk : ↘ organoleptic (rancid taste) and technological properties of products.



Ewe's milk production in the EU is dominated by Greece, Spain and Italy. France, Cyprus, Turkey, Bulgaria, Portugal and Romania are also producers.

↗ use for highly-fresh products (yogurts) from which rancid flavor is not acceptable.



Major part is processed in cheese (e.g. in France Ossau-Iraty and Roquefort). Foaming problems can occur.



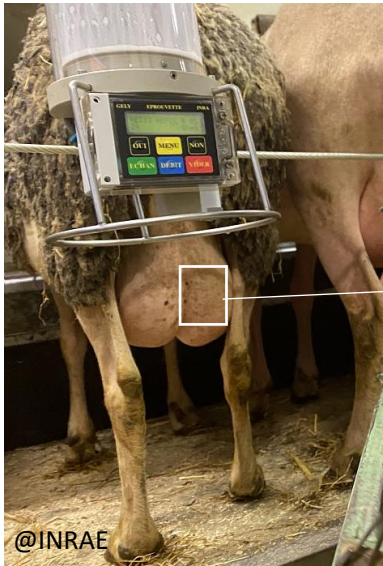
FranceAgriMer, (2019, 2021)

The lipolysis of milk is of three types:

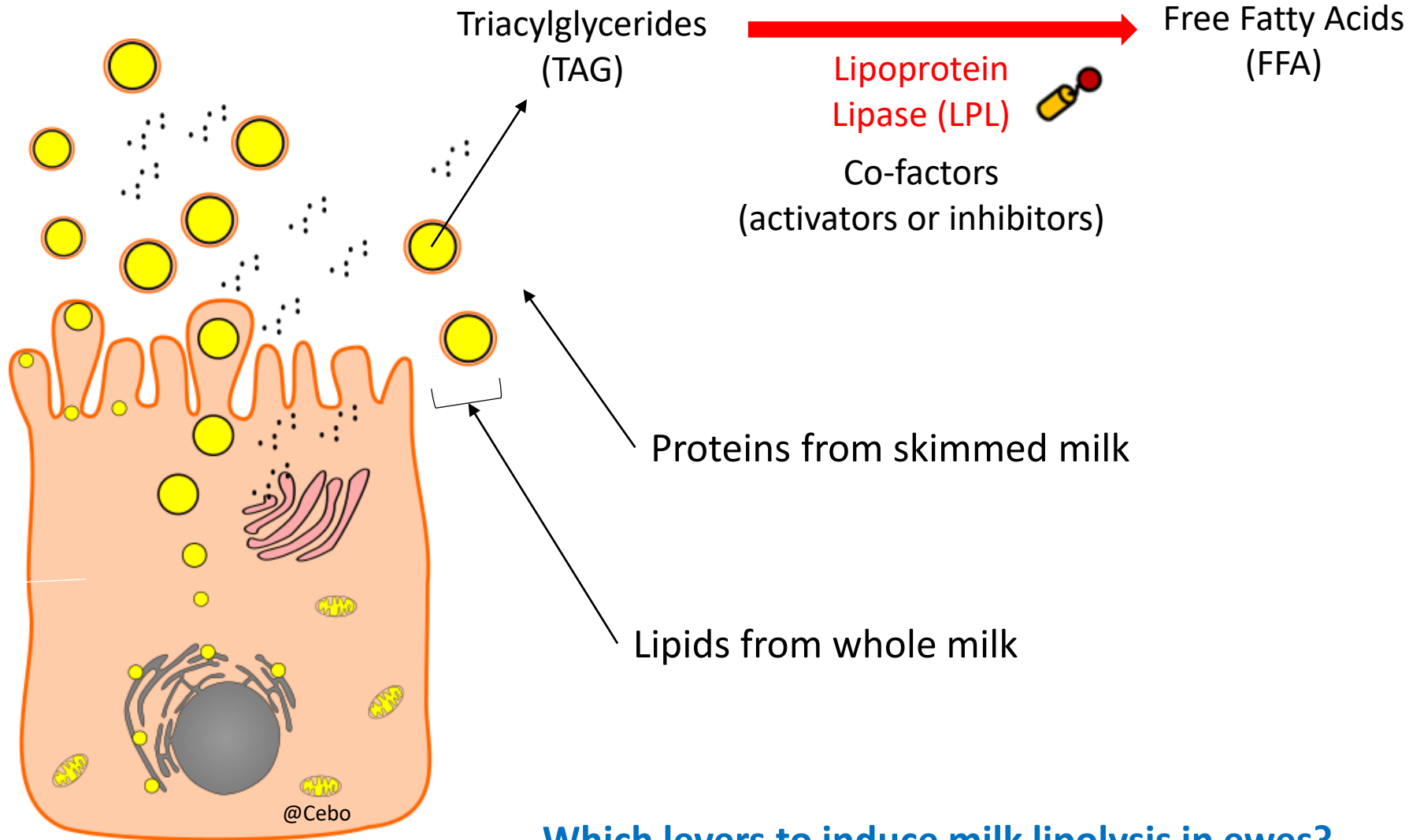
- « spontaneous lipolysis » in cold milk is due to the action of milk native lipoprotein lipase (LPL)
- « induced lipolysis » is due to LPL action increased by milk agitation, foaming or temperature changes
- « microbial lipolysis » occurs during long-term storage of products

Chilliard and Lamberet (1984)

➤ Milk spontaneous lipolysis



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Which levers to induce milk lipolysis in ewes?



