

## B-CAROTENE SUPPLEMENTATION TO NON-LACTATING DAIRY COWS CAN RESTORE B-CAROTENE AVAILABILITY IN THE FOLLICULAR ENVIRONMENT UNDER NEGATIVE ENERGY BALANCE CONDITIONS

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**Implications** Daily  $\beta$ -carotene supplementation in dairy cows in negative energy balance can lower oxidative stress to levels similar to not supplemented healthy cows and can significantly increase the availability of  $\beta$ -carotene in plasma and follicular fluid, the micro-environment of the growing and maturing oocyte.

**Introduction** High energy demands during lactation result in a negative energy balance (NEB), which is typically associated with elevated serum non-esterified fatty acid (NEFA) concentrations. These elevated NEFA concentrations are reflected in the micro-environment of the maturing oocyte, the follicular fluid, and have been associated with increased oxidative stress (OS). We previously showed that bovine oocytes, exposed to elevated NEFA concentrations, have a reduced developmental competence leading to a deviating embryo physiology. Gene expression and functional assays pointed to pathways related to oxidative metabolism, REDOX status and OS in NEFA exposed oocytes, cumulus cells and subsequent blastocysts (Van Hoeck *et al.*, 2013). Therefore, strategic antioxidant supplementation such as  $\beta$ -carotene (bC) can be a promising solution. However, very little is known about the availability of bC in the follicular fluid (FF) and how oral supplementation may affect this. Furthermore, it can be hypothesized that a NEB status may negatively affect this availability in the follicular fluid due to a higher systemic use. To investigate this we aimed to 1) determine the effect of the NEB on bC concentrations in serum and follicular fluid, and 2) how this effect could be altered by dietary bC supplementation. In this first study, a NEB was induced in non-lactating dairy cows by a reduction in dry matter intake.

**Material and methods** After 6 weeks of acclimatisation, all 6 non-lactating Holstein Friesian cows were subjected to the same order of 4 consecutive dietary treatments, 28 days each: 1) 1,2x maintenance (M) (= positive EB, PEB-bC), 2) 1,2xM with daily 2000mg bC similar to the level of bC intake at grazing (Rovimix 10% bC, DSM) (=PEB+bC), 3) 0,6xM with 2000mg bC (=NEB+bC) followed by a 6 week acclimatisation period and 4) 0,6xM (=NEB-bC). Rations consisted of hay, straw and concentrates. Weight was monitored weekly. In the second half of each dietary period, cows were synchronised by means of a progesterone releasing intravaginal device (PRID, 1,55g P<sub>4</sub>, CEVA) for 7 days and a PG F2 $\alpha$  injection on day 6 after PRID insertion. Together with blood sampling, FF of the dominant follicle was collected by transvaginal follicle aspiration 2 days after PRID removal. Blood and FF samples were analysed for bC, NEFAs, estradiol (E<sub>2</sub>) and progesterone (P<sub>4</sub>). Additionally, serum total antioxidant status (TAS) was determined as well as intra-erythrocyte glutathione (GSH) concentrations. Data were statistically analysed using a paired samples T-test, analysing the effect of NEB or bC supplementation (IBM SPSS Statistics 20). Data are presented as means  $\pm$  standard deviation.

**Results** All cows on average lost  $11.44 \pm 1.80\%$  of their body weight during both energy restriction (0,6xM) periods. Fasting resulted in a significant increase in serum NEFA concentrations ( $0.21 \pm 0.11\text{mM}$  vs.  $0.36 \pm 0.18\text{mM}$ ). All follicles punctured displayed a E<sub>2</sub>/P<sub>4</sub> ratio  $> 1$  ( $18,20 \pm 10,95$  on average). Overall, bC concentrations in FF correlated well with serum concentrations ( $R=0.645$ ;  $P=0.001$ ). NEB significantly reduced bC in serum ( $1.02 \pm 0.91\mu\text{g/ml}$  vs.  $0.44 \pm 0.18\mu\text{g/ml}$ ;  $P=0.046$ ) and FF ( $0.21 \pm 0.12\mu\text{g/ml}$  vs.  $0.05 \pm 0.02\mu\text{g/ml}$ ;  $P=0.02$ ). However, bC supplementation drastically increased bC availability in serum and in FF in NEB (X8 in serum  $P<0.001$  and X10 in FF  $P=0.001$ ) as well as in PEB (X3 in serum  $P<0.001$  and X2 in FF  $P=0.034$ ). Remarkably, in bC supplemented animals (PEB+bC vs. NEB+bC), no negative effect of fasting (NEB) in bC levels in serum and FF could be detected ( $P>0.05$ ). Fasting significantly reduced GSH content ( $657.21 \pm 121.11\mu\text{g/ml}$  vs.  $466.64 \pm 122.26\mu\text{g/ml}$ ;  $P=0.003$ ) as well as TAS ( $1.21 \pm 0.09\text{mM}$  vs.  $1.14 \pm 0.07\text{mM}$ ;  $P=0.027$ ), but bC supplementation to cows in NEB could restore these GSH concentrations in red blood cells ( $591.47 \pm 104.36\mu\text{g/ml}$  vs. PEB-bC conc;  $P>0.05$ ). Only in PEB the diameter of the dominant follicle was significantly larger when supplemented with bC ( $20.40 \pm 5.12\text{mm}$  vs.  $13.60 \pm 2.47\text{mm}$  in PEB-bC;  $13.15 \pm 1.80\text{mm}$  in NEB+bC and  $14.08 \pm 1.07\text{mm}$  in NEB-bC;  $P<0.05$ ), but bC supplementation did not affect serum and FF E<sub>2</sub> concentrations.

**Conclusion** Fasting associated NEB has a negative effect on bC concentrations in serum and in the oocyte's micro-environment, leading to higher OS levels. bC supplementation was able to significantly increase the bC availability in the FF irrespective of the energy status and could restore the oxidative status as in PEB conditions. Further research will investigate the validity of these intriguing findings in lactating dairy cows early *post partum*.

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**References** Van Hoeck, V., Leroy, J.L.M.R., Arias Alvarez, M., Rizos, D., Gutierrez-Adan, A., Schnorbusch, K., Bols, P.E.J., Leese, H.J. and Sturmey, R.G. 2013. *Reproduction*. 145,33-44