

Introduction

- Negative energy balance (NEB) in dairy cows is a critical period that may lead to excessive lipid mobilization and fatty liver (Drackley, 1999).
- Mitochondrial dysfunction is involved in fatty liver pathogenesis (Day and James, 1998).
- There are several post-translation modifications that could explain mitochondrial dysfunction, however, lysine acetylation (AcK) has been shown to be relevant in fatty liver pathology (Kendrick et al., 2011).
- Acetylation may inhibit pathways such as β -oxidation, Krebs cycle and ketogenesis, among others (Anderson and Hirschey, 2012).

Objetive

Our objective was to assess negative energy balance markers and study AcK levels in mitochondrial proteins in liver biopsies.

Materials and methods

Animals and treatments

Twenty-four Holstein-Friesian multiparous cows (spring calving, 664±65 kg BW and 3.0±0.4 BCS) were assigned to a non-grazing (G0) or grazing treatment (G1) in a randomized block design:

- G0:** 100% total mixed ration (TMR) offered *ad libitum* from 0 to 180 days postpartum (DPP).
- G1:** From 0 to 113 DPP cows grazed *Festuca arundinacea* (30 kg DM.cow⁻¹.d⁻¹ in two 7-h sessions) and were offered 5.4 kg DM of a commercial concentrate. Due to heat stress conditions, grazing was reduced to one pm session *Mediticago sativa* (20 kg DM.cow⁻¹.d⁻¹) and cows were offered 50% of G0 offer.

After 180 DPP both groups grazed *Festuca arundinacea* (20 kg DM.cow⁻¹.d⁻¹) and were offered 50% of G0 offer.

Sample collection

Liver biopsies and plasma samples were collected during 35 and 250 DPP, representative of negative and positive energy balance.

NEB markers

Plasma metabolites were determined using commercial kits for non-esterified fatty acid and β -hydroxybutyrate quantification. For **liver triglyceride** determination, lipids were extracted from homogenates and separated using thin layer chromatography. An internal standard was added for quantification.

Mitochondrial respiration

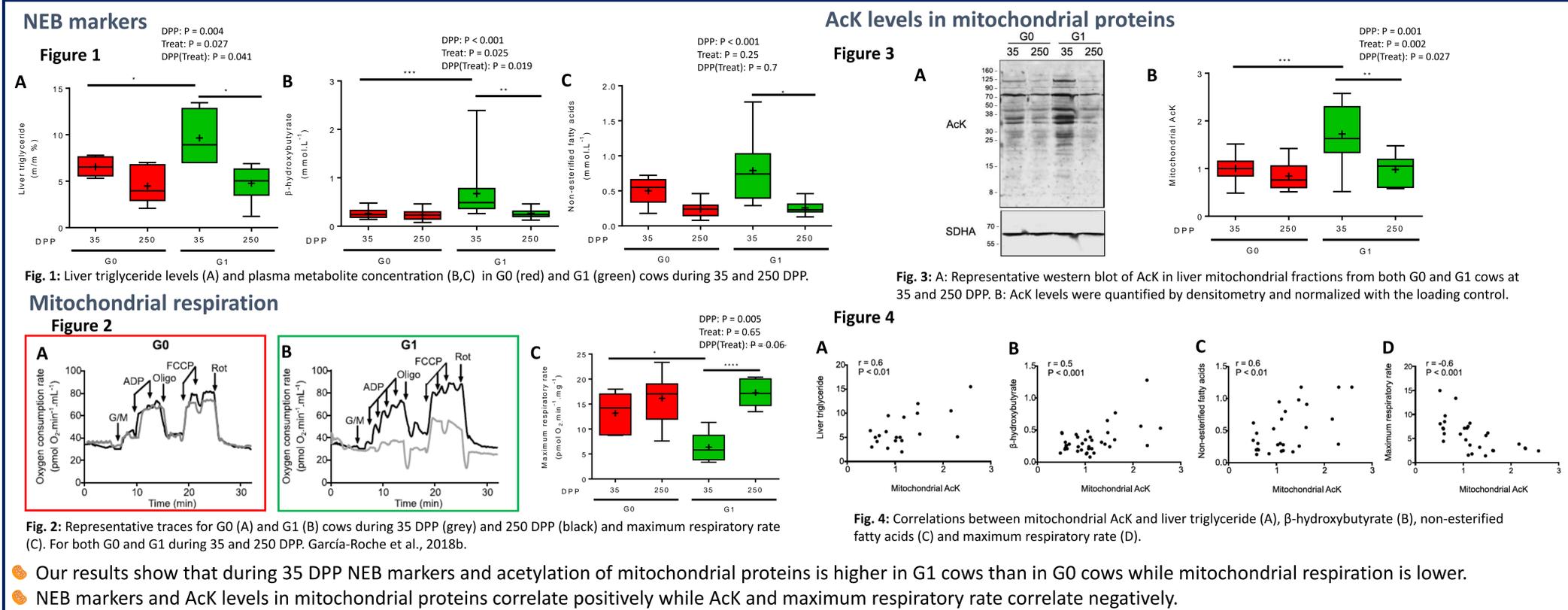
Mitochondrial respiration was studied in cryopreserved liver biopsies using an Oroboros 2k High Resolution Respirometer and maximum respiratory rate was calculated as described in García-Roche et al., 2018a.

AcK levels

To study **AcK levels in mitochondrial proteins**, subcellular fractionation was performed and mitochondrial fractions were resolved in an SDS/PAGE gel and western blots were performed using antibodies against acetylated lysine and loading controls for quantification.

Data was analyzed as repeated measuring using the MIXED procedure that included treatment, DPP and their interaction (SAS Academic Edition).

Results



Conclusions

- Acetylation of mitochondrial proteins occurs during early lactation negative energy balance in cows in the grazing system.
- Acetylation may be responsible for impaired mitochondrial respiration in cows in the grazing system.

References

- Anderson KA and Hirschey MD. Mitochondrial protein acetylation regulates metabolism. *Essays Biochem.* 2012;52: 23–35.
- Day CP and James OFW. Steatohepatitis: A tale of two "Hits"? *Gastroenterology.* 1998;114: 842–845.
- Drackley JK. Biology of Dairy Cows During the Transition Period: the Final Frontier? *J Dairy Sci.* 1999;82: 2259–2273.
- García-Roche M et al. Respiratory analysis of coupled mitochondria in cryopreserved liver biopsies. *Redox Biol.* 018;17: 207–212.
- García-Roche M et al. Mitochondrial function of cryopreserved liver biopsies during early and late lactation of dairy cows. *ADSA Annual Meeting.* Knoxville, TN, USA; 2018.
- Kendrick AA et al. Fatty liver is associated with reduced SIRT3 activity and mitochondrial protein hyperacetylation. *Biochem J.* 2011;433: 505–14.

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