➤ Feeding factors affecting milk spontaneous lipolysis



Lipid supplements	+/-	-	?
Restriction	+	- / 0	?
Grass	-	-	?

Vanbergue et al. (2017, 2018)
 Hurtaud et al. (2023)
 Chilliard et al. (2014)
 Bernard et al. (2005, 2016)

Inter-species differences

Lack of knowledge in ewes

Does feed restriction affect milk spontaneous lipolysis in ewe's milk?

➤ Objectives

To characterize the effects of dietary restriction on milk lipolysis in ewe

To explore proteomes and lipidomes of ewe's milk to better understand the mechanisms of lipolysis

➤ Experimental design

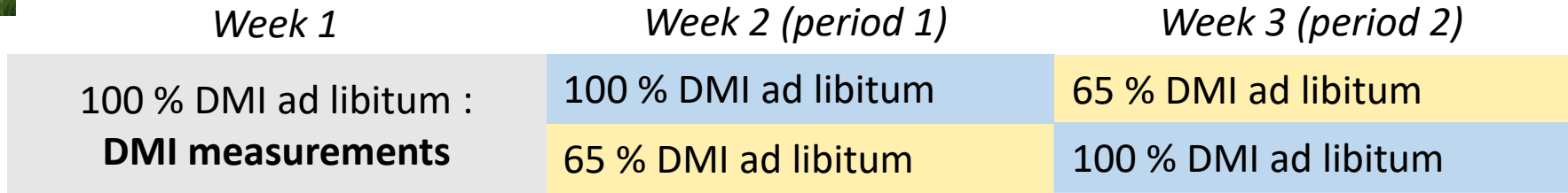


48 dairy ewes *Lacaune*
 102 ± 2 DIM
 Parity 4 ± 1

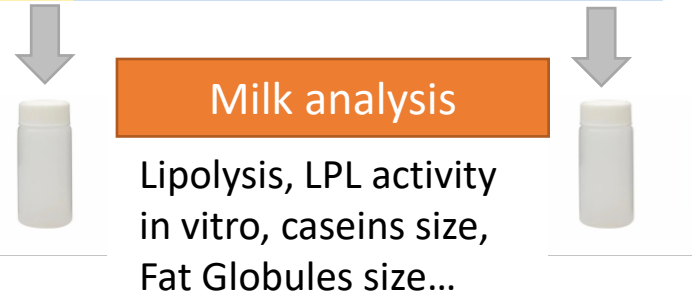


72% forages (Grass silage, hay, alfalfa)
 28% concentrate

**2 x 2
 cross-over
 design**



**Zootechanical
 performances**



Morning milk sampling
 at the end of each
 week (n=48)

Multi-omics data analysis

Statistical analysis

PCA, differential analysis, MFA,
 multiblocks... with R software
 GO with ProteINSIDE
 webservice

Proteome

Semi-quantification of
 proteins from **skimmed milk**
 by label-free nanoLC-MS/MS

Lipidome

Semi-quantification of lipids
 from **milk** by SFC-HRMS

**Selection of milk with
 extreme lipolysis levels
 (n=20)**

➤ Feed restriction effects on performances and lipolysis on « omics ewes » (n=20)

Dairy ewes (n=20)	Non-Restricted (NR)	Restricted (R)	P-values
DMI, kg/d	2.8	1.9	<0.001

68% of DMI *ad lib* near the expected 65%

Feed restriction affects dairy performances in ewes

Milk yield, L/d	1.9	1.4	0.01
Milk fat content, g/L	79.5	82.7	NS
Milk protein content, g/L	58.3	59.3	NS
Milk lipolysis, Meq/100 g of fat	0.83	0.33	0.07
In vitro milk LPL activity, nmol/mn/ml	461.6	456.1	NS

Feed restriction modulates spontaneous lipolysis

(With a statistical difference at $P < 0.05$ on the 48 ewes)

