

# Effects of nutrient restriction on mammary cell turnover in lactating dairy cows

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Feeding level acts on lactation performances in dairy cows: more precisely a nutrient restriction results in decreased milk yield (MY). However, little is known about the effects of nutrient restriction on mammary epithelial cells and tissue remodeling. The aims of the study were to investigate the effects of nutrient restriction on mammary tissue morphology and on mammary epithelial cell turnover in lactating dairy cows. We hypothesized that nutrient restriction reduces MY by affecting mammary cell turnover.

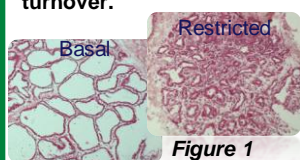


Figure 1

## Material & methods

- 2 groups submitted to different feeding levels from calving to week 11 postpartum :

- ✓ Basal Diet : 55 % maize silage, 15 % dehydrated alfalfa and 30 % concentrate
- ✓ Restricted Diet : 60 % grass silage and 40 % hay

- 2 groups of 7 Normande x Holstein dairy cows

- Twice daily milking

- Milk composition
- Mammary DNA concentration and histology
- Proliferation (qPCR and Western Blot)
- Apoptosis (qPCR and Western Blot)
- Metalloproteinases activity (Zymography)

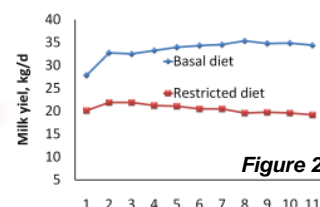


Figure 2

## Results-Discussion

### Zootechnical performances

- The cows of the Restricted diet-group had a lower 11-week average daily MY from calving to slaughter than Basal diet-group cows (20.5 vs. 33.5 kg/d,  $P<0.001$ , **Table 1** and **Figure 2**).
- Feed restriction decreased milk protein content ( $P<0.001$ ) and milk lactose content ( $P<0.002$ ), without modifying milk fat composition (**Table 1**).
- Mammary glands from Restricted diet-group were less heavy than those of Basal diet-group (16.7 vs. 26.9 kg,  $P<0.001$ ).
- The total amount of DNA in the mammary gland were lower in the Restricted diet-group (**Table 1**).

### Physiologicals results

- The size of the mammary acini was lower ( $P<0.01$ , -41%) in the Restricted diet-group (**Table 1** and **Figure 1**).
- Mammary cell proliferation did not vary significantly with feed restriction whereas TUNEL assay showed a higher level of apoptosis in the mammary gland of Restricted diet-group ( $P<0.001$ , **Table 1**).
- Gelatin zymography showed a higher level of MMP-9 and MMP-2 proteolytic activity in the Restricted diet-group (**Table 1**).
- The expression levels of BAD, PTEN, Caspase-3, Cathepsin B, IGFBP-5 and Calpain were significantly more important in mammary glands of the cows subjected to a restrictive diet (**Figure 3**).
- Expression of  $\alpha$ -LAC and  $\kappa$ -CAS were repressed in the udders of the cows subjected to the restricted diet (respectively, -38% and -46%,  $P<0.01$ , **Figure 3**).

Table 1	Basal diet	Restricted diet	P
Milk yield (kg/d)	33.5	20.5	<0.0001
Fat content (%)	4.2	4.2	NS
Protein content (%)	3	2.7	<0.0001
Lactose content (%)	5	4.8	<0.001
Total mammary DNA amount (mg)	52	42	0.003
Proliferation (%)	22	23	NS
Apoptosis (%)	0.14	0.3	0.02
MMPs activity (Integrated Density)	1.97	2.32	0.004
Acini size ( $\mu\text{m}^2$ )	22505	10587	0.02

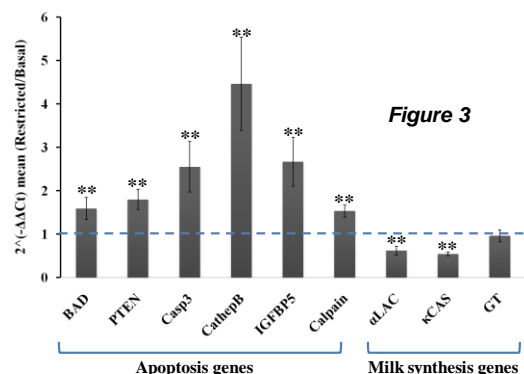


Figure 3

In conclusion, the lower MY induced by nutrient restriction in lactating dairy cows was partly due to a lower number of mammary cells. This was a consequence of a higher level of apoptosis but also to a mammary remodelling by the extracellular matrix. The future objective will be the study of the influence of nutrient restriction on mammary cell activity.

