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Influence of roughage intake on free fatty acid receptors mRNA abundance in bovine adipose tissues

P. Friedrichs, L. Locher, S. Dänicke, B. Kuhla, H. Sauerwein, and M. Mielenz

Session 13 "Appetite control - mechanisms and comparative aspects„

EAAP

25/08/2014

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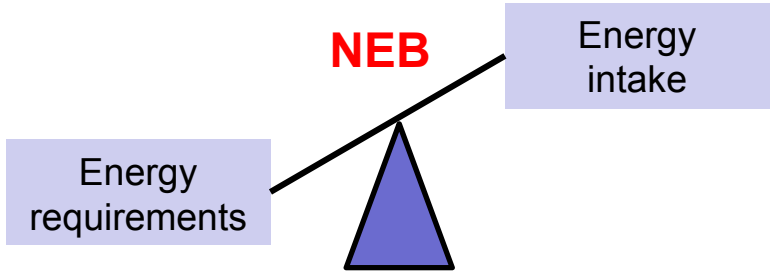
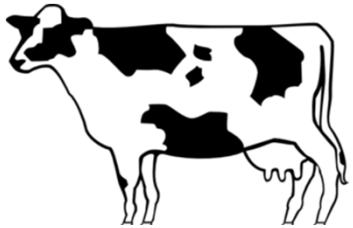
Introduction I



- Negative energy balance (NEB) at the onset of lactation
  - Primarily compensated by increased lipolysis in adipose tissue (AT)

→ Nutrient sensors: Regulation of energy homeostasis



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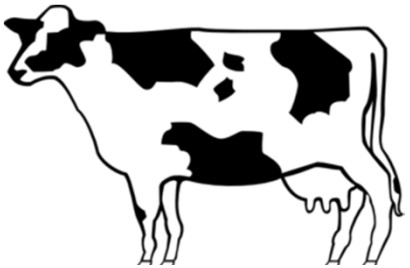
Introduction II

- Free fatty acid receptors (FFAR) 2 and FFAR3: receptors for free fatty acids
- In adipocytes:
  - Activation of FFAR2 (aka GPR43) inhibits lipolysis (Hong et al., 2005)
  - Activation of FFAR3 (aka GPR41) stimulates leptin secretion (Xiong et al., 2004)



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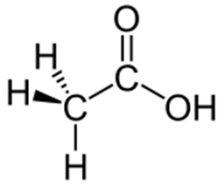
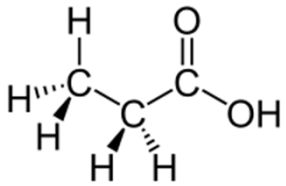
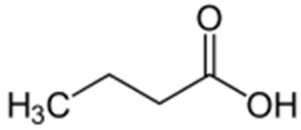
Introduction III

- Acetate (C2), propionate (C3) and butyrate (C4):
  - Produced by enteric microbial fermentation
  - Provide the major energy supply in ruminants (Bergman, 1990)
  - EC_{50} values in µmol*liter^{-1} (and fold-change to C4)

	C2	C3	C4
bFFAR2	7000 (**175-fold**)	550 (**14-fold**)	40 (**1-fold**)
bFFAR3	4500 (**17-fold**)	150 (**0.5-fold**)	250 (**1-fold**)

(Hudson et al., 2012)

- LR/high energy content in the diet increases the propionate production in the rumen (Baumann et al., 1971; Rabelo et al., 2003; Sutton et al. 2003)

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Hypothesis

Different extends of NEB induced by unequal proportions of roughage in the diet will affect the **expression of FFAR2 or 3** in **subcutaneous (SC)** and **retroperitoneal (RP)** AT of dairy cows during the transition period.