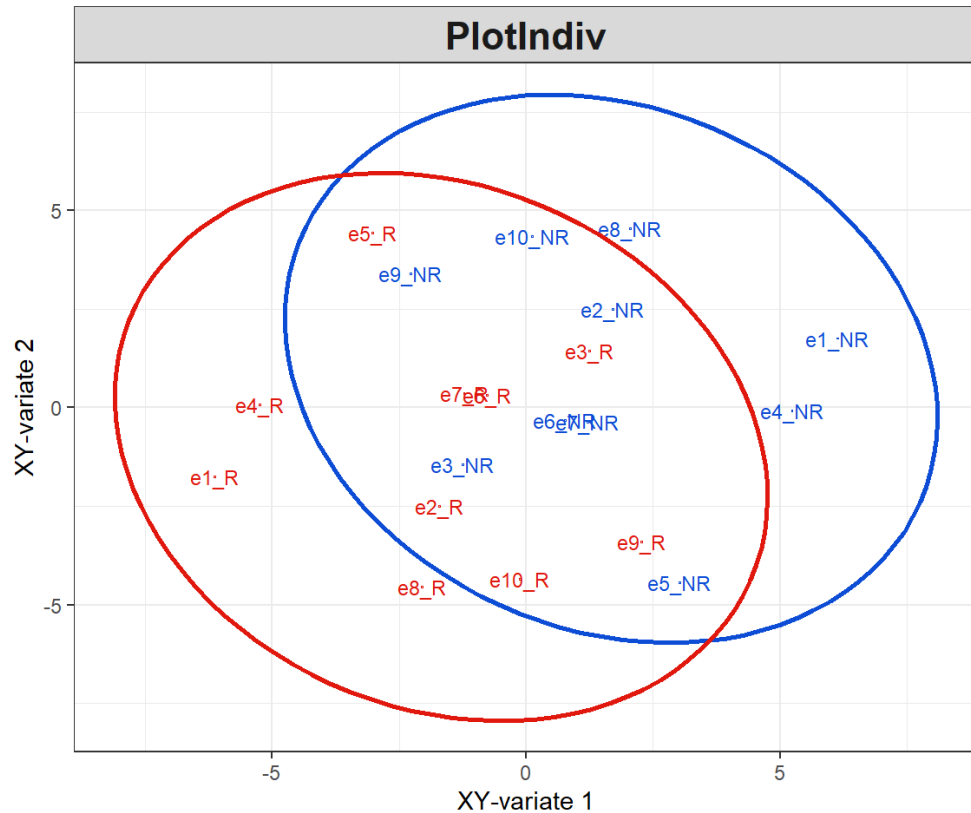
Bernard et al.
(submitted)



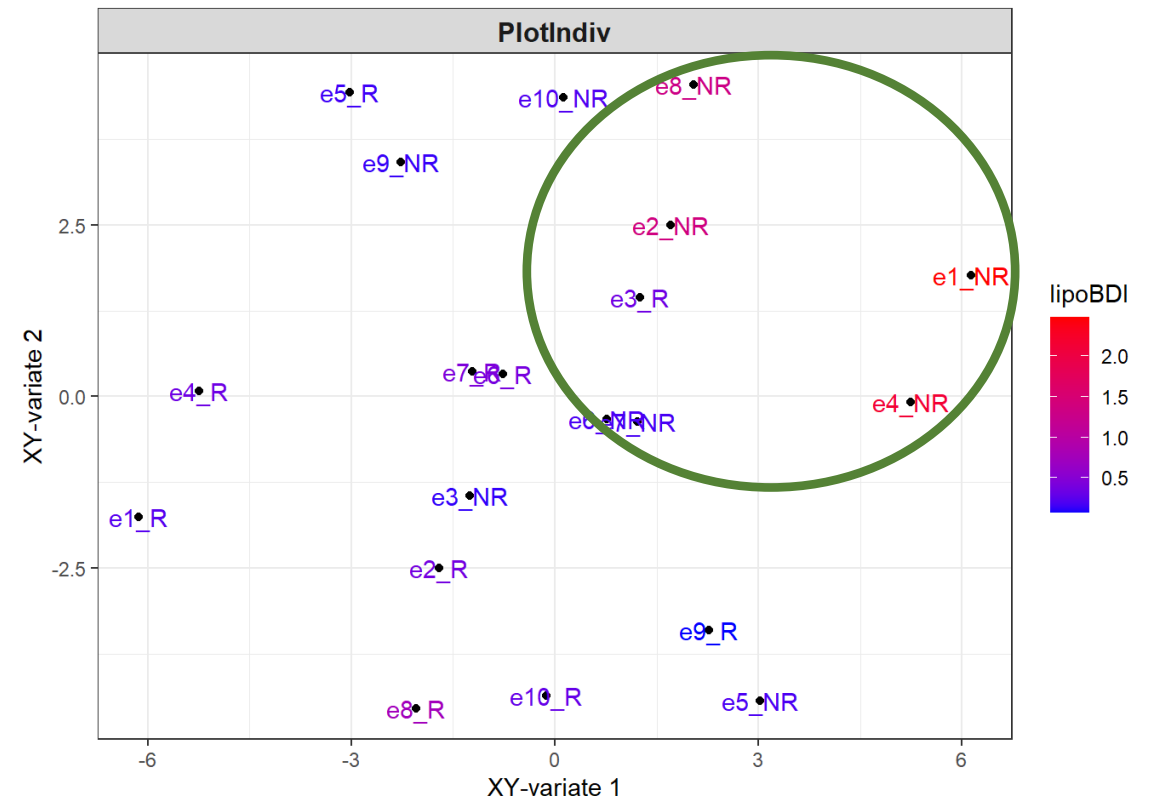
➤ Skim milk proteome according to feed restriction and lipolysis

328 proteins were identified in skim milk from ewes

Proteome projection of individuals according to sparse Partial Least Squares regression (block X = proteins; block Y = zootechnics)



