Milk microRNA composition depends on dairy cow breed

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MILK MICRORNA COMPOSITION DEPENDS ON DAIRY COW BREED

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CONTEXT

Mother's reflect

- Direct link between microRNAs expression in the mammary gland and their level in milk

Le Guillou *et al.*,2012; Laubier *et al.*, 2015; Alsaweed *et al.*, 2016

Healthy food

- Milk is a source of nutrients for neonate and adult
- Milk composition influences consumer health in both short and long terms

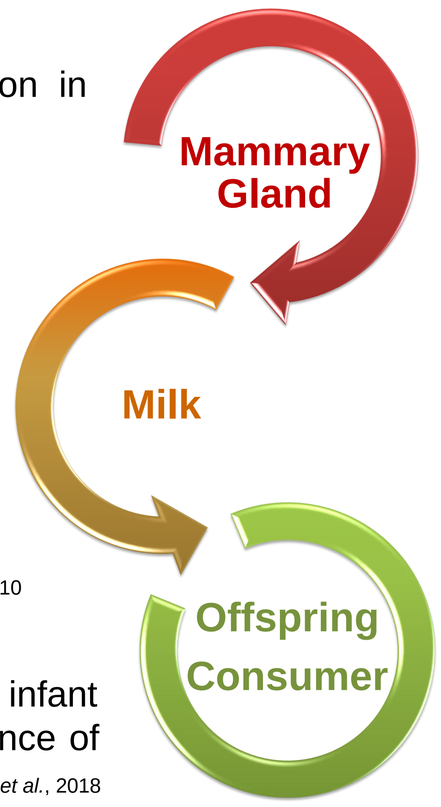
Kongerslev Thorning *et al.*, 2016; Mosca *et al.*, 2017

microRNAs

- Affect functions such as immunity, growth, development or cell proliferation Kosaka *et al.*, 2010
- Present in large quantities in milk Weber *et al.*, 2010

Impact

- microRNAs are transmitted from mother to infant
- Potential action across species and influence of milk effects on consumer health Title *et al.*, 2015; Manca *et al.*, 2018



Factors for microRNA composition variation

- *Nutrition*
- *Breeds*
- *Lactation stage*

Does milk microRNA composition (miRNome) vary according to the genetic background?

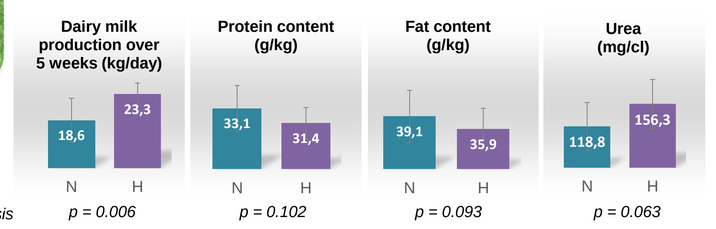
Comparison of milk miRNomes in Normande vs. Holstein dairy cows

EXPERIMENTAL MATERIAL

Primiparous cows raised together in the experimental farm

Normande (N)
n = 8

Two breeds with contrasted lactation performances



Mann & Whitney analysis — *p = 0.006* — *p = 0.102* — *p = 0.093* — *p = 0.063*

Holstein (H)
n = 9

METHODS

Milk collection → Total RNA extraction → Small RNA libraries Illumina → Small RNA-seq Illumina → Sequences processing: adaptors removing (CutAdapt) and sizing (17-28 nt) → Bio-informatic analysis miRDeep2 : genome blasting (BosTau8), annotation (miRBase), microRNA prediction and quantification. Redundancies filter (parse_miRDeep2_output.pl) (Le Guillou *et al.*,2014) → Statistical analysis: PCA (ade4 package), counting threshold identification (Bioconductor HTSFilter), differential analysis *via* Limma (voom)

RESULTS

The list of microRNAs in milk is common to both breeds

with variable abundancies, and consists of 957 annotated and 1,422 predicted microRNAs