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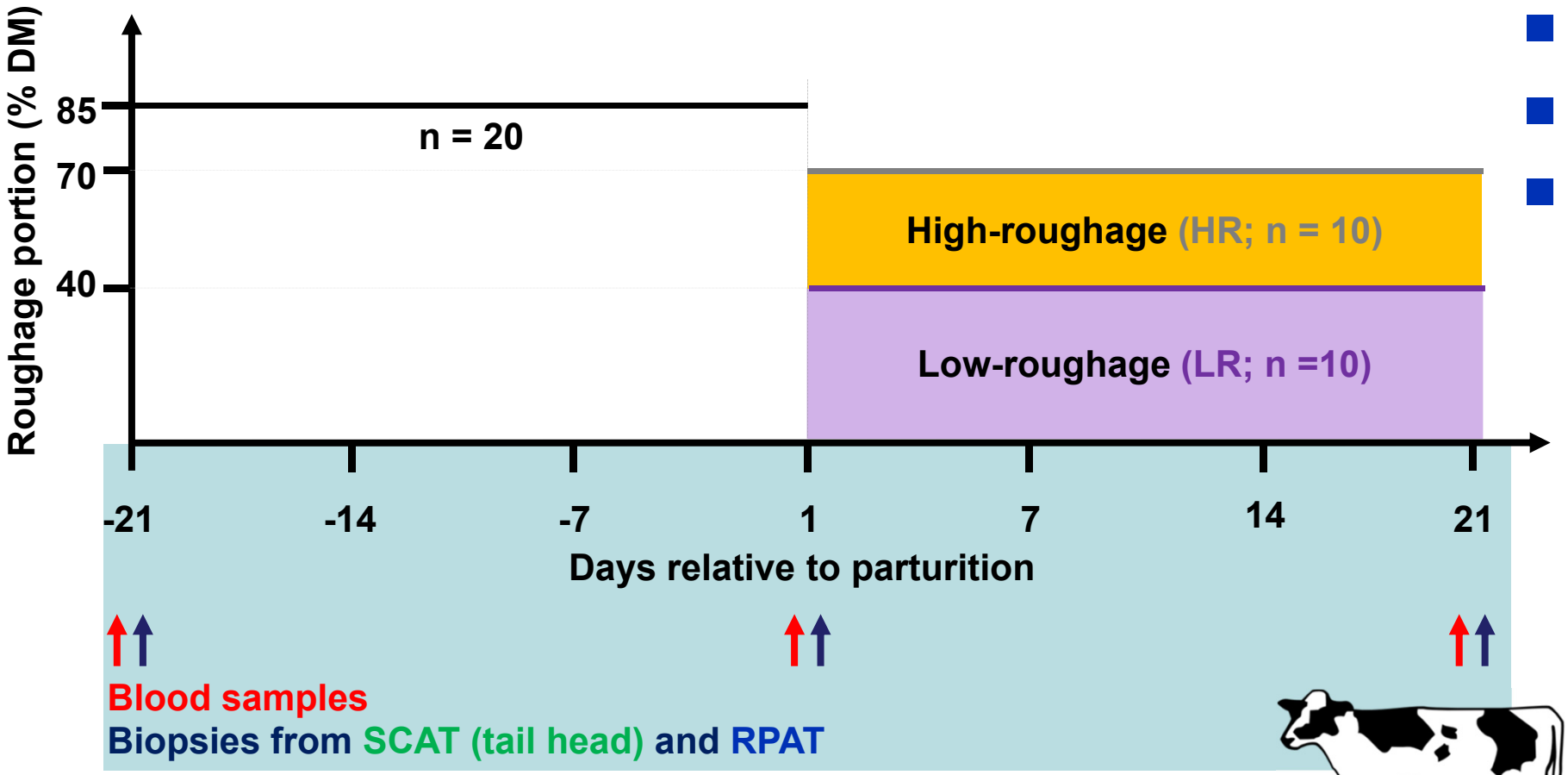
Materials & Methods I



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Materials & Methods II

DMI, EB, serum BHBA and triglycerides in the 3rd week of lactation / at d 21 after parturition

Characteristics	HR	LR	*P*-value
DMI [kg/d]	16.32 ± 0.42	18.93 ± 0.74	< 0.01
EB [MJ/d]	**-33.7 ± 5.8**	**-15.3 ± 6.0**	**< 0.05**
BHBA [mmol/L]	**0.76 ± 0.05**	**0.47 ± 0.11**	**< 0.05**
Triglyceride [µmol/L]	0.22 ± 0.06	0.13 ± 0.01	< 0.05

(Locher et al., 2011)