A light feed restriction effect

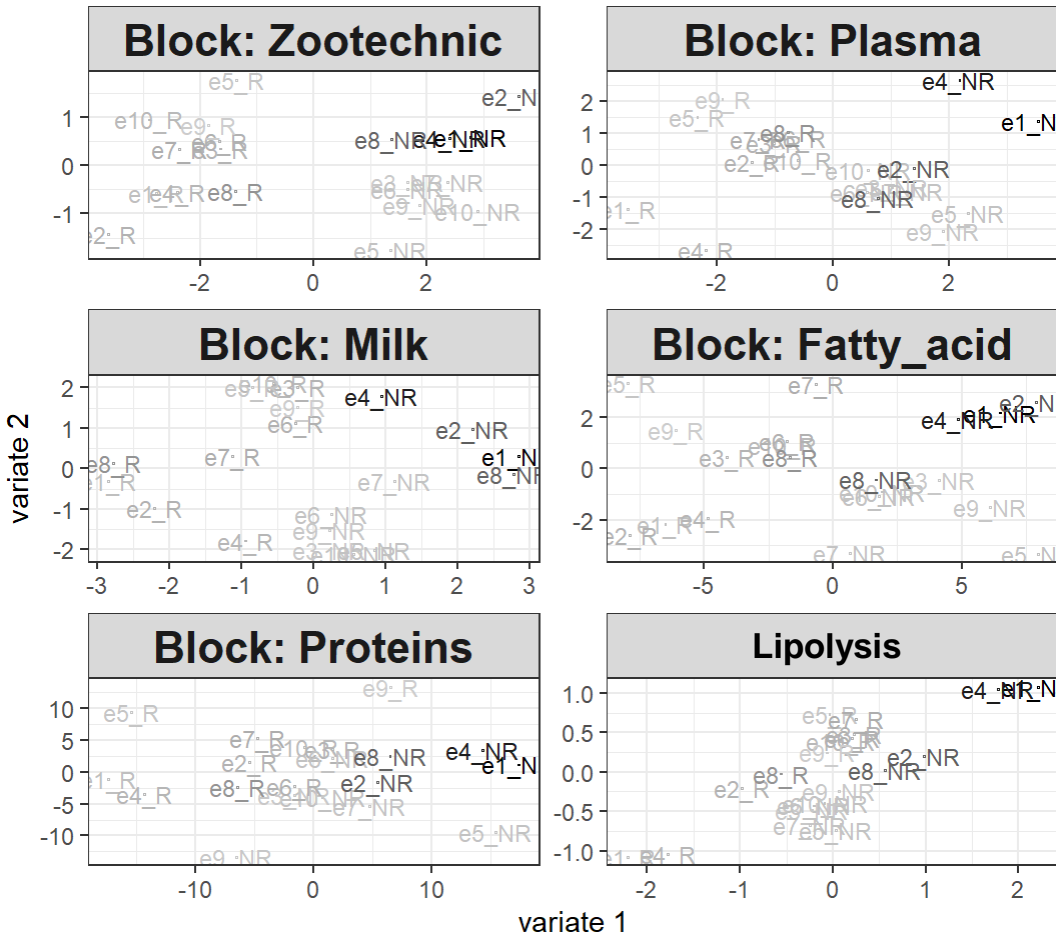


A highest lipolysis group

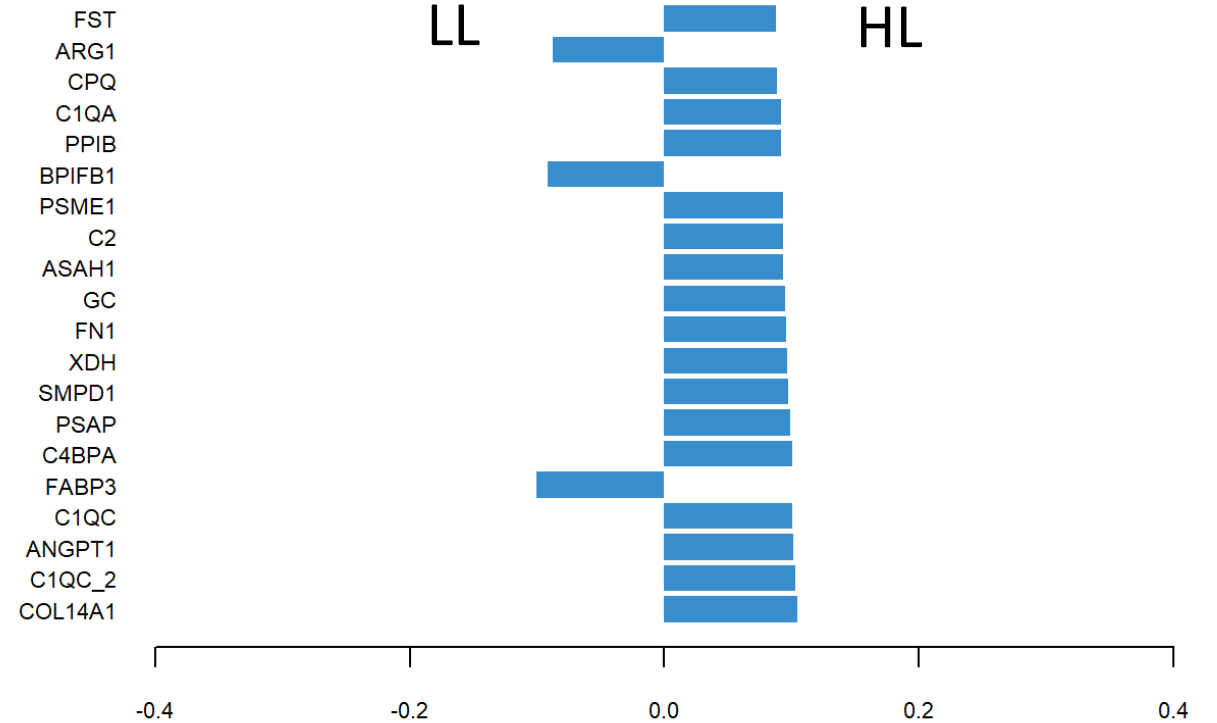
Which are the proteins discriminating lipolysis?

