

# Book of Abstracts

of the 75<sup>th</sup> Annual Meeting  
of the European Federation of Animal Science



**Book of Abstracts No. 34 (2024)**  
**Florence, Italy**  
**1-5 September, 2024**

Undernutrition and hydroxytyrosol supplementation during the final third of gestation modulate immune response and redox balance in newborn beef calves

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Hydroxytyrosol (HT) is a polyphenol present in olive leaves that can enhance antioxidant capacity and may mitigate the negative impact of prepartum maternal nutrient restriction in beef cattle. This study assessed the effects of maternal undernutrition (100% vs. 60% of nutritional requirements) and HT supplementation during the last third of pregnancy (Control vs. HT, 178±36 mg/kg of feed) on the whole blood gene expression of key markers related to redox balance and immune response in their offspring during the first week of life. Blood samples were collected from 43 calves in Tempus blood RNA tubes (Applied Biosystems) at week 1 after birth. Gene expression of SOD1, CAT, GPX1, NRF2, NFKB, TLR4, and TNF $\alpha$  was analysed using qPCR. A general linear model including fixed effects (nutrition and HT supplementation) and their interactions was used to compare gene expression with JMP software. A higher CAT and GPX1 expression were observed in calves born from HT-cows compared to those born to Control-cows ( $P < 0.05$ ). Meanwhile, higher TNF $\alpha$  expression was observed in the calves born to 60%-cows compared to 100%-cows ( $P < 0.05$ ). Our results suggest that maternal HT supplementation enhances antioxidant defences responsible for the control of intracellular H<sub>2</sub>O<sub>2</sub> levels of the newborn offspring. In addition, maternal undernutrition increased proinflammatory cytokines and therefore might contribute to immune disturbances postnatally. Overall, undernutrition and HT supplementation modulated immune response and redox balance in newborn beef calves. Funded by PID2020-113617RR-C21/22 FETALNUT.

Interplay between pre-partum nutrition and hydroxytyrosol supplementation on calf behavior

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Pre-partum maternal diet restriction and polyphenol supplementation may have carry-over effects on neonatal calf behavior. The role of dietary restriction (100 vs. 60% of the cow nutrient requirements during the last 3 months pre-partum) and hydroxytyrosol (HT) addition (0 vs. 178±36 mg/kg of feed) on beef calves' behavior was evaluated. Twenty-nine ( $\pm 7$ )-day old calves ( $n=48$ ) from four pre-partum maternal dietary treatments (100%-CTR, 100%-HT, 60%-CTR, 60%-HT) were housed in individual pens, with visual and olfactory contact, between suckling periods (08:00-14:00h). Each pen was provided with a warm milk replacer and water bucket with nipple. Observations were performed in three 1-h sessions (9:00-10:00; 10:30-11:30; 12:00-13:00). Calf posture and behavior was recorded by scan sampling every 2 min. The data were analyzed with non-parametric Wilcoxon tests; means were separated with a t-Student test. Calves from 60%-HT cows remained lying for shorter than calves from 100%-HT (33.0 vs. 42.4±2.47 min/h,  $p < 0.05$ ). This led to lower inactive time in 60%-HT than in 60%-CTR (29.7 vs. 37.6±2.41 min/h,  $p < 0.05$ ), more time spent on oral or olfactory manipulation of the pen fixtures (8.2 vs. 5.1±0.97 min/h,  $p < 0.05$ ) and higher number of vocalizations per session (1.5 vs. 0.3±0.37,  $p < 0.05$ ). The 60%-HT calves explored for longer the milk and water bucket than the 100%-HT calves (2.1 vs. 0.6±0.41 min/h,  $p < 0.05$ ), but calves did not perform any nutritive activity therein. In conclusion, maternal nutrient restriction, together with HT supplementation, enhanced calf response to environmental stimuli. Funded by PID2020-113617RR-C21.