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Materials & Methods III

C2, C3 and C4 quantification in serum

- C2, C3 and C4 were derivatized and measured on a GC-FID

mRNA quantification in SCAT and RPAT biopsies

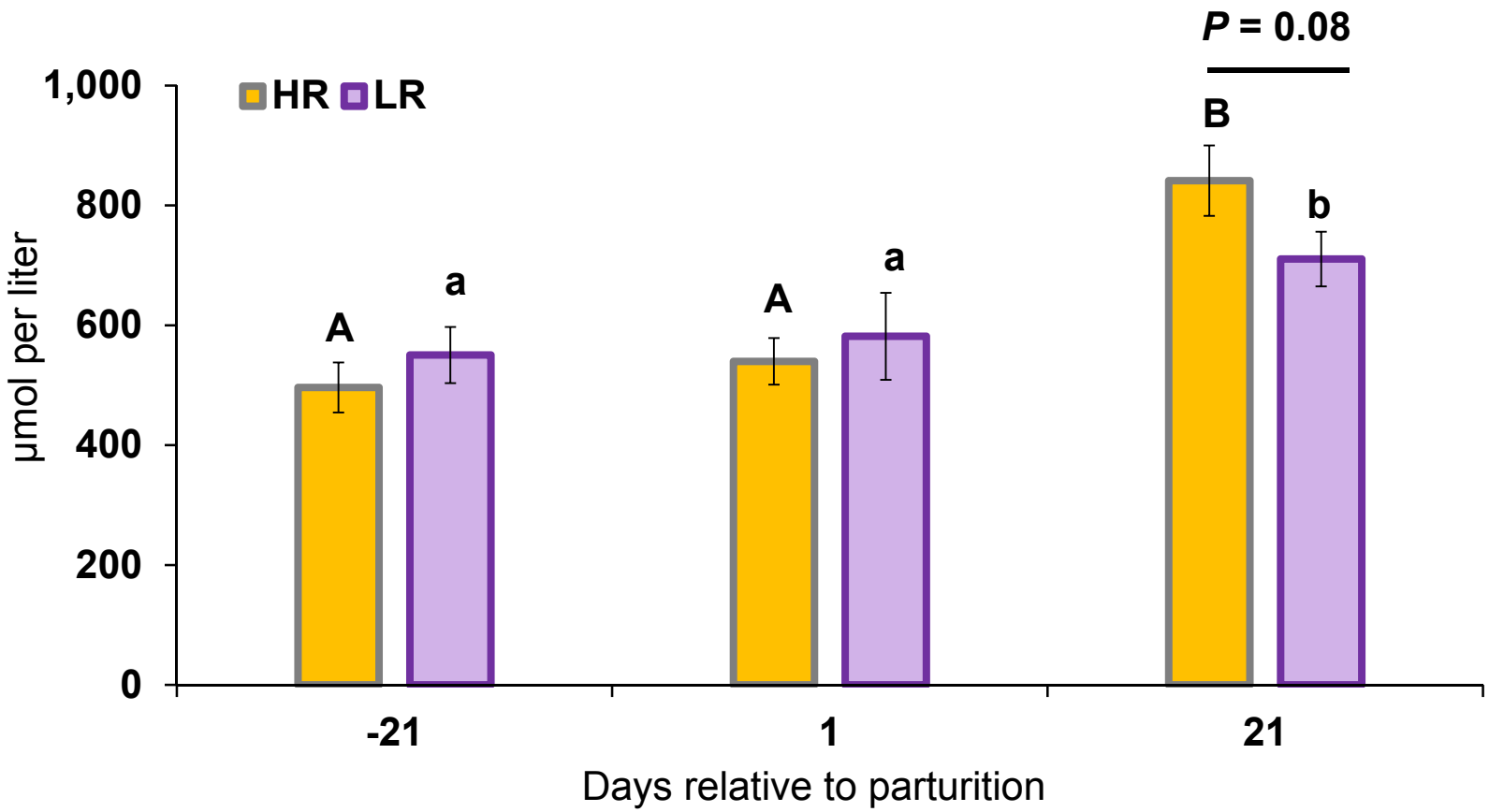
- Tissue homogenization
- RNA extraction and purification
- cDNA synthesis
- mRNA quantification by qPCR

Statistical analyses

- SPSS 21.0 (SPSS Inc., Chicago, IL)
- Mann-Whitney test to compare both diet groups (and both tissues)
- Wilcoxon test to compare the sampling dates with following Bonferroni correction
- $P < 0.05$

