NU-01

Influence of ketosis prevalence during first lactation and subsequently on the cow's performance through its following lactations

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Objectives: Ketosis, defined as hyperketonemia (high levels of beta-hydroxybutyrate (BHB) in blood, milk or urine) during first weeks postpartum, is a common and costly disease. The aim of the present study was to evaluate the impact of first milk test ketosis prevalence on first lactation performance as well as on the subsequent lactations' ketosis prevalence and lactation performance.

Materials and methods: Cow information, test-day production data, and milk BHB concentrations were obtained from the Africor Lugo database (Lugo –Spain), including 22018 animals with first calving occurring during 2015. Information from these animals was obtained until May 2019, including farm name and cow ID (coded), date of birth, date of culling, and for each lactation: calving data, lactation number, total milk, fat and protein yield (kg), 305d corrected milk (305d-milk), fat and protein yield (kg), lactation length (days) and days in milk (DIM) and BHB (µmol/L) content at first test. Energy-corrected milk (ECM, kg/d, Santichi et al., 2016 (JDairySci. 99:9263–9270)), for both total ECM (T-ECM) and 305 days ECM (305d-ECM). Milk samples were analyzed by Fourier-transform infrared (MilkoScan FT6000, Foss Electric, Hillerød, Denmark) and ketosis was determined when milk BHB values were ≥ 0.10 mmol/mL (Viña et al., 2017, JAnimPhysiol An N, 101:835–845).Only cows with DIM at first test <26 days were considered, excluding 9445 animals (Santichi et al., 2016, JDairySci. 99:9263–9270). All statistical analysis were performed using JMP 13.1.0 (SAS).

Results: Retrospective analysis of data from 12573 heifers from 1326 farms and first time calving during 2015 indicated that 80.53% (n=9.220), 56.88% (n= 5.244), 10.51% (n=551) and 0.73% (n=4) of animals reached second (2ndL), third (3rdL), fourth (4thL) and fifth (%thL) lactation respectively during the observational period. Ketosis prevalence was 22.99% (n=2632), 19.91% (n=1836), 23.55% (n=1235), 22.87% (n=126) and 0% (n=0) for animals reaching first, second, third, fourth and fifth lactation respectively. Animals developing ketosis had longer lactation period (353.1 vs 346.9 days, p=0.013), yielded less total milk (9836 vs 10114 kg, p<0.001), total protein (318 vs 333 kg, p<0.001), 305d-milk (7909 vs 8286 kg, p<0.001), T-ECM (10481 vs 10759 kg, p=0.003) and 305d-ECM (8315 vs 8703 kg, p<0.001) and tended to lower total fat yield (384 vs 390 kg, p=0.080) during 1stL.

Compared to non-ketotic cows, positive cows on 1stL had higher odds ratio of developing ketosis during 2nL, 3rdL and 4thL (OR= 1.94, 95%CI: 1.73-2.17; 2.19, 95%CI:1.90-2.; and 2.39, 95%CI:1.54-3.71, respectively). During 2ndL, positive cows at 1stL yielded less milk (11138 vs 11506 kg, p<0.001), protein (363 vs 379 kg, p<0.001), fat (354 vs 372 kg, p<0.001), 305-d milk (9195 vs 9579 kg, p<0.001), T-ECM (11891 vs 12374 kg, p<0.001) and 305d-ECM (9712 vs 10193 kg, p<0.001) regardless those cows developed ketosis during 2nL or not. Ketosis prevalence during 2ndL was higher for those cows developing ketosis during 1stL (28.79 vs 17.26%, for ketotic and healthy cows during 1stL respectively, p<0.001). Multinomial regression model, including 1stL 305d-ECM and ketosis prevalence at 1stL and 2ndL (r^2=0.17, p<0.001), indicated that 2ndL 305d-ECM was impaired in a bigger extent when ketosis occurred in 1stL than in 2ndL (9776^{b}; 10121^{a,b}; 10254^{a}; 10122^{a} kg for ketotic animals in 1stL, 1stL and 2ndL, 2ndL and healthy animals, respectively, p<0.001). Contingency analysis indicated that ketosis prevalence during 3rdL was higher for those animals developing ketosis during 1st and 2nd calving compared with animals developing ketosis during first or second and healthy animals in previous lactations (47.4, 31.6, 31.3 and 17.9% respectively, p<0.001). In the same way, 1stL ketotic cows yielded less total milk (10639.6 vs 11178.7 kg; p<0.001), protein (344.3 vs 366.5 kg; p<0.001), fat (412.8 vs 440.6 kg; p<0.001), 305-d total milk yield (9436.5 vs 10076.7 kg; p<0.001), T-ECM (11303.2 vs 12000.4 kg; p<0.001) and 305d-ECM (9962.6 vs 10759.6 kg; p<0.001) during 3rdL than non-ketotic 1stL cows.

Conclusions: Many DHI organizations offer clients an infrared test to detect milk BHB as a herd surveillance test and it has been recommended to monitor ketosis on a herd level when evaluating nutritional management or preventative medicine strategies in herds (Renauld et al., 2018. JDairySci. 102:1–5). Current results indicate a strong impact of a positive result in the first test of the 1stL on cow's performance, not only on the current but also future lactations.

Keywords: Ketosis, Transition, Management.

NU-02

Evaluating plasma methionine in response to feeding three rumen-protected methionine products

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Objectives: Rumen protected (RP) amino acid products offer the opportunity for precision feeding of limiting amino acids to ruminant animals. The efficacy of such products is dependent on survival in the rumen and availability in the intestines, necessitating estimates of bioavailability. Plasma methionine (Met) is linearly related to intestinal Met absorption, and, thus, can be used as a tool to assess RP-Met bioavailability. However, rates of ruminal passage and absorption may differ among products, so time of blood sampling relative to feeding may be an important consideration.

The primary objective of this work was to compare the plasma methionine levels of cows fed with three different

RP-Met supplements under similar feeding and experimental conditions. This study examined the comparability of a new RP-Met product to two existing and well-studied products. The comparison was not made against a control diet but again each other as internal controls. The second objective was to determine whether time of blood sampling relative to feeding impacted relative differences among products.

Materials and methods: Ten multiparous Holstein cows, 280 ± 73 days in milk, were used in a replicated 3x3 Latin square design (three complete squares, one incomplete square), with 7-day experimental periods. Treatments consisted of a control diet plus 12 g/d of either RP-Met K (KESSENT® M, Kemin Animal Nutrition and Health, Herentals, Belgium), RP-Met S (Smartamine® M, Adisseo Inc., Antony, France), or RP-Met M (Mepron®, Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany). Amount of Met contained in each of the three products was similar. Cows were fed ad-libitum with 33% of their daily feed allotment and RP-Met treatment provided every 8 h. Milking occurred at twice daily with milk samples collected on days 5-7 of each period. During d 5-7 of each experimental period, blood samples were collected from jugular catheters at 2, 4, 6, and 8 h after the morning feeding. At the end of the experiment, samples were sent to Missouri Agriculture Experiment Station Chemical Laboratories, USA, for amino acid analysis by cation-exchange chromatography.

Prior to statistical analyses, plasma methionine was converted to percentage of total amino acids minus methionine as calculated below:

methionine, % of total amino acids minus methionine = methionine (µg/mL) / (sum of all amino acids [µg/mL] – methionine [µg/mL]) * 100%

Treatment effects on plasma free methionine were evaluated using the GLIMMIX procedure of SAS (SAS Institute Inc., 2011). The model included fixed effects of treatment, period, square, hour of sampling (2, 4, 6, or 8), day of sampling (5, 6, or 7), and all interactions among treatment, hour of sampling, and day of sampling. Plasma free methionine prior to the start of the experiment was included as a covariate, and cow was included as a random effect. Significance was declared at $P < 0.05$.

Results: There was no significant effect of treatment on dry matter intake or production parameters. Plasma Met as a % of total amino acids minus Met was 1.5085, 1.5267, and 1.3622% for RP-Met K, RP-Met S, and RP-Met M, respectively. RP-Met K and RP-Met S were not found to be different (P=0.3420), however RP-Met K and RP-Met M were different (P<0.0001), with RP-Met K yielding greater plasma Met Levels. There was a significant effect of time of sampling on plasma Met as a percentage of amino acids minus Met (P=0.002), due to higher Met at 2 h (1.508%) than 4, 6, and 8 h (1.439, 1.447, and 1.469% respectively). However, the relative differences among treatments remained consistent at all time points.

Conclusions: The relative bioavailability of a new RP-Met product was assessed by comparing plasma Met response to that of existing products. Similarities in plasma Met levels between RP-Met K and RP-Met S treatments would suggest comparative bioavailabilities and bioavailability greater than that of RP-Met M. Differences among products were consistent at all blood sampling times, suggesting that time of sampling relative to feeding is relatively unimportant.

Keywords: Rumen protected methionine, plasma methionine response.

