lated for each group to meet the nutrient requirement of NRC (2001). Diets included corn silage and alfalfa pasture and the difference was given by the supplementation offered: flaxseed (1.2 kg DM/d, 273 g/d of n-3 polyunsaturated fatty acid (n-3 PUFA), FLAX) and cottonseed (3 kg DM/d, COTT). Regarding reproductive management, ovulations were synchronized by using an Ovsynch protocol preceded by a G6G presynchronization (PGF: d 0; GnRH: d 2, 8; PGF: d 15; GnRH d 17). Cows were not inseminated. Color Doppler ultrasound with a linear transductor (ESAOTE MyLab OneVET) was used to measure LA and LBFA on d 7 after ovulation (D7) (color mode: F:5 MHz, G:28%; PRF:1.0 MHz). Three different Doppler images of each clip were used to calculate LA (mm^2) and LBFA (mm^2). Images were processed with the computer software Image J (version 1.52p; National Institutes of Health; Maryland, USA) to quantify the pixels off-line. In addition, P4 concentration was measured at D7. We found that LBFA was 2.2 times higher in FLAX than in COTT group (23.60 ± 15.07 mm^2 vs. 13.25 ± 10.74 mm^2, $P = 0.1204$) respectively. Conversely, we did not find any difference in LA (295.97 ± 116.87 mm^2 vs. 298. 94 ± 53.34 mm^2, $P = 0.9487$) for FLAX and COTT, respectively) or in P4 concentration (3.43 ± 2.39 vs. 3.58 ± 2.02 ng/dl; $P = 0.8880$) for FLAX vs. COTT, respectively. We concluded that flaxseed supplementation increases LBFA probably through the stimulation of inflammatory mediator synthesis and/or luteal angiogenesis. This may be due to higher inclusion of n-3 PUFA in FLAX diet.

Key Words: PUFAs, luteal blood flow area, luteal area

P381 Pyruvate carboxylase knockdown alters lactate oxidation in Madin-Darby bovine kidney cells. L. M. Beckett*, J. Laguna, S. Hilger, and S. S. Donkin, *Purdue University, West Lafayette, IN.*

Pyruvate carboxylase (PC) is a key enzyme at a critical control point between the tricarboxylic acid cycle and gluconeogenesis in the liver of dairy cattle, and catalyzes the conversion of pyruvate to oxaloacetate. Determination for the role of PC in energy metabolism in bovine is hampered by the lack of a metabolic model that precisely controls PC expression. The objective of this study was to develop a model to precisely reduce PC expression and to determine the effect on carbon flux. We hypothesized that a targeted reduction in PC expression would reduce lactate oxidation to CO_2, but propionate oxidation to CO_2 would not be affected. Bovine MDBK cells were cultured and transduced with lentiviral human PC short hairpin RNA (shRNA). Cells containing the PC shRNA knockdown (PCshRNA) or scrambled RNA (SRM) were selected using puromycin resistance. Based on Western blotting, PC abundance was reduced by approximately 50%. Wild type MDBK (wtMDBK) cells, SRM cells, and PCshRNA (n = 3) were grown to 90% confluency in 35 mm dishes. Cells were incubated with either 2.0 mM U-[^{14}C] lactate or 2.0 mM 2-[^{14}C] propionate for 3 h. Incubations were terminated, $^{14}CO_2$ was trapped, and samples analyzed by liquid scintillation counting. Rates of metabolism (nmol of substrate converted to $CO_2 \cdot plate^{-1} \cdot 3\ h^{-1}$) were analyzed using the PROC Mixed procedure in SAS and Tukey means separation test with significance at $P < 0.05$. The rate of lactate oxidation to CO_2 was significantly reduced for PCshRNA in comparison to wtMDBK control (8.2 vs 12.4, respectively, $P = 0.007$). There were no differences between SRM and PCshRNA cells ($P = 0.70$). There were no significant effects of PC knockdown on propionate metabolism to CO_2. The data indicates that PC knockdown specifically reduces metabolism of lactate to CO_2. Knockdown of PC in MDBK cells appears to be a useful model in characterizing the impact of precise changes in PC gene expression on lactate metabolism in bovine. This bovine model appears to have utility in understanding shifts in cellular metabolism that occur when PC expression is uniquely impaired.