➤ Milk proteins discriminates high lipolysis group

Projection of the blocks of variables (multiblock sPLS)



Protein's contributions to high lipolysis group



3 proteins contribute to low lipolysis group
17 proteins contribute to high lipolysis group



➤ 17 skimmed milk proteins contribute to high lipolysis group

Gene Ontology reported significant terms

BP: Complement activation ; Immune system ; Ceramide and sphingolipid catabolic process

CC: Lytic vacuole, lysosome

MF: Acid sphingomyelin phosphodiesterase activity; ceramidase activity

PSAP	Prosaposin
SMPD1	Sphingomyelin phosphodiesterase
ASAH1	Ceramidase

3 proteins implicated in degradation of sphingolipids (SM) and ceramides (Cer)

- Sphingolipids and ceramides are located in the rafts of fat globule membranes (Lopez et al., 2010; Wei et al. 2023).
- The lipid rafts are suggested as break-down point of MFGM (Wei et al., 2023).

=> Follow up with the lipidome

➤ First results from the milk lipidome analysis

Raw data	NR (HL group)	R (LL group)
<i>Sphingomyelin (SM)</i>	41 826	110 606
<i>Ceramide (Cer)</i>	7.4	164.3

⇒ Analysis workflow on lipidome and lipolysis effect is still in progress ...

SM and Cer are modulated by feed restriction (to be confirmed)

3 proteins from high lipolysis skim milk could participate in the hydrolysis of SM and Cer of MFGM...

➤ Conclusions and perspectives for the study on ewes

- Feed restriction affects dairy performances in ewes and modulates spontaneous milk lipolysis
- The 3 proteins contributing to the high level of milk lipolysis (PSAP, SMPD1, ASAH1) could participate to hydrolyze Cer and SM and fragilize the MFGM (to be confirmed by lipidomic analysis)

A first combination of proteins and lipids results allow to propose a mechanism explaining lipolysis => a fat globule membrane degradation allows LPL access to TAG substrate



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- What about the lipolysis and the proteome in the milk of cows ?

➤ Feeding factors affecting milk spontaneous lipolysis



Lipid supplements	+/-	-	?
Restriction	+	- / 0	-
Grass	-	-	?

Vanbergue et al. (2017, 2018)
 Hurtaud et al. (2023)
 Chilliard et al. (2014)
 Bernard et al. (2005, 2016)
 Bernard et al. (submitted)

Inter-species differences

Lack of knowledge in ewes

Feed restriction increases milk spontaneous lipolysis in cow's milk

➤ Objectives

To identify biomarkers of spontaneous lipolysis and putative regulators of LPL enzyme in bovine skim milk.

=> we used feed restriction as a lever to generate highly contrasted samples with regard to milk lipolysis and to study skim milk proteome.

➤ Experimental design

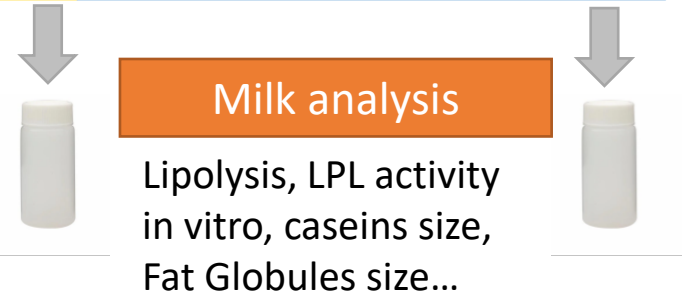
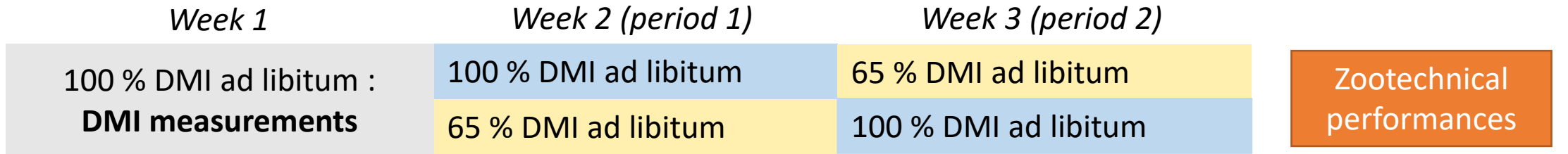