NU-03

Bioavailability of rumen-protected choline chloride sources based on in situ rumen degradability and in vitro intestinal digestibility criteria

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Introduction: Choline is a vitamin-like substance that interacts very closely with methionine and vitamin B12 metabolism as a methyl donor. It plays an important role in animal production, reproduction, and health (Jayaprakash et al., 2016, Morrison et al., 2018). Choline is rapidly degraded in the rumen, so effective supplementation requires the use of rumen-protected forms that are digestible in the small intestine (Grummer, 2008). There are several commercial protected products in the market, but the amount of choline delivered to the small intestine for absorption (bioavailability) may vary largely between products (Sales et al., 2010, Humer et al., 2019). Therefore, it is important to determine their rumen solubility, potentially degradable fraction, rate of degradation, effective degradation in the rumen and intestinal digestibility. However, this information is often not available. The objective of this project was to evaluate rumen degradability and intestinal digestibility of three different rumen protected choline products.

Mateiral and methods: The study was conducted at the Universitat Autonoma de Barcelona, following approved standard protocols. Rumen-protected choline products were CholiGEM (Kemin Animal Nutrition and Health), product R and product N containing 5.75, 3.23 and 2.71% N (% DM), respectively. Rumen microbial degradation was determined using the in situ nylon bag technique. Samples (2.020.018 g) of each rumen-protected choline product were weighed into nylon bags and incubated in the rumen of a Holstein dry-cow for 0, 2, 4, 8, 16, 24 and 48 h, in duplicate bags and in two consecutive periods. Ruminal degradability was calculated with the exponential function: Y=a+b*(1-exp^(-ct)); where a was the amount of N disappearing from the bag at 0 h; b was the potentially degradable fraction; and c was the degradation rate. The effective degradability of N (EDN) was calculated as: EDN,%= a+[(b*c)/(c+k)]; where k (estimated at 10%/h; Dufreneix et al., 2019) was the rate of outflow from the rumen, and a, b, and c were the same parameters described earlier. For the determination of the intestinal digestion, the in vitro three-step procedure (Gargallo et al., 2006) was used. The undegraded residue after 12 h incubation in the rumen was incubated in vitro in a HCl-pepsin buffer at pH 2.0, 38oC and 1h, followed by a phosphate-pancreatin buffer incubation at pH 7.0, 38oC and 24h.

Results: Fraction a was 27.6, 0.4 and 24.7%; fraction b was 57.1, 5.8 and 73.6%, and fraction c was 0.032, 0.002 and 0.081 /h for CholiGEM, product R and product N, respectively. Effective ruminal microbial N degradation was very low in product R (0.5%), moderate in CholiGEM (41.4%) and relatively high in product N (57.6%). However, intestinal digestion was low in product R (12.2%) compared with CholiGEM (98.4%) and product N (80.9%) resulting in the highest bioavailability for CholiGEM (57.6%), intermediate for product N (34.3%) and lowest for product R (12.1%).

Considering rumen degradation kinetics is not sufficient to define the bioavailability of protected choline chloride sources. Results indicate that intestinal digestibility is a critical step in the evaluation of bioavailability of rumen protected choline sources. There are considerable differences in rumen degradability and intestinal digestibility among rumen protected choline products in the market.

Conclusions: Evaluation of the quality of products requires determination of rumen degradability and intestinal digestibility. Results indicate that bioavailability was very different among commercial sources tested.

Keywords: Rumen protected choline, ruminal degradability, intestinal digestibility.

NU-04

Correlation between feed efficiency and mobilization of fat in dairy Holstein cows during the fresh cow period and early lactation

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Objectives: Feed efficiency (FE), a parameter to estimate the effectiveness of dairy production, gained importance for economic and ecological reasons in recent years. However, after parturition lipomobilization, a consequence of negative energy balance, may compromise estimates of FE. Thus, we aimed to examine the connection between FE and fat mobilization during the fresh cow (FCP) and early lactation period (ELP) in Holstein dairy cows with initially lower and higher condition.

Materials and methods: The study was performed at the Friedrich-Loeffler-Institute (FLI), Braunschweig, Germany. Thirty-one pluriparous German Holstein cows were used to determine the estimated depot mass (eDM) of subcutaneous (SCAT) and total abdominal (AAT) adipose depots by ultrasonography on day (d)-42, 7, 28 and 70 relative to parturition. Cows were allocated into two experimental groups according to the eDM of SCAT on d-42 relative to parturition (low body condition (LBC) group: n=16, mean eDM 8.61 kg; high BC (HBC) group: n=15, mean eDM 15.6 kg). Average daily change (aDC) of adipose mass was calculated for the FCP (d7 to d28) and ELP (d28 to d70). Additionally, dry matter intake (DMI) and lactation performance were recorded, and FE was calculated for FCP and ELP by dividing the energy-corrected milk yield (ECM) with DMI.

Results: The AAT depot had about 2 to 3 times higher mass than SCAT. The HBC cows had greater fat depot masses and mobilized more from both depots during FCP and ELP. The two groups did not differ in DMI, but the HBC group had greater ECM than LBC cows. As consequence, the HBC cows showed better FE compared to LBC cows. Correlation analysis revealed that during FCP, the more SCAT was mobilized the better the feed efficiency was (r^2: 0.18). However, in case of AAT no correlation was found (r^2: 0.01). On the other hand, mobilization of fat from both depots correlated positively with FE (r^2: 0.35 and 0.33, resp.) during the ELP.

Conclusions: During ELP and partly FCP, excessive lipomobilization increased estimated feed efficiency. Results indicate that the parameter feed efficiency may not be suitable for evaluation of performance efficiency in dairy cows during early lactation because it may lead to selection of cows for lipomobilization and possibly subclinical ketosis.

Keywords: Dairy cattle, feed efficiency, fat mobilization, fresh cow period, early lactation.

NU-05

Rumen degradability and intestinal digestibility of different rumen protected lysine products determined in situ and in vitro

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Introduction: The supply of metabolizable protein to dairy cows is important for optimizing milk and milk protein yields. However, excess MP in dairy cow diet results in a reduced efficiency of N utilization and an increase in the emission of N to the environment (St-Pierre and Thraen, 1999). This problem may be minimized by supplying dairy cows with the correct amount and proportions of essential amino acids. There is consensus that most current diets are deficient in Lysine and Methionine (Schwab and Broderick, 2017). There are several rumen-protected Lysine and Methionine products in the market, but their rumen degradability and intestinal digestibility may vary considerably (Whitehouse et al., 2017). The objective of the current study was to determine the bioavailability of the rumen-protected Lys products in dairy cows using the *in situ* ruminal degradation and the *in vitro* intestinal digestion methods.

Material and methods: The thee rumen-protected Lys products were LysiGEM and LysiPEARL (Kemin Animal Nutrition and Health), and AjiPro L3G (Ajinomoto). Rumen microbial degradation was determined using the *in situ* nylon bag tech-

nique. Samples (0.38±0.01 g) of each rumen-protected Lysine products were weighed into nylon bags. Duplicated bags were incubated in the rumen of a Holstein dry-cow for 0, 2, 4, 8, 16, 24 and 48 h. The process was repeated in two consecutive periods. Degradability of Lysine in the rumen was calculated as N disappearance from the rumen using an exponential function: Y=a+b*(1-⟦exp⟧^(-ct); where a was the amount of N disappearing from the bag at 0 h; b was the potentially degradable fraction; and c was the degradation rate (Mathers and Miller, 1981). The effective degradability of N (EDN) was calculated as: EDN (%)= a+[(b*c)/(c+k)]; where k (estimated at 10%/h; Dufreneix et al., 2019) was the rumen passage rate, and a, b, and c were the same as described earlier. The undegradaded sample residue after 12 h ruminal incubation was used to determine the intestinal digestibility using the *in vitro* three-step procedure (Gargallo et al., 2006): samples were incubated in a pepsin-HCL solution (Sigma 77160) at 39ºC and pH 1.9 for 1 h, followed by a second incubation in a buffer-pancreatin (Sigma P1750) solution at pH 7.75 at 39ºC for 24 h. The in vitro intestinal digestion was calculated as the amount of the sample N (rumen-exposed residue) minus the N remaining after pepsin-pancreatin incubation divided by the amount of sample N.

Results: The soluble fraction (%) was lower in AjiPro L3G (2.6) compared with LysiGEM (20.4) and LysiPEARL (24.6). The potentially degradable fraction (%) was larger in LysiPEARL (70.7) compared with LysiGEM (48.6) and AjiPro L3G (49.1). Rate of ruminal degradation (/h) was highest in LysiPEARL (0.05), intermediate in AjiPro L3G (0.013) and lowest in LysiGEM (0.006). The resulting effective degradation in the rumen (%) was highest in LysiPEARL (48.2), intermediate in LysiGEM (23.2) and lowest in AjiPro L3G (8.2). In contrast, intestinal digestibility was lowest in AjiPro L3G (49.6) compared with LysiGEM (87.3) and LysiPEARL, suggesting that AjiPro L3G was overprotected. Overall bioavailability (%) was highest for LysiGEM (67.1), intermediate for LysiPEARL (50.3) and lowest for AjiPro L3G (45.5). There were differences in ruminal degradation and intestinal digestion among different commercial products. Bioavailability is the result of the combination of the degree of rumen resistance to degradation and the intestinal digestibility of the undegraded fraction. The quality of rumen protected lysine products requires the determination not only of the degree of protection from ruminal degradation, but also the intestinal digestibility of the undegradable proportion of Lysine.

Conclusion: Results indicate that differences in bioavailability from the different Lysine sources may be important and needs to be considered in diet formulation.

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Keywords: Rumen protected lysine, rumen degradability, intestinal digestibility, bioavailability.