Key Words: knockdown, pyruvate carboxylase, bovine

P382 Mitochondrial function during early and late lactation, of Holstein cows under 3 different productive systems. G. Cañibe*[1], M. Garcia-Roche[1], A. Casal[2], A. Jasinsky[2], and M. Carriquiry[1], [1]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay,* [2]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Estación Dr. Mario A. Cassinoni, Universidad de la República, Paysandú, Uruguay.*

The aim of the study was to assess the effect of 3 different productive systems on hepatic mitochondrial function in early and mid-lactation. Multiparous Holstein cows (n = 30), 698 ± 60 kg of body weight, 3.2 ± 0.3 body condition score (BCS) were assigned in a randomized block design to a total mixed ration (TMR) offered in a freestall facility (ad libitum; 29.4 kgDM/d, 40:60 forage to concentrate ratio), or to intensive grazing based systems (mixed pastures, forage allowance of 19.3 kgDM/d supplemented with 14.7 kgDM/d of TMR) in a freestall facility (PFS) or an open-sky paddock with access to water and shade (POS). Liver biopsies were collected and cryopreserved at 34 ± 13 and 171 ± 19 d postpartum (DPP). Mitochondrial function was assessed measuring oxygen consumption rates sustained by complex-I (glutamate/malate; C-I) and complex-II (succinate; C-II) substrates. Data were analyzed as repeated measures with a mixed model that included DPP, treatment and their interaction as fixed effects and block as a random effect. Energy-corrected milk yield was greater ($P < 0.05$) for TMR than PFS cows and intermediate for POS cows (38, 33 and 35 ± 1 kg/d for TMR, PFS and POS, respectively), while BCS increased ($P < 0.01$) from early to mid-lactation for TMR cows and decreased for PFS and POS cows. Oligomycin sensitive respiration –ATP synthesis-linked respiration–for C-I was affected by the treatment and DPP interaction as this increase was observed for TMR and PFS but not for POS cows (0.59 vs. 2.5 ± 0.4; 0.67 vs. 1.8 ± 0.3 and 1.1 vs. 1.5 ± 0.4 $pmolO_2 \cdot min^{-1} \cdot mg^{-1}$ for TMR, PFS and POS respectively, $P < 0.01$). In addition, for C-II, it increased from early to late lactation (2.22 ± 0.42 vs. 3.77 ± 0.55 $pmolO_2 \cdot min^{-1} \cdot mg^{-1}$, $P < 0.05$). In contrast, C-I and C-II non-mitochondrial respiration decreased from early to mid-lactation (3.94 ± 0.31 vs. 0.54 ± 0.11, 4.86 ± 0.39 vs. 0.75 ± 0.15 $pmolO_2 \cdot min^{-1} \cdot mg^{-1}$, for C-I and C-II respectively, $P < 0.05$). Our results confirm ATP synthesis is decreased while non-mitochondrial respiration is increased during early lactation and that plane of nutrition may impact mitochondrial function especially when C-I substrates are used.

Key Words: grazing vs tmr, mitochondrial function

P383 Predicting dry matter intake in dairy cows from ear tag-based estimates of chewing activity. L. M. Campos*[1], V. L. Daley[2], A. G. Morales[1], J. M. Prestegaard[1], and M. D. Hanigan[1], [1]*Dairy Science Department, Virginia Tech, Blacksburg, VA,* [2]*Purina Animal Nutrition Center - Land O'Lakes, Gray Summit, MO.*

Diet formulation is reliant on accurate predictions of dry matter intake (DMI). However, DMI is usually estimated by group despite large animal to animal variation which often results in over- or under- feeding. Tailored individual DIM predictions would allow development of rations that optimize nutrient utilization and animal performance, and minimize nutrient excretion and financial losses. Determining individual animal DMI may be possible using pen DMI supplemented with behavior and