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The objective of this study was to determine the effects of the number of double bonds of dietary lipids on expression of genes related to lipid metabolism in milk somatic cells (MSC) in dairy cows. For this, 15 dairy cows (2ndand 3th lactation, 42 L milk/day, 195 ± 35 d in milk) were randomly assigned to a control diet (CD) containing no added lipid (n = 5 cows); and diets supplemented with soybean oil (SO) (n = 5 cows; unrefined SO; 3% DM) or fish oil (FO) (n = 5 cows; manufactured from salmon oil; 3% DM); cows were fed for 63 d. On d 21, 42 and 63, MSC were obtained 4 h after first milking from all cows. Milk production, milk fat, and milk protein were not affected by treatments. Relative abundance of mRNA from 17 genes involved in lipid metabolic functions: fatty acid (FA) importation into cells, FA synthesis and desaturation, acetate and FA activation, FA intra-cellular transport, triacylglycerol synthesis, and lipid droplet formation regulation. Products of transcription (mRNA) from MSC were obtained by qPCR. The mRNA from cows fed CD on d 42 and 63 were compared with mRNA relative abundance at d 21 to evaluate fold-changes. Those genes from CD group without changes over the time (*ACACA*, *PPARGC1*, *LPIN1*, *INSIG1*, *DGAT1* and *FABP3*) were selected to analyze effects of SO and FO. The relative abundance software tool (REST) was used to analyze qPCR results. This software incorporates PCR efficiency correction and reference gene normalization. Compared with CD, SO downregulated ($P < 0.01$) *ACACA*, *INSIG1*, and *DGAT1*. Compared with CD, FO downregulated ($P < 0.01$) *ACACA*, *PPARGC1*, *LPIN1* and *FABP3*. Overall, data indicated that there are differential transcriptomic effects of lipid-related genes in MSC and that will depend on the number of double bonds of dietary lipids.

Key Words: oils, mammary gland, gene expression

256 Differential fates for gluconeogenic precursors in diverging Holstein genotypes. M. Garcia-Roche*[1,2], G. Cañibe[1], D. Talmón[3], A. Mendoza[1], C. Quijano[2], A. Cassina[2], and M. Carriquiry[1], [1]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay,* [2]*Departamento de Bioquímica, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay,* [3]*INIA La Estanzuela, Colonia, Uruguay.*

The aim of the study was to assess the effect of diverging Holstein genotypes in hepatic mitochondrial function and gene expression in a grazing system. Multiparous cows of New Zealand (NZH, n = 10) and North American origin (NAH, n = 10) (512 ± 19 vs. 563 ± 29 kg BW, 3.1 ± 0.1 body condition score (BCS), fall calving) were evaluated during the Spring of 3 consecutive years (2017, 2018 and 2019) to ensure maximum pasture allowance. Cows grazed a mixed pasture of *Medicago sativa* and *Dactylis glomerata* (15.4 ± 1.0 kg DM/d ha in 2 grazing sessions and were supplemented with 6.9 ± 0.5 DM/d of concentrate). Liver biopsies were collected 180 ± 17 d postpartum (DPP) and mitochondrial function was assessed measuring oxygen consumption rates using complex I (glutamate/malate: G/M) and complex II (succinate: succ) substrates; gene expression of pyruvate carboxylase (*PC*) and phosphoenolpyruvate carboxykinase (*PCK*) was measured by real-time PCR. Data were analyzed using a repeated model that included the genotype as the fixed effect and year and cow as random effects. In average, no differences were observed in solid-corrected milk yield (25.7 vs. 26.4 ± 1.2; $P = 0.38$) or BCS did not differ between genotypes NZH vs. NAH (2.6 ± 0.09, $P = 0.18$). The maximum respiratory capacity - related to the potential reserve capacity that could be used in cases of very high demands or insult - was higher for NZH than NAH (14.3 vs. 11.5 ± 1.9, for G/M and 50.2 vs. 26.7 ± 9.0 pmolO_2/min/mg for succ respectively, $P < 0.05$). Similarly, oligomycin-sensitive respiration - representing ATP-linked respiration - was higher for NZH than NAH (6.1 vs. 4.3 ± 1.9, for G/M and 9.3 vs. 5.2 ± 1.1 pmolO_2/min/mg for succ respectively, $P < 0.05$). Both parameters were 3-fold greater for succ than G/M-driven respiration. Both, hepatic *PC* and *PCK* mRNA were higher for NAH than NZH cows (1.4 vs. 0.6 ± 0.15 and 1.79 vs. 0.74 ± 0.24, $P < 0.05$). Negative correlations were found between succinate-driven respiratory parameters and expression of gluconeogenic genes ($r \geq -0.55$, $P < 0.05$). These results indicate that gluconeogenic precursors may have different metabolic fates: ATP synthesis vs. gluconeogenesis in NZH and NAH cows, respectively.

Key Words: grazing, mitochondria

419 Characterization of fatty acid esters of hydroxy fatty acids, a novel class of bioactive lipids, in milk fat of cows supplemented with stearic and palmitic acid. C. Matamoros*[1], B. Harsch[2], I. Salfer[3], R. Shepardson[1], G. Shearer[2], and K. Harvatine[1], [1]*Department of Animal Science, The Pennsylvania State University, University Park, PA,* [2]*Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA,* [3]*Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Fatty acid (FA) esters of hydroxy FA (FAHFA) are classified as estolides and have been characterized as potential anti-inflammatory and antidiabetic bioactive FA. FAHFA are classified into families according to the FA and hydroxy FA makeup [i.e., palmitic acid (PA) esters of hydroxystearic acid are PAHSA] and within each family multiple regioisomers exist depending on the location of the hydroxy group in the hydroxy FA (i.e., 9-PAHSA). The objective of this study was to characterize 5 FAHFA families made of PA, stearic (SA), palmitoleic (PO), or oleic (OA) acid (PAHSA, SAHSA, POHSA, PAHPA, OAHSA) in a retrospective analysis of milk fat samples from a fat supplement experiment. Briefly, 12 multiparous Holstein cows were arranged in a 4 × 4 Latin square. Treatments were a no supplement control (CON) or a 2% DM inclusion of either a highly enriched PA or SA or a combination of both (PA/SA). FAHFA families were detected by LC-TQMS using multiple reaction monitoring and quantified using a standard curve for each FAHFA regioisomer. Data were analyzed with a mixed model with cow and period as random effects and treatment as a fixed effect and a regression analysis was done between FAHFA and production traits and plasma metabolites. PA increased 9- and 12-PAHPA concentration 2.1- and 5.5-fold ($P \leq 0.01$ for both) and tended to decrease 10-PAHSA ($P = 0.07$), when compared with CON. There was no effect of treatment on 9-SAHSA, 9-OAHSA, 9-POHSA, 9-PAHSA, 12-PAHSA. Interestingly, the combination of PA/SA abolished the effect of PA on PAHPA levels. Notably, 12-PAHPA was positively related to milk 16 carbon FA ($R2 = 0.12$, $P = 0.02$) and plasma glucose ($R2 = 0.10$, $P = 0.04$) and negatively related to milk de novo FA ($R2 = 0.10$, $P = 0.03$). Additionally, 10-PAHSA was positively related to plasma nonesterified FA ($R2 = 0.26$, $P < 0.001$). The results, to the best of our knowledge, are the first characterization of FAHFA in bovine milk, suggest a role of dietary FA on FAHFA concentrations, and show that some FAHFA are correlated with production traits and plasma metabolites in Holstein dairy cows.

Key Words: fatty acid esters of hydroxy fatty acids (FAHFA), functional lipids, milk fat