NU-06

Production performance of lactating dairy cows fed two rumen protected methionine supplements

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Intorduction: The new formulation systems have refined requirements and supplies of digestible protein and amino acids through the description of different feed protein fractions and digestive processes, and the incorporation of dynamic adaptation (NRC, 2001; INRA, 2018, CNCPS, Van Ambourgh et al., 2015). Despite all these improvements, the efficiency reported at 23.7% 50 years ago (Stone et al., 1960) remains virtually unchanged at 24.0% (Hristov and Huhtanen, 2008). This low efficiency represents a production cost and the emission of excessive N to the environment. Recent evidence indicates that at least part of this inefficiency is associated with the unbalanced supply of essential amino acids (Doepel and Lapierre, 2010). The supply of intestinally available essential amino acids is currently achieved through the use of rumen-protected forms of these amino acids. However, there are large differences in bioavailability of different protected methionine sources (Whitehouse et al., 2017) which may affect performance. The objective of this research is to determine the effect of feeding two different sources of Met on milk production and composition of lactating Holstein dairy cows.

Material and Methods: Ninety-four multiparous lactating Holstein dairy cows from 50 to 110 days in milk were divided in 3 groups to determine the effect of feeding different rumen protected Methionine sources on milk production and composition. Cows were fed a 46:54 forage to concentrate based on corn silage once daily formulated to meet current NRC (2001) recommendations (17.5% CP, 28.4% NDF, 33.2% starch and 4.6% fat and balanced for Lysine). Treatments were the control diet (CTR), and the same diet supplemented with 11.4 g of metabolizable methionine from either KES (KESSENT® M, Kemin Animal Nutrition and Health) or SMT (Smartamine® M, Adisseo Inc.). Experimental animals were blocked by previous milk production, assigned to three different lots and supplied with one of the three treatments. Cows were milked three times daily. After 30 days on treatment, milk production and composition were determined in weeks 6 and 10 postpartum (milk samples taken 3 consecutive days). Data were analyzed using the PROC GLM procedure of SAS as a completely randomized model.

Results: Milk yield (kg/d) was higher (P<0.002) in KES (46.7) than CTR or SMT (43.9 and 44.5, respectively). The 3.5% fat corrected milk (kg/d) was numerically higher in KES and SMT (51.3 and 50.6, respectively) compared with CTR (48.8), but did not reach significance (P<0.11). Milk fat content (%) tended to be higher (P<0.06) in SMT (4.38) than in CTR or KES (4.16 and 4.14, respectively). Milk protein content (%) was higher (P<0.04) in KES and SMT (3.09 and 3.11, respectively) compared with CTR (3.04). Similar effects (P<0.02) were observed for casein (%) (2.40, 2.43 and 2.45 for CTR, KES and SMT, respectively). Milk fat yield (kg/d) was similar among treatments (1.90), but protein yield (kg/d) was higher

(P<0.01) in KES (1.43) compared with CTR and SMT (1.33 and 1.38, respectively). Casein yield (kg/d) was also higher (P<0.01) in KES (1.13) compared with CTR and SMT (1.05 and 1.09, respectively).

Conclusions: Both rumen protected Methionine supplements improved dairy cow performance compared with control, but also significant differences between commercial supplements were observed.

Keywords: Rumen protected methionine, dairy cow performance.

NU-07

Ketosis monitoring in Spain using testday samples

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Objectives: Ketosis (KET) is one of the most impactful diseases in dairy farming and it represents a good indicator of a poor adaptation to high energy demand during the transition period (Duffield T., 2009). β-hydroxybutyrate (BHB) and KET can be monitored in milk (Carrier J., et al., 2004) using testday milk samples (de Roos A. et al., 2007). The main goal of this project was to map KET prevalence across Spain and identify risk factors and consequences.

Materials and Methods: Data from 4 DHI associations across Spain have been joined into one dataset: Cantabria (CANT), Castilla y Leon (CYL), Cataluña (CAT) and Galicia (GAL). The dataset includes 337,759 first tests occurring during 2018 and 2019. Since BHB monitoring best performs in cows ≤25 days in milk (DIM) (Renaud D. L. et al., 2019), samples outside the interval of 5-25 DIM were excluded, so that the final dataset contains 200,593 first tests belonging to 2242 farms. A BHB 0.1 mmol/liter cut-point has been choosen for KET (Viña C. et al., 2017). Variables used to investigate KET risk factors were: fresh season, parity, milk production and somatic cell count (SCC) at first test. First calving age in categories (<24mo; 24-27mo;>27mo), calf outcome (Male, Female, Twins) were also available except for GAL. Mean number of cows controlled per farm and test was divided into quantiles as a proxy of farm size. Calving season effects were evaluated via 2 classes: Atlantic climate regions (GAL and CANT) and the rest of Spain (CYL and CAT). Descriptive statistics were used to describe current KET prevalence across Spain. Multivariate models were built to evaluate specific risk factors for KET and to assess its relationships with milk production and udder health. Statistically significant results were identified using a p<0.05.

Results: 32% of cows were lact=1, 27% lact=2 and 40% lact≥3. 31% of lact=1 calved with <24mo, 41% between 24-27mo and 27% >27mo. Twinning rate was 3%. Overall KET prevalence was 21%: 15% for lact=1, 19% for lact=2 and 27% for lact≥3. Possibly, due to on demand KET monitoring, prevalence for CANT was significantly lower than other regions with 15.25% vs. 21.23% for GAL, 21.58% for CAT and 22.09% in CYL. Running the analysis by farm, 37.1% (n=763) of all farms with at least 20 tests each (n=2055) had a Ketosis prevalence >25%; this percentage varied by region with CANT 17.1%, CYL 40.3%, CAT 38.0% and GAL 39.7%. Mean number of cows controlled per farm and test had a significant impact on KET prevalence: in the Atlantic area the 1st quartile (<5 cows) had a KET prevalence of 27.09%, 2nd quartile (5-7 cows) 20.47%, 3rd quartile (7-11 cows) 17.88% and 4th quartile (>11 cows) 16.62%. In the rest of Spain KET prevalence was: for 1st quartile (<12 cows) 25.7%, 2nd quartile (12-24 cows) 22.13%, 3rd quartile (24-51 cows) 19.66% and 4th quartile (>51 cows) 19.96%. In the Atlantic area, Winter and Spring had the highest KET prevalence, conversely in the rest of Spain Summer and Fall had the highest KET prevalence. Statistically significant differences for KET risk were found across parities (3+ vs. 2, Relative Risk (RR)=1.54 95%CI=1.50-1.58; 3+ vs. 1, RR=2.03 95%CI=1.97-2.08; 2 vs 1, RR=1.31 95%CI=1.27-1.35) and across age at first calving for lact=1 (>27mo vs. <24mo, RR=2.15 95%CI=1.98-2.33; >27mo vs. 24-27mo, RR=1.48 95%CI=1.28-1.59; 24-27mo vs. <24mo, RR=1.44 95%CI=1.34-1.56). Cows with a singleton male had slightly more risk of having KET than cows calving singleton female (M vs F RR=1.05 95%CI=1.01-1.10). Ketotic cows were more prone to have, at the same time, subclinical mastitis (>200 000 cell/ml, Ruegg P. 2017) (KET Yes vs No RR=1.69 95%CI=1.63-1.75). Finally, accounting for parity, farmcode, DIM 1st test, month and year fresh, Ketotic cows yielded 1.29 kg less milk at first test compared to cows without KET (p<0.0001). As it was not possible to account for previous lactation milk production, this milk loss might represents an underestimate of the true milk loss attributable to KET.

Conclusions: KET prevalence and proportion of high risk herds is higher than perceived by much of the dairy industry. Elevated BHB was associated with lower milk production at first test and higher prevalence of subclinical mastitis. The idenfied risk factors can help design better prevention strategies.

Keywords: Ketosis, testday, Spain, dairy.

NU-08

Associations of serum calcium and subclinical hypocalcemia at calving with productive, reproductive and health outcomes in multiparous Jersey cows

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The most appropriate blood calcium (Ca) threshold to define subclinical hypocalcemia is still under study. Currently, there is a wide range of suggested definitions, and variability on its reported associations with productive, reproductive and health outcomes, which have not been described in Jersey

cows. Our aim was to evaluate the associations of serum Ca concentration and subclinical hypocalcemia at calving with: subsequent lactation milk and energy-corrected milk yield, fat%, protein%, somatic cells count linear score, mastitis, herd removal, and pregnancy at 1st service and by 150 days in milk (DIM), in 609 multiparous Jersey cows from 2 commercial herds fed acidifying prepartum diet.