44 dairy cows *Holstein*
156 ± 20 DIM



72% forages (Grass silage, hay, alfalfa)
28% concentrate

**2 x 2
cross-over
design**



Morning milk sampling at the end of each week (n=44)

Multi-omics data analysis

Statistical analysis
PCA, differential analysis, MFA, multiblocks... with R software
GO with ProteINSIDE webservice

Proteome

Semi-quantification of proteins from **skimmed milk** by label-free nanoLC-MS/MS

Lipidome

Semi-quantification of lipids from **milk** by SFC-HRMS

Selection of milk with extreme lipolysis levels (n=18)

➤ Feed restriction effects on performances and lipolysis on « omics cows » (n=18)

Dairy cows (n=18)	Non-Restricted (NR)	Restricted (R)	P-values
DMI, kg/d	21.1	15.8	<0.001

75% of DMI *ad lib* less near the expected 65% reached in ewe's experimentation

Milk yield, L/d	18.5	16.7	0.03
Milk fat content, g secreted	710.7	611.5	0.05
Milk protein content, g secreted	583.4	486.1	<0.001
Milk lipolysis, mEq/100 g fat	0.32	0.84	0.002
In vitro milk LPL activity, nmol/mn/ml	514.8	744.2	0.002

Feed restriction increases spontaneous lipolysis in cow's milk

Hurtaud et al., 2023

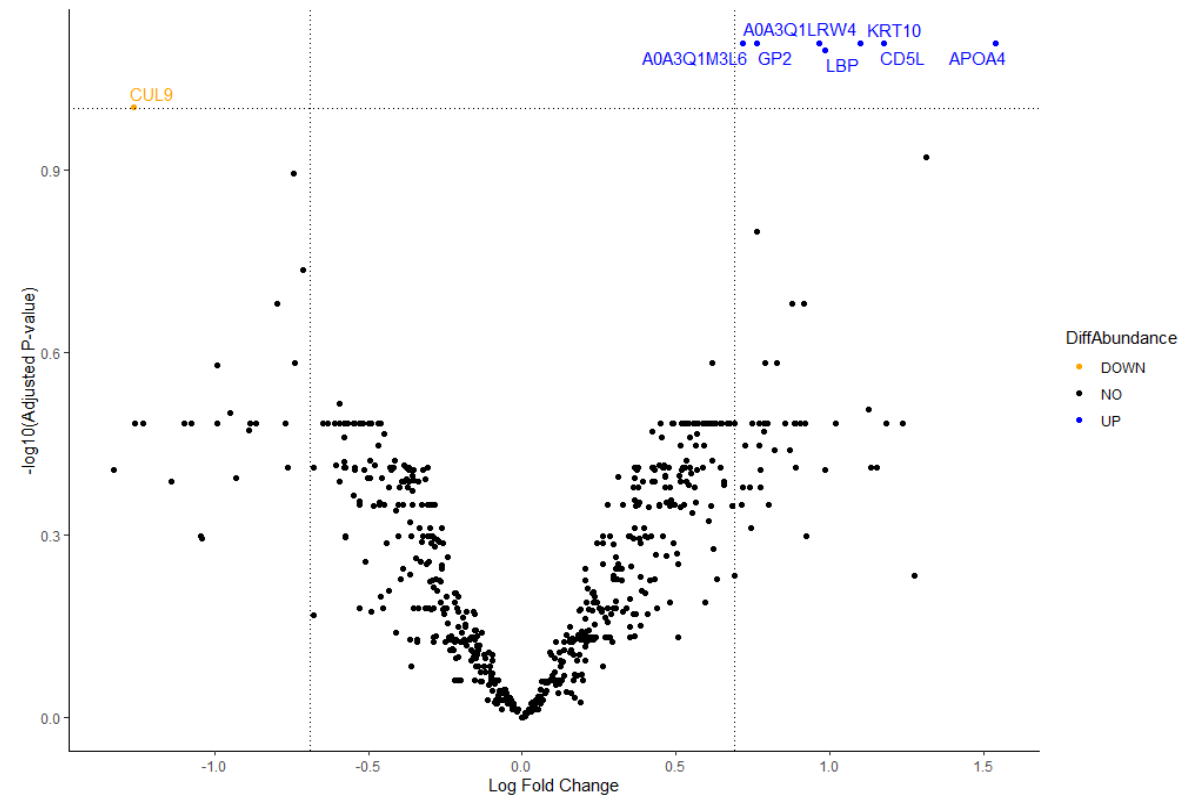
Effect on cow milk's proteome?