Results I

Acetate (C2) in serum

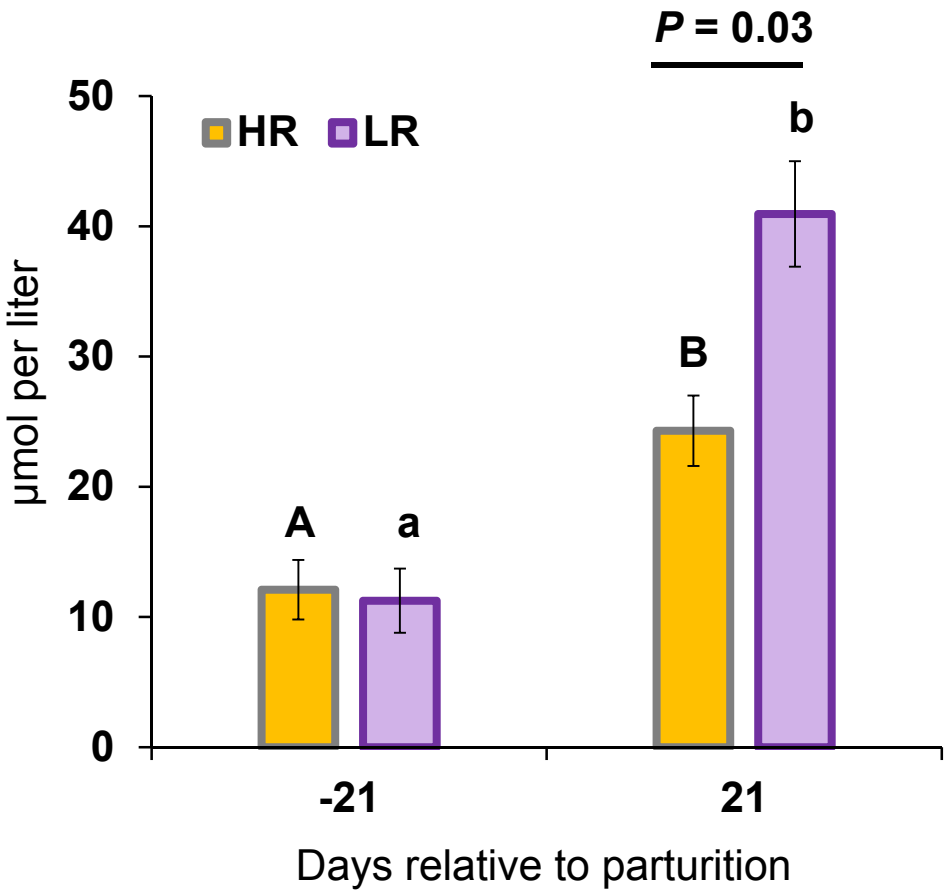


Different letters indicate differences between the sampling dates ($P < 0.05$).

Means ± SEM

Results II

Propionate (C3) in serum

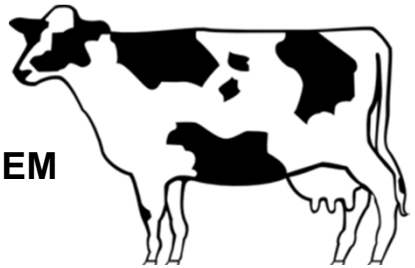


Butyrate (C4) in serum

- No diet effect
- No time effect

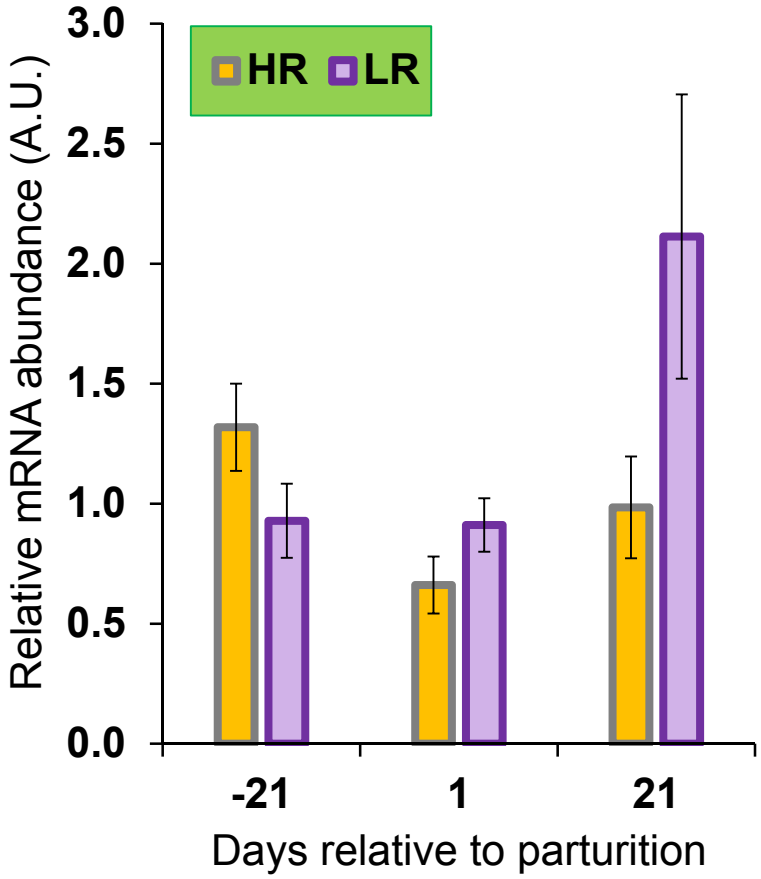
- Mean ± SEM:
  24 ± 4 µmol per liter

Different letters indicate differences between the sampling dates ($P < 0.05$).**Means ± SEM**