Blood samples for total serum Ca concentration determination were collected from the coccygeal vessels at 3 h 10 min (±2 h 17 min) after calving. Monthly test milk yield, fat%, protein% and somatic cells count information up to the 10th test was obtained from the Dairy Herd Improvement Association. Additional information was obtained from herd records. Statistical analyses were conducted with multiple linear, Poisson, log-binomial, and Cox's proportional hazards regression using SAS (version 9.4). Considered explanatory variables for all outcomes were: parity, herd, previous lactation length and 305-days mature equivalent milk yield, dry period length, calving body condition and locomotion scores, calving easiness, and oral Ca supplementation. Additional variables considered were: somatic cells count linear score at test for milk yield, fat%, and protein%; milk yield at test for fat%, protein% and somatic cells count linear score; and DIM at 1st service and breeding code (timed artificial insemination and heat breeding) for pregnancy at 1st service. Serum Ca thresholds among 1.80 and 2.20 mmol/L (7.2 and 8.8 mg/dL) at 0.02 mmol/L (0.08 mg/dL) intervals were used to define subclinical hypocalcemia when serum Ca concentration (in a continuous scale) was associated with the outcome. Serum Ca thresholds that better predicted the outcome (continuous outcomes: smallest *P*-value and most extreme estimate; categorical outcomes: maximized sensitivity and specificity on receiver operating characteristic curve analyses) were chosen to define subclinical hypocalcemia.

Subclinical hypocalcemia (Ca ≤2.18 mmol/L; 8.7 mg/dL) was associated with 1.52 and 1.88 kg/d more of milk and energy-corrected milk yield, respectively ($P < 0.001$). Milk fat% was 0.12 units of percentage higher and milk protein% was 0.06 units of percentage lower per day for cows with subclinical hypocalcemia (Ca ≤1.96 and ≤1.80 mmol/L, respectively; 7.9 and 7.2 mg/dL, respectively), compared to normocalcemic cows during the subsequent lactation ($P = 0.01$ and $P = 0.03$, respectively). Subclinical hypocalcemia was associated with lower 1st service pregnancy risk [Ca ≤2.08 mmol/L (8.3 mg/dL); risk ratio = 0.70; $P = 0.03$] and hazard of pregnancy by 150 DIM [Ca ≤1.90 mmol/L (7.6 mg/dL); hazard ratio = 0.50; $P < 0.001$]. No association was observed among serum Ca concentration, mastitis and herd removal. Similar effects were observed for additional thresholds evaluated.

Establishing a single serum Ca threshold for subclinical hypocalcemia definition based on productive, reproductive and health outcomes doesn't seem feasible. Further studies are needed to elucidate the applicability of a subclinical hypocalcemia definition.

Keywords: Dairy cow, hypocalcemia, transition cow.

NU-09

A prospective cohort study on periparturient muscle tissue mobilisation in high producing dairy cows

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Objectives: Dairy cattle at the onset of lactation experience a period of Negative Energy Balance (NEB). Adipose tissue is mobilised to meet energy demands for milk production. There is evidence that some cows will also begin to mobilise muscle during the dry period prior to fat mobilisation[1]. 3-methylhistidine (3-MH) is a very specific indicator of muscle metabolism in cattle which is not further metabolised nor produced through any other metabolic pathways[2]. The main objective of this study was to investigate the changes in the thickness of the longissimus dorsi muscle in high producing dairy cows in the periparturient period and the possibility to use a commercial ELISA kit for 3-MH measurements as a correlate of changes in muscle thickness.

Materials and Methods: We enrolled 455 cows from three farms with data collected for 500 lactations (312 from Farm 1, 75 from Farm 2, and 113 from Farm 3). Data were collected from each animal on three occasions per lactation: 3-4 weeks before the expected date of parturition (Pre-calving (PC)), 0-10 day's post-partum (Fresh (FR)) and approximately 60-80 days post-partum (Early Lactation (EL)). At each time point blood samples were collected from the coccygeal vein, Body Condition Score (BCS), Muscle Thickness (MT), and Back Fat Thickness (BFT) were also recorded. Muscle and fat thickness were measured using an Easi-Scan ultrasound machine. Longissimus dorsi depth was measured perpendicular to the skin at the fourth lumbar process. Genomically estimated breeding values (GEBVs) were also available for these animals. A commercially available ELISA kit (Abbexa Ltd, 96 test kit) was also evaluated for measurement of 3-MH concentration in bovine serum. Descriptive and univariable analyses were undertaken between variables, before multivariable regression models were constructed. Cox Proportional Hazards Analysis was used for analysis of time to first service and time to conception.

Results: Explanatory variables using MT as an outcome found assessor had a significant effect on MT ($P < 0.0001$). Muscle thickness decreased in the period between the PC and FR measurement. Cows in the lowest Milk PTA tercile had higher MT measurements comparing to the other two terciles but there was no significant Milk PTA tercile by time interaction. Interesting Farm*Time-point interactions were also observed. Higher MT PC was associated with increased MT loss (Estimate -0.39 ± 0.04, $P < 0.0001$). Higher BFT PC was associated with decreased MT loss (Estimate 0.23 ± 0.06, $P < 0.0001$). A longer period between the PC and FR measurement was also associated with increased MT loss (Estimate -0.1 ± 0.03, $P = 0.0006$). Cows in the MT Pre-EL 3rd tercile (cows that had minimal loss of MT or gained MT during the studied period) were served earlier than cows in the 1st tercile

(cows with the greater loss of MT) (Hazard ratio: 1.39, CI = 1.004-1.94, P = 0.046). Additionally, cows in the top fertility index tercile (better genetics for fertility) were served earlier than cows in the lowest fertility index tercile (Hazard ratio: 1.58, CI = 1.17-2.13, P = 0.003).

The 3-MH ELISA produced a good standard curve using assay buffer but the co-efficient of variation was large in duplicate serum samples potentially suggesting an interfering substance within the serum. The polyclonal antibody in the kit was found to be raised against 3-MH conjugated to bovine-serum-albumin (BSA) and the BSA in the serum may have been competing with the 3-MH. Despite trying two different methods of extraction (acetone and 5% sulfosalicyclic acid) previously published for separation of 3-MH from plasma or serum proteins, none gave reliable results, therefore the kits were deemed unsuitable for use.

Conclusions: Although US measurements are an accessible way to measure MT, a more specific method which would avoid assessor effect, such a looking at 3-MH, would have provided further insight into the amount of MT loss occurring. Until a more cost-effective way can be found to monitor muscle catabolism it will be hard to reliably use the current methods of monitoring it on a commercial setting. This is an area of study that requires further investigation.

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Keywords: Dairy Cow, Muscle Mobilsation, Periparturien.

NU-10

Magnesium butyrate is a readily available magnesium source in dairy cow nutrition

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Objectives: Magnesium (Mg) is an essential nutrient for cows. This means that dairy rations need to supply a sufficient amount of absorbable Mg in order to safeguard the cow's health, for example decreasing the risk of milk fever and grass tetany. Currently, Mg-oxide is widely used to supplement dairy cows with Mg. In dry cow nutrition, however, Mg-butyrate can be considered of interest as an alternative source of supplemental Mg. This is because the use of Mg-butyrate, instead of Mg-oxide, is potentially advantageous. Butyrate, but not oxide, may stimulate the growth of rumen papillae which is instrumental to prevent rumen acidosis. However, the bioavailability of Mg from Mg-butyrate is not known. Therefore, an experiment was conducted with dairy cows to measure the apparent absorption of Mg from Mg-butyrate.

Materials & Methods: Six mid-lactation Holstein Friesian dairy cows were used in an experiment which had two dietary treatments arranged in a cross-over design. The experiment consisted of 2 experimental periods of 14 days each, preceded by a 14-day pre-experimental period to allow the cows to become adapted to the experimental rations. Two different diets were fed during the experiment, with the basal rations being identical and consisting of a low Mg diet without Mg-butyrate (L-Mg, 3.1g Mg/kg dry matter) and a high Mg diet with Mg-butyrate (H-Mg, 3.9 g Mg/kg dry matter). The magnesium content of the diets was modified via a pelleted experimental beet pulp. For the H-Mg group, the experimental beet pulp contained Mg-butyrate (Rumen-Ready®, Palital Feed Additives, Velddriel, The Netherlands) whereas the experimental beet pulp for the L-Mg diet did not. The Mg-butyrate from Rumen-Ready® is completely released and solubilized within the rumen, i.e., it is 100% available for absorption. Both types of experimental beet pulp contained TiO_2, which was used as an inert marker for determination of fecal output. Feed refusals and milk yield were recorded daily during each experimental period, and any feed refusals stored at -18 °C. During the last 4 days of each experimental period, all spontaneously voided feces and urine were collected between 9:00 and 17:00. At the end of each collection day, the individual feces collections were stored at -18 °C whereas the individual urine collections were stored at 5 °C. All individual feces, urine and feed refusal collections were pooled per cow and mixed thoroughly prior to chemical analysis. The Mg content of pooled samples of the feces, urine, feed and feed refusals was measured by means of inductively coupled plasma mass spectrometry, and the titanium concentration of the experimental beet pulp and feces was determined using a spectrophotometer.

Results: Cows offered the L-Mg diet ingested 54.7 g Mg/day while the cows fed the H-Mg diets ingested 66.3 g Mg/day ($P < 0.001$). The fecal excretion of Mg, however, was similar between the two experimental diets ($P = 0.174$). Consequently, apparent Mg absorption was found to be 7.9 percentage units greater ($P = 0.038$) when the cows were fed the diet supplemented with Mg-butyrate. The greater Mg absorption after feeding the H-Mg diet was, however, not reflected by a greater urinary Mg concentration ($P = 0.228$). These results indicate that the availability of Mg from the Mg-butyrate supplemented diet was high (34.1% of intake). The absolute fractional Mg absorption from Mg butyrate (i.e. 8.3 g/d) was at least 1.5 times greater than that of similarly derived values for the fractional Mg absorption from a highly soluble MgO (using previously published data). Thus, it appears that Mg butyrate, relative to MgO, is superior in supplying Mg available for absorption. The fractional Mg absorption from Mg-butyrate was calculated to be 71.6%.