➤ Skim milk proteome according to lipolysis

635 proteins were identified in skim milk from cows

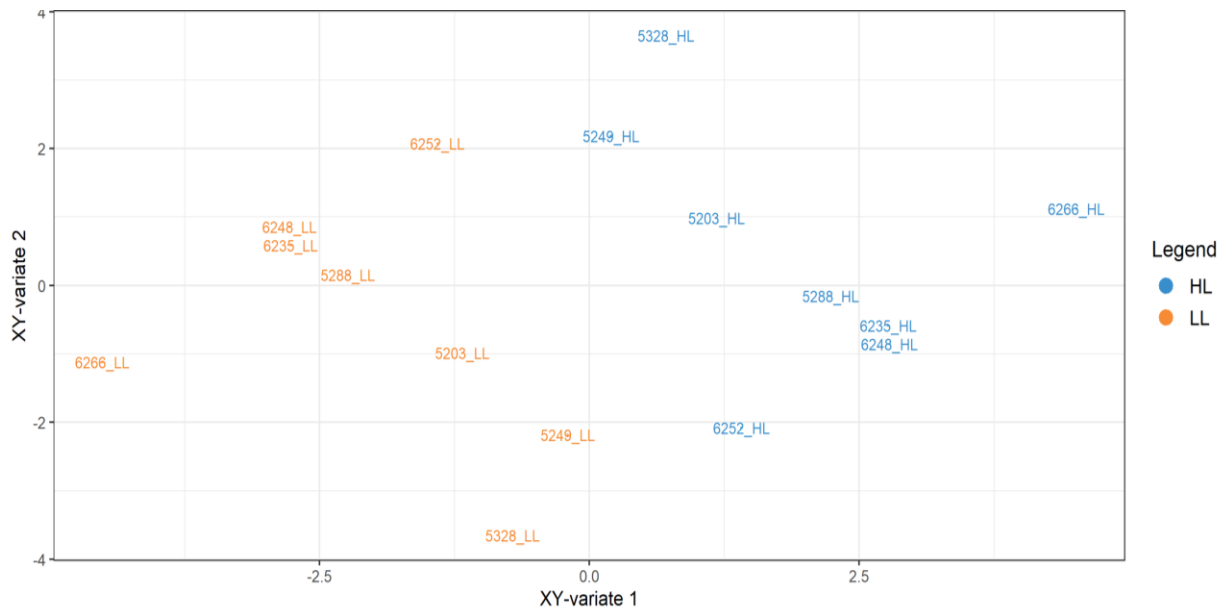
Projection of differential abundances of milk's proteins (Volcano plot representation)



List of 7 proteins contributes to HL

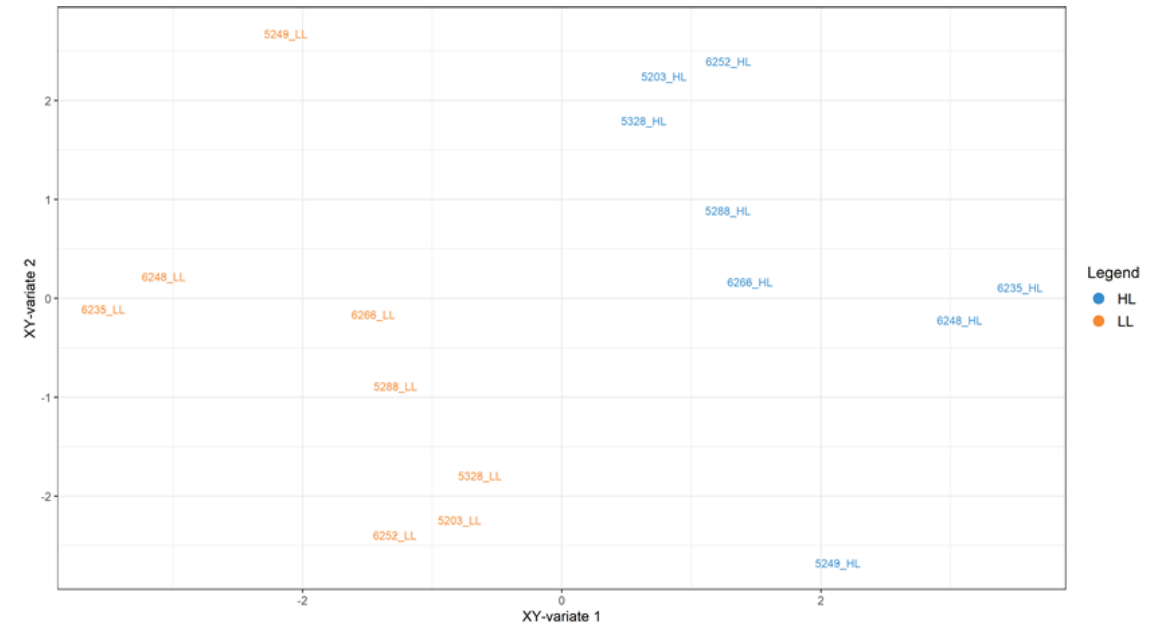
➤ Skim milk proteome according to lipolysis

Proteome projection of individuals according to sparse Partial Least Squares regression-Discriminant Analysis (block X = proteins; block Y = lipolysis)



List of 15 proteins contributes to HL

Proteome projection of individuals according to sparse Partial Least Squares regression-Discriminant Analysis (block X = proteins; block Y = LPL activity)



List of 17 proteins contributes to HL

Common proteins contributing to HL ?