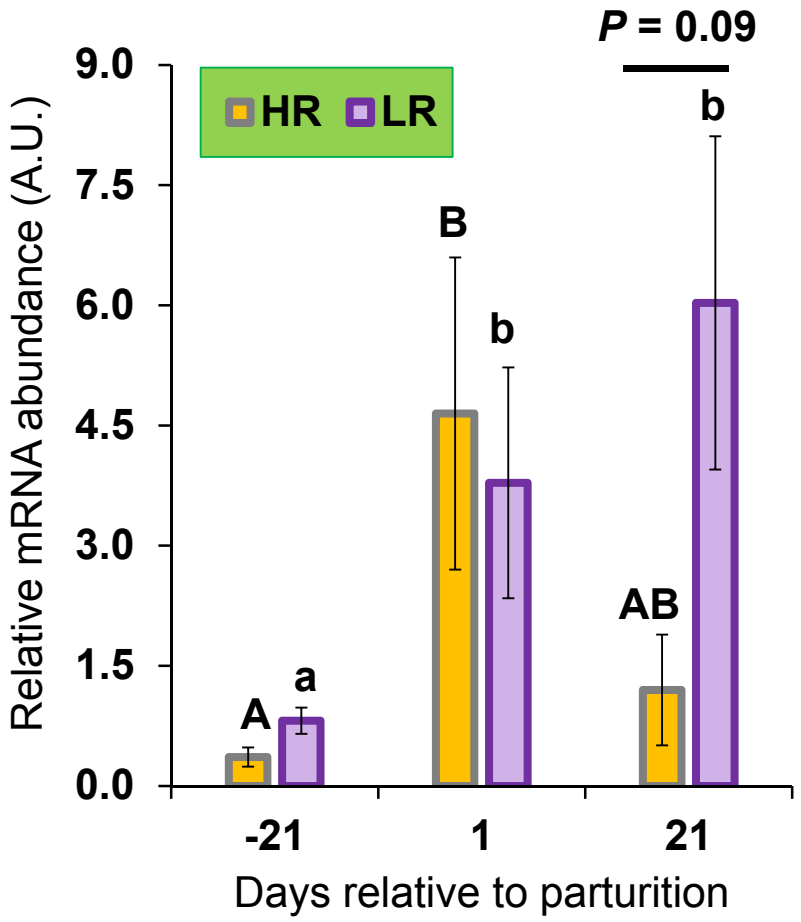


Results III

FFAR2 expression in SCAT



FFAR3 expression in SCAT

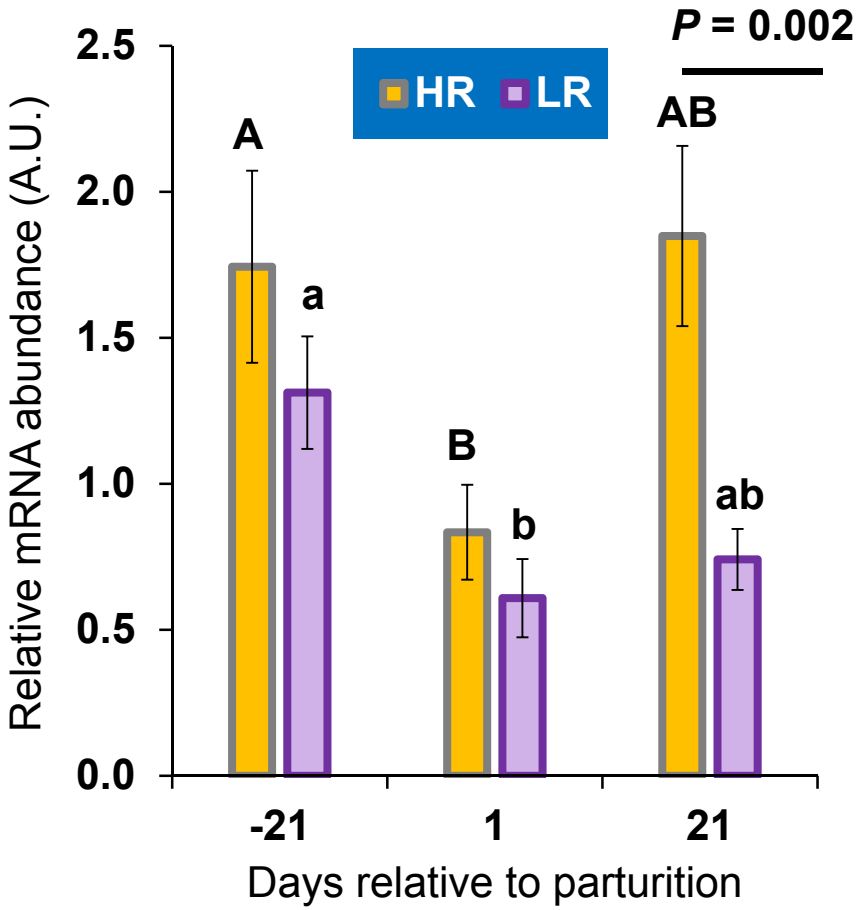


Different letters indicate differences between the sampling dates ($P < 0.05$).

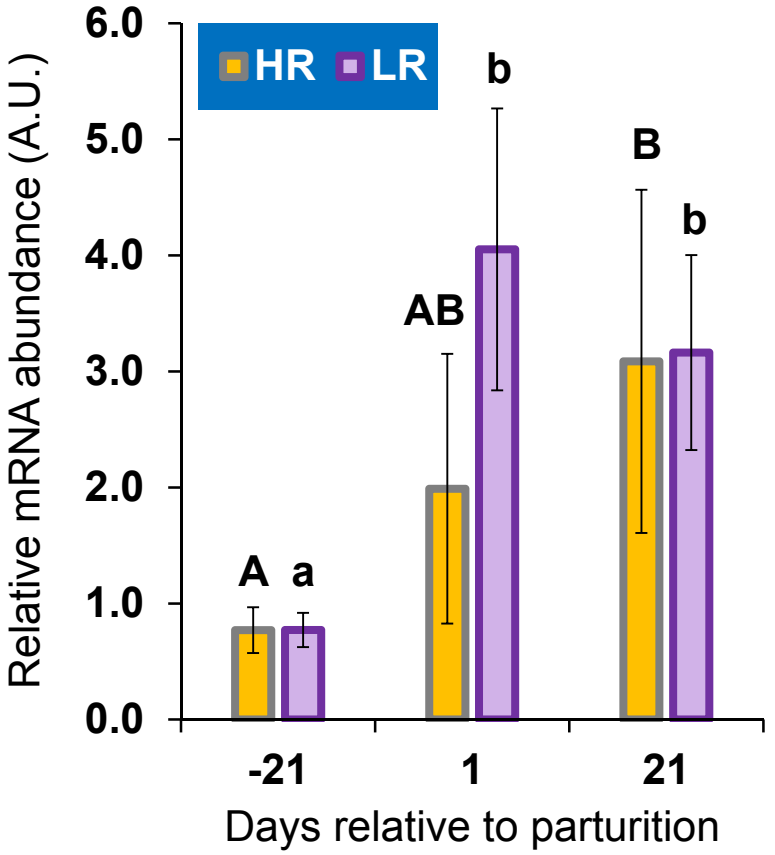
Means ± SEM

Results IV

FFAR2 expression in RPAT



FFAR3 expression in RPAT



Different letters indicate differences between the sampling dates ($P < 0.05$).

Means ± SEM

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Results V

mRNA abundance in SCAT *vs.* RPAT

FFAR2: no difference between both tissues
FFAR3: higher in **RPAT** at d-21 compared to **SCAT**

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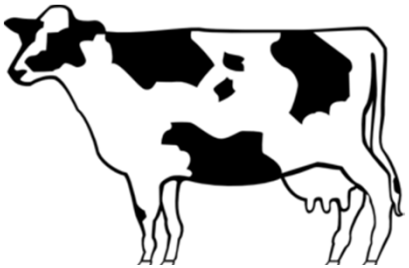
Discussion & Conclusion I

- Higher circulating propionate concentrations due to LR diet
- FFAR2 expression in **RPAT**: inverse regulated to propionate concentrations in the circulation



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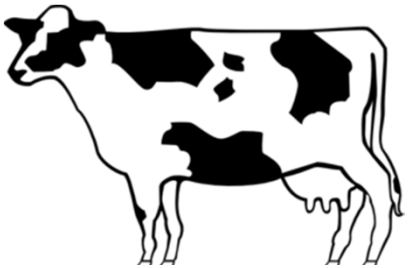
Discussion & Conclusion II

- Down-regulation of lipolysis rate in **RPAT** of HR animals
  - counteract an extensive mobilisation of this tissue?
  - Strictly regulated in a more NEB?