Conclusion: Mg-butyrate is an attractive alternative to supplement dairy rations with Mg.

Keywords: Rumen, ruminant, apparent absorption.

NU-11

Utilising infra-red thermography to evaluate rumen development in dairy youngstock

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Objectives: Timing of weaning is a critical issue for the efficient management of dairy youngstock. Successful transition to a solid diet as soon as possible provides economic advantages due to the high cost of milk or milk replacer products when compared with solid foods. However, successful transition to a solid diet requires sufficient ruminal function to be present before weaning off milk. Accurate determination of ruminal development in calves is therefore an important measure in calf management. Calves with increased solid feed consumption, and therefore more rumen development before weaning show greater weight gain postweaning (1). Currently available rumen function evaluation methods involve direct sampling of rumen fluid or tissue which is both costly and invasive. Infra-red thermography of the left flank has been used to quantify rumen activity in adult cattle with flank temperatures rising 21% after feeding due to increased heat production through fermentation (2). This study evaluated the use of flank temperature to measure rumen function over time in calves between 6 and 105 days of age.

Materials and Methods: The study was conducted in 30 Holstein heifer calves born over a 3-month period at Cambridge University Farm, UK. Calves were housed in individual pens immediately after birth and bottle fed 3l of milk replacer twice daily for one week. Calves were then moved to group housing at an average age of 10±4 days and fed 8l milk replacer per day by free access to a machine feeder (Vario Smart Feeder, Volac) for 40 days before beginning a reduction of 0.5l/day until complete weaning at 56 days of age. Hay and concentrate pellets (18% crude protein) were provided *ad libitum*. After weaning calves were housed in groups of 8 and fed 2.5 kg concentrate pellets per head per day and *ad libitum* hay.

Calves were weighed every 7 days from entry to group housing (T0) for 42 days until weaning (T6) and 42 days post weaning until average age 101±4 days (T12). During weighing the mean maximum surface temperature of the left flank was measured in each calf using an infra-red camera (T335, FLIR Systems, UK).

All data were analysed using RStudio 2022.02.0. Univariate regression was used to quantify the relationship between flank temperature and bodyweight at each time point pre-weaning (T0-T6) and growth rate post-weaning (T6-T12). Variables with a p-value ≤0.1 in the univariate analysis were included in a multivariate model of post-weaning growth rate simplified by stepwise selection based on Akaike Information Criterion (AIC). Recorded cases of pneumonia and diarrhoea were included in the multivariate model to examine the influence of these diseases on growth rate.

Results: Univariate regression demonstrated significant positive correlations between growth rate post-weaning and flank temperature at T0 (r^2=0.146, p<0.05), T1 (r^2=0.336, p<0.01) T2 (r^2=0.208, p<0.05) and a tendency for a positive correlation at T3 (r^2=0.08, p<0.1) and these variables were therefore included in the multivariate model. Stepwise selection based on AIC retained flank temperature at T0, T2 and T3 and occurrence of pneumonia in the final model which accounted for 47.6% of the variance in post-weaning growth rate (adjusted r^2= 0.476, p<0.01).

Conclusions: Measurement of flank surface temperature in calves shortly after introduction of solid feed was a significant predictor of growth rate after weaning - with measurement at one week after the introduction of solid feed (T1) being the largest contributor variable and therefore the best single time for this prediction.

It is suggested therefore that infra-red thermography of the left flank in dairy calves represents a viable method of identifying differences in rumen function between calves of similar age. As the differences in rumen function soon after introduction of solid food identified using infra-red thermography had significant impacts on growth rate post-weaning, this method offers considerable promise as a non-invasive method to identify individual differences in young calves that will have significant commercial implications.

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Keywords: Dairy, Calves, Weaning, Rumen, Thermography.

NU-13

Iron deficiency anemia in whole milk fed calves

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Objectives: This is one of the first studies to assess the prevalence of iron deficiency anemia in whole milk fed dairy calves on farms in the United Kingdom and the effect of a single parenteral iron supplementation on haemoglobin (Hb) levels and daily liveweight gain (DLWG).

Materials and methods: 268 whole milk fed dairy calves from seven farms in the south of England were recruited in the study. Six of the farms were organic, one was non-organic. All calves were blood sampled for total protein and Hb levels and weighed from one to ten days after birth. They were then randomly assigned to either receive or not receive 1 gram of iron as iron dextrane (Uniferon™, Virbac Animal Health) by intramuscular injection immediately after thist first blood sampling and weighing. Calves were again weighed and blood sampled for Hb at around six weeks of age and weighed only at around 12 weeks of age. Hb levels and DLWG were compared between treated and untreated animals.

Results: There were no reported adverse effects to the iron injection in any of the treated calves.

Parenteral iron dextran had a significant effect on DLWG from one to six weeks with an average 52g per day increase

in the treated calves compared to the control group. Iron had a significant effect on Hb concentrations at six weeks and the difference between Hb at week one and week six, with calves in the treated group having a higher average Hb concentration than calves in the control group (112.2 vs 97.0g/l). Over that period Hb levels in the treated group increased by 4.5 g/l while levels in the control group decreased by 10.1 g/l. Calves with higher DLWG and calves in the control group were significantly more likely to have Hb levels below 90 g/l at six weeks: 35 % of calves in the control group (42 out of 120) and 3.4 % of the treated group (4 out of 118) showed Hb values below this level.

There was farm variation in both Hb levels and DLWG differences between groups. The size of the effect on DLWG could not be predicted for any particular farm from either Hb measurement at week one or six.

However, there was a consistent effect of iron across all farms, suggesting a generalized finding.

Conclusion: The current industry recommendations are to feed higher volumes of milk for longer to achieve higher growth rates. On a significant proportion of organic and non-organic farms in the United Kingdom whole milk is fed to to dairy replacement calves, and iron supplementation should be considered in these calves.

Keywords: Calves, anemia, iron, whole milk.

NU-14

Epidemiology of hyperketonemia in first parity Holstein cows. Is subclinical ketosis a problem of fresh cows, only?

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Objectives: Subclinical ketosis (SCK) is a common disorder of dairy cows, associated with several clinical diseases during the post-partum period. Although negative energy balance lasts on average up to 8-10 weeks, commonly recommended herd monitoring programs are mostly limited to the first 3 weeks of lactation. The objective of this study was to explore stage-of-lactation related epidemiologic parameters of SCK.

Materials and Methods: This retrospective observational study was based on two data sets established for two published genetic studies. Data were collected on a large commercial free-stall dairy farm located in northern Greece. The first data-set (A) included β-hydroxybutyrate (BHBA) measurements from 362 primiparous Holstein cows that calved between January 2005 and July 2006 and the second (B) BHBA measurements from 287 primiparous Holstein cows that calved between January 2008 and April 2010. Management conditions were greatly improved during the second period (mainly regarding feeding and housing conditions during the transition period). On both time periods, all cows were blood-sampled weekly, during the first 13 weeks of their lactation. Blood was drawn from the coccygeal vein and samples were left to clot at room temperature for approximately 30 min and then centrifuged at 2,000 ×g. The serum concentration of BHBA was assayed with the use of an enzymatic kinetic method based on the oxidation of BHBA to acetoacetate by β-hydroxybutyrate dehydrogenase; a total of 8,437 samples were analyzed. Subclinical ketosis was defined as a BHBA concentration ≥ 1.2 mmol/L. Prevalence of SCK was calculated for each of the 13 weeks by dividing the weekly number of cows with SCK by the total number of cows tested. Incidence of SCK was calculated as the number of cows with at least one positive BHBA test result, either for the first 3 weeks of lactation (3W) or over the entire 13-week sampling period (OV). Median first positive BHBA test and number of repeated positive test results per cow were also calculated for 3W and OV.

Results: Prevalence of SCK in data-set (A) was 12.2%, 9.7%, 8.0%, 8.6%, 6.9%, 4.8%, 5.7%, 3.3%, 3.0%, 5.2%, 5.0%, 2.5% and 3.0% for the 13 weeks, respectively. Prevalence of SCK in data-set (B) was lower, at 2.1%, 3.5%, 2.8%, 4.9%, 3.5%, 2.1%, 1.4%, 3.1%, 1.7%, 0.3%, 0.3%, 0.3% and 0.0% for the 13 weeks, respectively. Incidence of SCK in data-set (A) was 23.2% (84/362 cows) for 3W, while it was 46.7% (170/362 cows) for OV. Incidence of SCK in data-set (B) was 9.1% (26/287 cows) for 3W and 19.2% (55/287 cows) for OV. Median first positive BHBA test was at week1 and week2 for 3W and data-sets A and B, respectively; it was at week4 for OV for both data-sets. When overall incidence of SCK was high (data-base A) % of cows with ≥2 positive tests were also high, both in 3W and OV (42.9% and 39.4%, respectively). When overall incidence of SCK was lower (data-base B), cows with ≥2 positive tests was only 3.8% in 3W but a considerable 27.3% in OV.