Major milk microRNAs are the same for both breeds

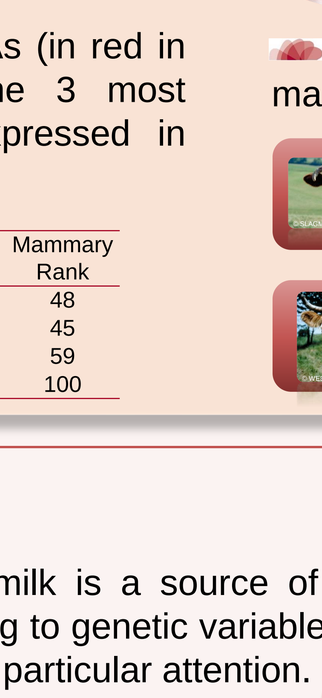
microRNA abundance in Holstein (H) and Normande (N) milk

microRNA	 % of reads	H Rank	 % of reads	N Rank
bta-miR-148a-3p	19.4%	1	19.8%	1
bta-miR-30a-5p	14.4%	2	16.4%	2
bta-miR-22-3p	8.1%	3	7.8%	3
bta-miR-26a-5p	4.5%	4	4.2%	4
bta-miR-27b-3p	3.9%	5	3.3%	7
bta-miR-186-5p	3.5%	6	3.1%	9
bta-miR-21-5p	3.2%	7	3.7%	6
bta-miR-181a-5p	3.0%	8	3.1%	8
bta-miR-141-3p	3.0%	9	4.1%	5
bta-let-7a-5p	2.5%	10	2.1%	11
bta-chr10_2883_mt-5p	2.3%	11	2.3%	10
bta-miR-30d-5p	2.2%	12	1.7%	12
bta-chr20_18762_mt-3p	2.1%	13	1.3%	15
bta-miR-92a-3p	1.6%	14	1.4%	13
bta-miR-191-5p	1.6%	15	1.4%	14
bta-miR-375-3p	1.6%	16	0.6%	20
bta-let-7f-5p	1.5%	17	1.3%	16
bta-miR-660-5p	1.2%	18	0.9%	18

> 40 % of milk reads

Milk miRNome differs between breeds

- 182 milk microRNAs are significantly different between breeds (p<0.05)



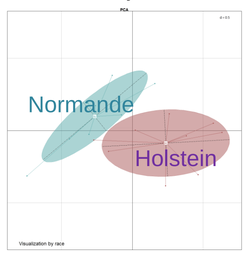
58 microRNAs are more abundant in Holstein milk *(53 known, 5 predicted)*, including:

miR-26a-5p, miR-27b-3p, let-7a-5p, let-7c-5p, miR-30d-5p, miR-375-3p, and miR-660-5p

124 microRNAs are more abundant in Normande milk *(17 known, 107 predicted)*, including:

miR-10a-5p, miR-142-3p & -5p, miR-221-3p, miR-222-3p, miR-223-3p & -5p, and miR-451-5p

Principal component analysis



Genes and pathways targeted by these microRNAs are particularly related to the regulation of milk fat synthesis and metabolism, and protein processing in endoplasmic reticulum

Comparison with bovine mammary miRNomes

Holstein cow
Le Guillou *et al.*, PLoS One 2014

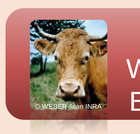
- 12 of the major milk microRNAs (in red in the previous table), of which the 3 most significant, are also the most expressed in Holstein lactating mammary gland
- 4 microRNAs show a significant enrichment in milk compared to mammary gland

microARN	Milk Rank	Mammary Rank
bta-miR-30d-5p	12	48
bta-miR-191-5p	15	45
bta-miR-375-3p	16	59
bta-miR-660-5p	18	100

- Variations according to breed in milk are also observed in mammary gland for 5 microRNAs

Dairy cow
Billa *et al.*, IMGC 2018 Poster

Between Holstein and Montbéliarde breeds: *miR-30c-5p, miR-143-3p* and *miR-340-3p*



Beef cow
Wicik *et al.*, J. Anim. Breed. Genet. 2018

Between Holstein and Limousine breeds: *miR-375-3p* and *miR-2285t-3p*

CONCLUSION

This study allowed to evaluate genetic variations of the bovine milk miRNome according to the breeds, highlighting 182 microRNAs with significantly different levels between Holstein and Normande milk.

As milk is a source of nutrients and may influence health, the variation of its miRNome according to genetic variables, such as breeds, may have several impacts on consumers and thus requires particular attention.

MicroRNAs' evaluation in milk opens a field of investigation, as they can used as biomarkers, for downstream consequences on health, physiological or metabolic status, in animals and humans.

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