- Butyrate concentrations could stimulate FFAR2 (Hudson et al., 2012)



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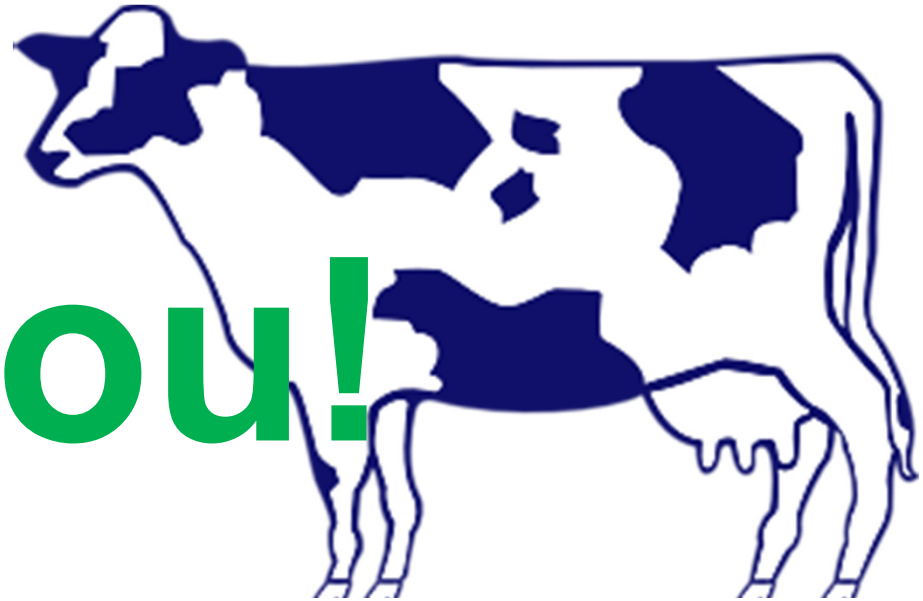
Acknowledgements

- Inga Hofs, Isabella Israel, Birgit Mielenz and Marlies Althaus
- H. Wilhelm Schaumann Foundation
- Theodor-Brinkmann-Graduate School

Thank you!



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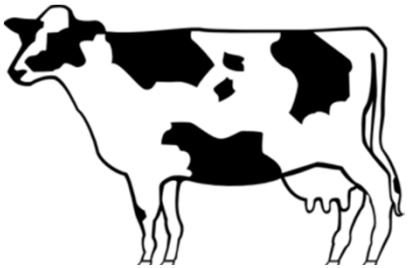
- Potency of FFAR2/3 for SCFA (Hudson et al., 2012)

	Human	**Bovine**
FFAR2	C3 = C2 > C1 = C4 > C5	**C6 > C5 > C4 = C7 > C3 > C2**
FFAR3	C5 = C7 > C3 = C6 = C4 > C2	**C5 = C6 > C3 = C7 > C4 > C2**



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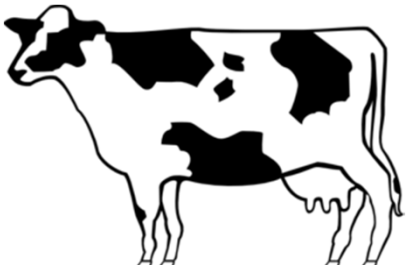
- Reference genes

SCAT	RPAT
• Low density lipoprotein receptor-related protein 10	• Low density lipoprotein receptor-related protein 10
• RNA Polymerase II	• RNA Polymerase II
• Emerin	• Emerin
	• Marvel domain containing 1