Conclusion: Subclinical ketosis is present well beyond the first 21 days of lactation; actually, overall incidence doubles when the monitoring period extents to 13 weeks post-partum. Therefore, more than half of positive cows would be misclassified as non-ketotic if monitoring was limited to the first 3 weeks of lactation. Testing cows for SCK during the post-fresh transition period may be useful in finding associations with clinical diseases prominent during the first month of lactation, like metritis and displaced abomasum. However, the effects of SCK on reproduction and milk yield would be better evaluated if monitoring continues beyond the post-partum transition period, especially when considering the remarkable presence of repeated positive tests.

Keywords: Dairy cow, subclinical ketosis.

NU-15

Risk factors associated with excessive negative energy balance in commercial United Kingdom dairy herds

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Objectives: Numerous studies have shown that excessive negative energy balance (eNEB) in dairy cows, characterized by elevations in blood biochemical parameters such as β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA) and reductions in glucose concentrations in either late pregnancy and/or early lactation, is associated with an increased risk of transition cow diseases (such as ketosis, metritis and displaced abomasum), an increased risk of culling in early lactation, decreased milk production and poorer herd fertility. The objectives of this study were to use the results from a commercial laboratory service specializing in dairy cow biochemical analyses to 1) assess the individual cow risk factors for eNEB in UK dairy herds 2) assess the dietary risk factors for eNEB and 3) assess the relative importance of these different risk factors.

Materials and methods: Between April 2006 and March 2015, blood samples were analysed for BHB, NEFA and glucose from a commercial nutritional monitoring service provided by the Dairy Herd Health and Productivity Service (DHHPS) at the Royal (Dick) School of Veterinary Studies at The University of Edinburgh, UK. For each cow that was blood sampled, additional details were collected of the calving date (milking cows), predicted calving date (dry cows), lactation number, body condition score (BCS) and daily milk yield. Information was standardized for each herd using identical data collection forms. In addition, data was collected of the ration being fed to the cows (in kg fresh weight fed each day).

Results: Following removal of all potential duplicate cows, a final dataset of 69,161 unique individual cows was obtained including biochemical results, individual cow information and feed data. Use of generalized linear mixed-effect models and multivariable classification tree-based models showed that individual cow risk factors for eNEB included: days relative to predicted calving date (dry cows); Days In Milk (lactating cows); Body Condition Score (BCS: lactating cows ≥ BCS 4; OR 2.1); milk yield (around 40 litres per day); parity (first lactation heifers OR 0.46 compared to older cows during lactation); chronic inflammatory conditions as assessed by globulin levels ≥ 50 g/l (OR 0.79 for elevated NEFA values). There was a higher prevalence of eNEB during April to October (OR 1.19), with the lowest prevalence in November. Feeding grass silage and wholecrop (silage made from cereal crops) to dry cows was associated with a reduced prevalence of eNEB, whereas access to grazed grass was associated with a higher eNEB prevalence in both the dry period (OR 1.32) and lactation (OR 1.33).

Conclusions: This study has shown that prevalence of eNEB (as indicated by three separate biochemical measures of energy balance: elevated BHB and/or NEFA values as well as low plasma glucose) in dairy cows is associated with cow level risk factors such as BCS, parity and milk yield. This study has also highlighted a number of dietary risk factors associated with the occurrence of eNEB in dairy cows including feeding of conserved forages such as maize silage and wholecrop to dry cows, and access to grazed grass for both milking and dry cows. Understanding the risk factors associated with eNEB in commercial dairy herds assists in both the implementation of herd monitoring programs and reduction of eNEB in dairy herds, with consequential benefits that could potentially include reductions in transition cow diseases, increased performance and improvements in herd fertility.

Keywords: Dairy cow, BHB, NEFA, glucose, ketosis.

NU-16

Effect of postpartum milking strategy on plasma calcium concentration and risk of subclinical hypocalcemia in multiparous dairy cows

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The aim of the present study was to evaluate the effect of different postpartum milking strategies on plasma calcium (Ca) concentration and risk of subclinical hypocalcemia in multiparous dairy cows. Additionally, effects of the evaluated milking strategies on colostrum quality and yield, and milk yield and somatic cell count (SCC) at 1st monthly test were assessed. A total of 83 Jersey and Jersey × Holstein crossbreed cows of 2nd to 8th parity, were enrolled in the study before 1st postpartum milking. The study was conducted in a commercial dairy where cows were fed a negative dietary cation-anion difference ration prepartum. Milking strategies implemented during the first 2 days postpartum were: once-a-day milking (M1; cows were milked every 24 h; n = 24), twice-a-day milking (M2; cows were milked every 12 h; n = 21), delayed milking (MD; cows were not milked for the first 24 h, and were milked every 12 h afterwards; n = 19), and restricted milking (MR; cows were milked 3 L every 12 h; n = 19). Blood samples for total plasma Ca analysis were collected from the coccygeal vessels into heparinized vacuum tubes starting before 1st postpartum milking, every 4 h up to 48 h and at 72 h postpartum. Colostrum and transition milk yield was recorded using clear buckets prior to manual homogenization and sample collection for IgG determination at each study milking. Plasma Ca concentration was determined by Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES). Colostrum and transition milk IgG concentration was determined by radio immunodiffusion. First monthly test milk yield and SCC information was obtained from the Dairy Herd Improvement Association. Multiple linear regression with Dunnett adjustment was used to evaluate plasma Ca concentration changes, 1st monthly test milk yield and SCC. Poisson regression was used to evaluate the risk of subclinical hypocalcemia (Ca ≤2.12 mmol/L; Ca ≤8.50 mg/dL).

Prevalence of subclinical hypocalcemia at enrollment was

48%. Overall, plasma Ca concentration was lower and tended to be lower for M2 cows (2.04 mmol/L; 8.18 mg/dL) compared to MD (2.16 mmol/L; 8.66 mg/dL) and MR cows (2.13 mmol/L; 8.54 mg/dL), respectively. Plasma Ca concentration was not statistically lower for M1 cows (2.11 mmol/L; 8.46 mg/dL). Risk of subclinical hypocalcemia during the study period was lower for MD compared to M1 [Risk ratio (95% confidence interval) = 0.63 (0.46 to 0.86)] and M2 cows [Risk ratio (95% confidence interval) = 0.54 (0.39 to 0.76)]. Good quality colostrum (>50 g of IgG/L) was harvested at 1st postpartum milking from ≥80% of the M1, M2 and MR cows, and from 17% of the MD cows. At 2nd postpartum milking, good quality colostrum was harvested form 68% of MR cows. Milk yield and SCC at 1st monthly test were not affected by the milking strategy.

Our results suggest that postpartum plasma Ca concentration and risk of subclinical hypocalcemia may be influenced by the postpartum milking strategy, without negatively affecting subsequent milk yield and SCC. Additionally, the implementation of once-a-day (M1) or restricted (MR) postpartum milking strategies allows to harvest enough good quality colostrum to feed the calves. Postpartum milking warrants further study as a prophylactic strategy for hypocalcemia. Project funded by USDA-NIFA (1013457 CFAH).

Keywords: Dairy cow, hypocalcemia, transition cow.

NU-17

The effect of monensin controlled release boluses on subclinical ketosis, consequent health and production in dairy cows

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Objectives: Subclinical ketosis is one of the most important hidden conditions in high yielding dairy cows. It is directly corelated with different management approaches for cows in dry period, transition period and also in later lactation. Subclinical ketosis is usually associated with negative energy balance that occurs in transition period and in first trimester of the lactation. It affects the outcome of transition together with occurrence of diseases typical for this period and is known to have an impact on the most productive stage of lactation. The aim of the study was to determine prevalence of subclinical ketosis (SCK) on a dairy farm and success of preventive intervention using ruminal monensin controlled release boluses. We monitored health status, production, reproduction and culling rate of 110 dairy cows included in this study, to determine the effect of monensin controlled release boluses.

Materials and methods: The study was conducted between years 2017 and 2019 on a dairy farm with about 300 Holstein cows. Total mix ration (TMR) was fed to all the cows according to NRC 2001 recommendations for dry and lactating cows. Average milk yield on the farm was 9926 ± 227 kg/standard lactation with 3,75 ± 0,03 % fat and 3,27 ± 0,03 % protein according to national milk recording control. Only healthy multiparous cows, with the prediction of at least one more lactation, were included in this study. Eighty-five cows were randomly assigned in the control group and 25 cows in test group. Cows in the test group received monensin controlled release boluses (*Kexxtone*®) three weeks prior to calving, because prevalence of SCK in the heard at the time of the study was unknown. Non-esterified fatty acids (NEFA) were measured in all cows three weeks prior to calving. First postpartum check-up was carried out 7-14 days and the second one 28-35 days after calving. Blood samples were taken from all the cows to measure β-hydroxybutyrate (BHB). Reproduction parameters, milk yield and number of culled animals were taken out of the national milk recording database.

Results: SCK threshold for BHB was set at 1,2 mmol/L and for NEFA at 400 µmol/L. NEFA was in normal ranges for all cows 3 weeks before parturition. The first check-up postpartum revealed that 6 % of cows in control group had SCK and none in the test group. At the second check-up 20 % of cows in control group and 8 % in test group had SCK. Two cows in the control group had SCK at both check-ups for BHB. During this study 29 cows were culled, 31 % of those were from control group and 12 % from the test group. Major reason for culled cows was failure to conceive (reproduction problems). There were 14 % of cows from control group and 4 % of cows from test group culled due to reproduction problems. From control group 3,5 % of cows were culled because of metabolic disease and none from the test group. Average days open in test group was 139 ± 72 and 156 ± 72 in control group. Average lactation energy corrected milk (ECM) was 32,0 ± 3,8 kg/day/cow in test group and 31,9 ± 3,9 kg/day/cow in control group.