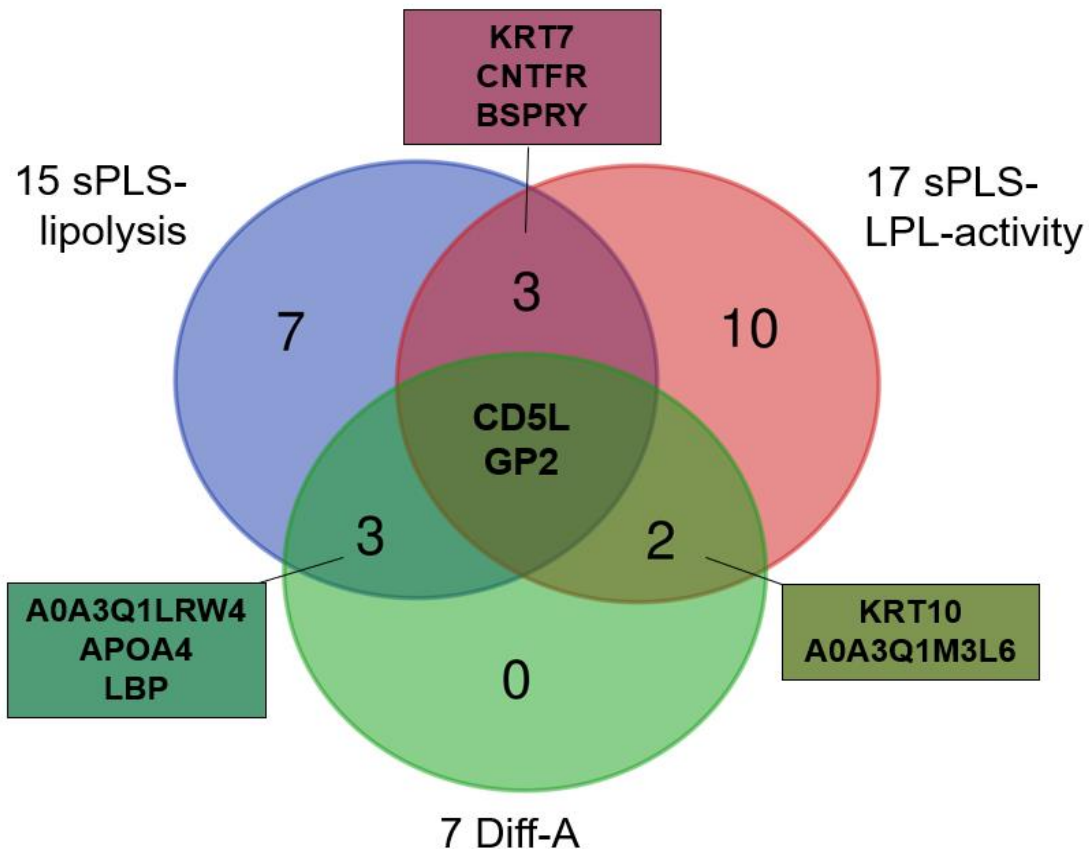
INRAE VetAgro Sup

UMR Herbivores

Journées Glандe mammaire, Lait / Delosière et al.

➤ 2 skim milk proteins are putative biomarkers of lipolysis

Combination of analyses: differential abundances, sPLS-DA on lipolysis and sPLS-DA on LPL activity



Putative biomarkers of high lipolysis in cow milk:

CD5 molecule like (CD5L) could bind and inhibit FASN, lowering de novo FA synthesis and favouring a higher FA uptake by both the mammary LPL and NEFA transporters.

Glycoprotein 2 (GP2) is a glycosylphosphatidylinositol-anchored glycoprotein may be involved in the link of LPL with MFGM.

+ Apolipoprotein A4 (APOA4) is required for efficient activation of LPL by APOCII.

Delosière et al., 2023

➤ Conclusions and perspectives for the study on cows

- Feed restriction affects dairy performances and increases spontaneous milk lipolysis
- We identified CD5L and GP2 as robust biomarkers of high spontaneous lipolysis in cow milk
- Lipidome is under analysis

➤ Acknowledgment



INRAE UE La Fage



INRAE UE Méjusseume

INRAE PFEM cp



INRAE UMR Herbivores - Biomarqueurs

LAURENCE BERNARD UMR Herbivores

CHRISTELLE CEBO
UMR GABI



LIPOMECH

CATHERINE HURTAUD
UMR PEGASE



Thanks for attention

➤ Cows : low lipolysis group

Study of Low lipolysis results report :

- HID1 (Chromosome 17 open reading frame 28 ortholog),
- SURF4 (Surfeit locus protein 4)
- CUL9 (Cullin 9)

as putative inhibitors of the lipolytic process in the milk of cows

➤ Comparing skim milk proteomes from ewes and cows