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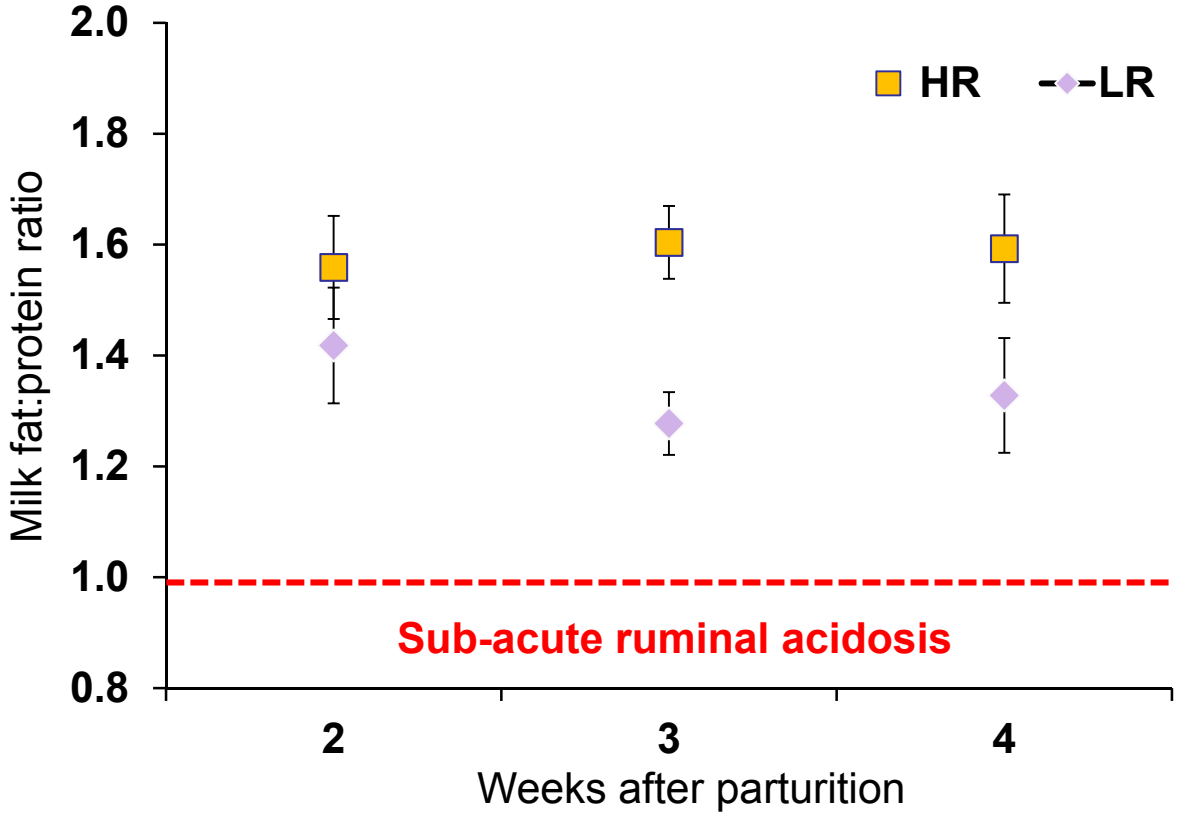
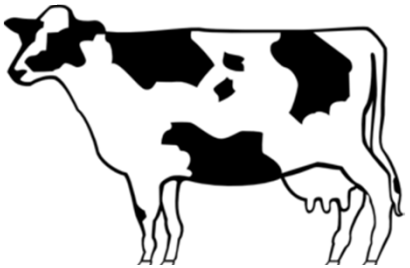
- Milk fat:protein ratio





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- Nutrient, fiber, and energy content of the feed ingredients as well as intended percentages of silage and concentrate in the diets fed to cows postpartum

	Feeding component			
Item	**Corn silage**	**Grass silage**	**HR**	**LR**
DM (g/kg)	368	289	883	885
Ash (g/kg of DM)	37	138	66	53
CP (g/kg of DM)	80	159	210	218
EE (g/kg of DM)	32	40	27	29
ADF (g/kg of DM)	223	311	53	52
NDF (g/kg of DM)	424	514	163	146
ME (MJ/kg of DM)	10.7	10.8	12.9	13.1
NE_L(MJ/kg of DM)	6.4	6.5	8.2	8.3

The HR diet comprised 42% and 28% and the LR diet 24% corn silage and 16% grass silage, respectively.

[1]EE = ether extract (crude fat); HR = high rouhage; LR = low rouhage.

(Locher et al., 2011)

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- Composition of the concentrate fed to the cows

Item	Feeding component in %
Wheat grain	50
Maize	21
Soybean meal	27
Soybean oil	1
Vitamin/mineral premix	1

[1]Per kg mineral feed: 140 g Ca; 120 g Na; 70 g P; 40 g Mg; 6 g Zn; 5.4 g Mn; 1 g Cu; 100 mg I; 40 mg Se; 5 mg Co; 1 000 000 IU vitamin A; 100 000 IU vitamin D3; 1500 mg vitamin E

The concentrates mostly consists of wheat, soybean meal, corn and a mineral and vitamin premix.

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- Sampling of the **SCAT**
  - 1.5-cm skin incision was made in the region of the tailhead on alternate sites to obtain SCAT
- Sampling of the **RPAT**
  - Skin incision (about 3-cm long) was made in the angle between the lumbar transversal processus (about 5 cm ventrally) and the iliac bone (about 5 cm cranially), muscles were dissected reaching the peritoneum, and adipose tissue biopsies were taken directly above the peritoneum
  - Biopsies of RPAT were obtained each time alternating from the left and right flank
  - Skin incisions were closed with U-stitches

(Locher et al., 2011)