Conclusions: SCK is present in the herd in ranges established also by other authors. The use of ruminal monensin controlled release boluses can decrease the number of cows with SCK and therefor attribute to better reproductive and metabolic health, which was reflected in the number of cows culled for reproductive failure and metabolic disease in the control group compared to test group. In cows that finished the lactation milk yields were not different between the groups and the average days open ware slightly lower in the test group. Even though ruminal monensin controlled release boluses can be successful in prevention of SCK, they cannot fully prevent it and should be used as an addition to good management and feeding practice.

Keywords: subclinical ketosis, monensin controlled release capsule, Kexxtone®.

NU-18

Expression of ADAMTS-7 in myocardial dystrophy associated with white muscle disease in lambs

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Objectives: White muscle disease (WMD), or nutritional muscular dystrophy, is an acute fatal disease in young farm animals. It generally leads to death associated with heart failure. A-Disintegrin and Metalloproteinase with Thrombospondin Motifs (ADAMTS) genes play an important role in the pathophysiology of chronic inflammations. And also, recent studies have specified the role of ADAMTS-7 gene in cardiovascular system disorders in humans. The aim of the present study was to investigate the role of ADAMTS-7 gene in the pathogenesis of myocardial dystrophy associated with WMD in lambs.

Materials and methods: A total of 341 cardiac tissue samples from lambs with WMD were used in the study. Western-blot, real-time PCR (rt-PCR) and immunohistochemistry were performed for ADAMTS-7 gene expression. Histopathological sections of the samples were stained with hematoxylin-eosin.

Results: RT-PCR revealed that the expression level of ADAMTS-7 was statistically significantly higher in cardiac tissue with WMD compared to the control group ($p<0.05$). Western blot analysis confirmed significantly increased ADAMTS-7 protein level in the hearts of WMD ($p<0.05$). The immunohistochemically a statistically significant in high density of immunopositive cells was found from the in myocytes in which degeneration and necrosis were detected by labeling with ADAMTS-7 ($p<0.05$). Histopathologically revealed myofibrils with irregular borders and significant swelling of cells. Diffuse hyaline degeneration was noticeable in myocytes.

Conclusions: The expression of ADAMTS-7 was significantly upregulated in myocardial dystrophy associated with WMD. Despite to this limited study, if the potential biological mechanisms of ADAMTS-7 in WMD is understood more clearly.

Keywords: ADAMTS-7, myocardial dystrophy, lamb, white muscle disease.

NU-19

On field evaluation of preweaning period growth impact on age at first insemination and survival risk at 500 and 1000 days in dairy heifers

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Previous studies indicated that nutrient intake and growth during the preweaning period can influence a cow's performance later in life[1]. Thus, Average daily gain during the preweaning period (ADGprew) has been stablished as a key parameter to measure the overall health of calves and a threshold of 0.820 Kg/day has been set up as "excellent"[2]. The aims of this study were: a) to evaluate if birth weight and preweaning length have an impact on ADGprew and b) to evaluate AGDprew impact on age at first insemination and survival.

A retrospective study was conducted on a commercial dairy farm in Carral (A Coruña, Spain). All rearing heifers born between March 2017 and November 2021 were included in the study and the following parameters were collected for each animal from the farm's software Gando (Gando Nuevas Tecnologías S.L.): calving date (CALVDATE), weaning date (WEANDATE), first artificial insemination date (1ªIADATE), first calving date (FSTCALDATE) and cull date (CULLDATE), as well as birth weight (BIRTHWGHT, Kg) and weaning weight (WEANINGWGHT, kg). ADGprew (kg/day) and preweaning length (PREWlenght, days) were calculated using Gando. Correlation between BIRTHWGHT and PREWlenght with ADGprew was calculated using a linear regression. Three groups were made based on ADGprew distribution: Bottom (GMD<0.83Kg/d, n=124, 25.75%), Medium (0.83≤GMD<1.02 kg/d, n=245, 50.83%) and Top (GMD≥1.02 kg/d, n=113, 23.44%). Cox Proportional-Hazards Model were performed to evaluate ADGprew group impact on survival at 500 and 1000 days, as well as risk of insemination during first 395 days of life.

BIRTHWGHT, WEANINGWGHT and ADGprew averages were 36.94 ± 4.32 Kg (range: 23 – 46), 95.80 ± 13.86 Kg (range: 56 – 126.5) and 0.912 ± 0.177 kg/d (range: -0.070 – 1.500) respectively. Animals in the Bottom group tended to have an 80% higher risk of elimination at 500 days, with no differences between Medium and Top groups (HR=1.81, 95%CI = 1.08-3.02). In same way, culling risk at 1000 days was higher for animals in the Bottom group compared with Medium group (HR=1.74, 95%CI=1.11-2.74) with no differences between Medium and Top groups. Animals in the Bottom group had a 38% lower risk of being inseminated in the first 395 days (HR=0.62, 95%CI=0.44-0.89) compared to those in the Medium group. Median time to first insemination was 379, 368 and 369 days for Bottom, Medium and Top groups respectively.

Even in a herd with birth-to-weaning growth rates better than those usually described in the literature, differences in survival and age at first insemination were observed as a function of growth during preweaning period.

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Keywords: preweaning, heifer, survival.

NU-20

Differences in the serum metabolome profile of dairy cows according to the BHB concentration revealed by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR)

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Abstract

Objectives: During the transition period, a higher nutrient demand and a reduction in dry matter intake induce to a negative energy balance (NEB). The nonesterified fatty acids (NEFA) are then mobilized by adipose tissue and they may be oxidized into ketone bodies (β-hydroxybutyrate (BHB), acetoacetate and acetone). This condition makes dairy cows more susceptible to metabolic diseases such as ketosis. The BHB concentration is commonly used for the diagnosis in high-yielding dairy cows, with a cutoff of blood BHB value above 1.0-1.4 mmol/L for subclinical ketosis or hyperketonemia without clinical signs. These conditions are not easily identifiable and are frequently related to other diseases that cause economic loss. Metabolomics is a new analytical approach that aims to measure simultaneously the entire metabolite profile of a biologic sample. The aim of this study was to analyze the serum metabolome using ^{1}H-NMR in dairy cows with different levels of BHB.

Materials and methods: Animal care and procedures were in accordance with the European Directive 2010/63/EU and the national law D.L. 2014/26. Furthermore, The Ethics Statement was approved by the Animal Care and Use Committee of the University of Padua (ID number 91/2019 - "BovineOmics" Projects). Forty-nine Holstein Friesian dairy cows between 15 and 30 days in milk were enrolled from a single high-yielding dairy farm. The same total mixed ratio (TMR) was used for all enrolled animals. A cross-sectional experimental design was used. Each animal received a clinical examination by veterinarians at the University of Padua and animals with clinical signs of disease were excluded from the study. The blood samples were collected into tubes containing clot activator to obtain serum for biochemical and metabolomic analysis. According to the serum BHB concentration, the animals were divided into three groups: Group 0 (G0; 12 healthy animals; BHB≤0.50 mmol/L); Group 1 (G1; 19 healthy animals; 0.51≤BHB<1.0 mmol/L); and Group 2 (G2; 18 hyperketonemic animals; BHB≥1.0 mmol/L). The statistical differences for biochemical parameters were performed by one-way ANOVA, whereas a t-test was used to evaluate differences in metabolite concentration. A post hoc pairwise comparison among metabolite concentrations was performed using Bonferroni correction. A robust principal component analysis (rPCA), and the metabolic pathways overrepresentation analysis (ORA) were generated to summarize the structure of the data and to highlight the metabolic pathways influenced by BHB concentration. A p-value<0.05 was accepted, whereas a 0.05≤p-value≤0.10 was considered as trend to significance.

Results: Among biochemical parameters, only NEFA showed a significant difference between groups, with a progressive increment according to BHB concentration. A total of fifty-seven metabolites were identified in serum samples: 27 amino acids and derivates, 10 organic acids, 5 alcohols, 4 carbohydrates, 2 amine and derivates, 2 fatty acids, 2 ketone bodies, 1 sulfone, 1 vitamin, 1 imidazole, 1 nucleoside, and 1 guanidine. The extreme groups (G0-G2) showed a statistical difference for thirteen metabolites, specifically: glutamate, proline, serine, aspartate, isovalerate, and choline showed a significant reduction in G2, whereas 3-hydroxybutyrate, 3-hydroxyisobutyrate, acetate, succinate, 2,3-butanediol, methanol, and methylsuccinate showed a significant increase. In addition, 11 metabolites showed a trend toward significance: lysine, alanine, arginine, formate, pyruvate, and dimethylsulfone were reduced in G2, whereas isoleucine, valine, ethanol, trimethylamine-N-oxide (TMAO), and acetone were increased. The rPCA analysis revealed three different structure of metabolome, with G1 values located between G2 and G0. The ORA analysis identified three metabolic pathways possibly responsible for changes in metabolome profile: lipid metabolism, synthesis of phosphatidylserine, and glycosaminoglycan metabolism.

Conclusions: Metabolomic analysis through ^{1}H-NMR is a useful tool to achieve knowledge about metabolic profiling related to serum β-hydroxybutyrate modifications during the transition period in dairy cows. The metabolic state of our hyperketonemic cows suggests the mobilization of body resources, increased anaerobic fermentation, alteration of lipid metabolism, a potential oxidative stress state, and a possible alteration of inflammatory and healing processes. This study demonstrates that the metabolomic approach can be considered a significant means to achieve knowledge about dairy cow diseases and their pathogenesis.

Keywords: Metabolomics; H-NMR; Ketosis; Dairy cows.

NU-21

The behaviour of dairy cattle in the transition period: Effects of blood calcium status

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Objectives: Low blood calcium concentrations at calving can have a detrimental effect on cow health, welfare, and productivity. Currently, there is no reliable method to predict if a cow will develop clinical hypocalcaemia at calving, nor if cows have subclinical hypocalcaemia without analysing blood samples.

This study aimed to use automated behavioural monitoring under commercial farm conditions to investigate the behaviour of both primiparous and multiparous cows 1) pre-partum, with

the aim of predicting calcium status at calving and 2) post-partum, to quantify the subsequent effects of hypocalcaemia on cow behaviour.

Materials and methods: Behavioural data and blood serum samples were collected from 51 multiparous and 21 primiparous Holstein dairy cattle. Blood samples from the coccygeal vein were taken within 24h of calving, and serum was analysed to measure total calcium concentration. Cows were classified into one of three categories: normocalcaemia (serum calcium concentration ≥ mmol/L), subclinical hypocalcaemia (serum calcium concentration below 2.0 mmol/L, absence of clinical signs), and clinical hypocalcaemia (clinical signs and treatment).

An activity sensor (IceQube, IceRobotics Ltd., United Kingdom) was fitted to the right hind leg of cows 3 wk prior to expected calving date. Data for lying time, standing time, number of steps, motion index (total motion), and the total number of standing and lying bouts (postural transitions) were automatically collected and summarised from the time of calving into two datasets: behaviour in 2h periods and 24h periods (behaviour per day). The bihourly dataset was used for the analyses of cow behaviour on the day of calving, and data was analysed in 2h periods from -24 h to 0 h (the time of calving). The dataset containing cow behaviour per day was used to create 2 experimental periods based on the time relative to calving: pre-calving (d −14 to −1), and post-calving (d 1 to d 21). Mixed-effect models were used to analyse cow behaviour in the 14 d before calving (d -14 to d -1), on the day of calving, and the 21 d post-calving (d 1 to d 21).

Results: Between d -14 to d -1, the step count (no./d) of primiparous cows with normocalcaemia decreased by 10.3% and their motion index (unit /d) decreased by 6.1% over the period. The step count and motion index of primiparous cows with subclinical hypocalcaemia remained constant. There were no behavioural differences between primiparous cows with normocalcaemia or subclinical hypocalcaemia on the day of calving. In the post-calving period, step count of cows with normocalcaemia decreased by 6.7% from d 1 to d 21, whilst the step count of cows with subclinical hypocalcaemia decreased by 16.4% ($P < 0.001$). Similarly, the motion index of cows with normocalcaemia decreased by 7.5% from d 1 to d 21, whilst the step count of cows with subclinical hypocalcaemia decreased by 15.5% ($P < 0.001$).

For multiparous cows, there were no behavioural differences between blood calcium status (normocalcaemia, subclinical hypocalcaemia, clinical hypocalcaemia) in the pre-calving period and on the day of calving (d 0). In the post-calving period, cows with clinical hypocalcaemia spent 88 min/d (1.5 h) and 125 min/d (2.1 h) more time lying down compared to multiparous cows with subclinical hypocalcaemia and normocalcaemia ($P < 0.001$). Cows with normocalcaemia had fewer daily postural transitions (18.4 ± 0.5 no. /d; $P = 0.01$) compared to cows with subclinical hypocalcaemia (21.1 ± 0.3 no. /d) and clinical hypocalcaemia (22.0 ± 0.5 no. /d). Cows with clinical hypocalcaemia had 10% fewer steps and 6% lower motion index compared to cows with normocalcaemia, and 6.7% fewer steps and 4% lower motion index compared to cows with subclinical hypocalcaemia ($P < 0.001$).

Conclusions: There was a decrease in activity across the pre-calving period for primiparous cows, and activity could potentially be used to predict subclinical hypercalcaemia in primiparous cows. No behavioural differences between blood calcium status categories were found within the pre-calving period for multiparous cows, suggesting that it would be difficult to predict blood calcium status at calving using lying and activity behaviours. Blood calcium status at calving affected lying time duration, the number of postural transitions, step count, and motion index in the post-calving period for multiparous cows illustrating the profound and long-lasting effects of clinical hypocalcaemia.

Keywords: Hypocalcaemia, transition period, dairy cow, calving.

NU-22

Preliminary results of a metabolic survey for plasma ionized Calcium and Magnesium in dairy herds from the south of Chile

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Ionized calcium is the biologically active form of calcium; therefore, total calcium might not be the best indicator for hypocalcemia in dairy cows because of the changes in blood pH and protein concentrations. Consequently, the objective of this study was to determine the concentration of plasma ionized calcium and magnesium in cows at parturition from representative dairy herds from the south of Chile. The study was conducted in the southern region of Chile covering an area of 20,544 km2 (214 km length, 96 km wide; -39.82 S, -73.23 W; -40.12 S, - 72.38 W; -41.39 S, -73.46 W; -41.43 S, -72.94 W). Mean temperature during autumn is 7°C while during spring is 10°C, while mean rainfall during autumn is 850 mm, but in spring is only150 mm. The study consisted of selecting at random 11 dairy herds from a pool of 100 herds and sampling 8 cows per herd during the first 12 hours after parturition. All herds were handled under grazing conditions and had a calving distribution of 30% in autumn/winter, and 70% in spring/summer. Herds consisted of Holstein cows (Australian, New Zealand, Europe, and US genetics), artificial insemination breeding, and milked twice a day. Predominant pasture was based on perennial ryegrass (Lolium perenne). Spring calvings received 70% of dry matter from pasture and the rest from a partial mixed ration previous to each milking and concentrate in the milking parlor. Between August and December 2019, a blood sample for plasma collection was taken from a total of 88 cows from the 11 herds. Samples were stored in a cooler and shipped during the same day to a certified veterinary clinical pathology lab (Cooprinsem, Osorno, Chile). Samples were centrifuged and plasma was stored in plastic vials and frozen at -20 °C until analysis. Samples were assessed for ionized Ca and Mg using the Stat Profile® PRIME Plus VET (Nova Biomedical Corporation, Waltham, MA 02454-9141 USA). From the total samples,

26.7% were from parity 1, 19.6% parity 2 and 53.6% parity 3 or more. Total mean ionized Ca concentration was 1.00 mmol/L (range: 0.54-1.24 mmol/L) and ionized Mg was 0.615 mmol/L (range=0.32 – 0.89 mmol/L). Within parity number, the concentration of ionized Ca was 1.064, 1.024, and 0.894 mmol/L for parity 1, 2, and ≥3, respectively. For ionized Mg was 0.63, 0.60, and 0.613 mmol/L, for parity 1, 2, and ≥ 3, respectively. Defining a cut-off value of ionized Ca for subclinical hypocalcemia of 1.10 mmol/L, the total prevalence of subclinical hypocalcemia at day 1 postpartum was 65% with a range of an intra-herd prevalence between 0% and 100%. From the total number of cows (n=88), the prevalence of subclinical hypocalcemia within parity number was 40%, 54.5%, and 86.7% for parity 1, 2, and ≥ 3, respectively. It is concluded that subclinical hypocalcemia based on ionized Ca determination, is a metabolic disorder more common than expected in Chilean southern dairy cattle handled under grazing conditions.

Keywords: Ionized calcium, ionized magnesium, dairy herds, Chile.

NU-23

Very low negative DCAD diet promotes severe metabolic acidosis and alters plasma and urine metabolomics in prepartum Holstein cows

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Several nutritional strategies to prevent clinical hypocalcemia in the US dairy industry and other countries have been focused on feeding anionic diets. These diets reduced urinary pH from 8.5 (normal) to as low as 5.5, which imposes a tremendous acid load on the kidneys. However, the impact of anionic diets on the metabolic status of both the cows and their calves is not well understood, and such levels of over acidification may have detrimental effects on both the dam and her offspring. Consequently, the objectives of this cross-sectional non-intervention observational study were to compare urine and blood parameters between cows consuming a positive DCAD diet (early dry cows, DCAD + 250 Meq/kg DM, n=15) with the same cows consuming a negative DCAD diet (-220 mEq/kg DM) 10 days after moving them from the early dry to the prepartum group. Urine pH and blood metabolites were analyzed by a one-way repeated measures ANOVA for paired samples using the PROC GLM of SAS (2017). Differences in metabolic classes between groups were compared using BH correction (FDR= 0.05; Graph Pad 7.0), and metabolomic analyses was conducted using Metaboanalyst 5.0. Urine pH and blood analytes for the early-dry and prepartum periods, respectively, were as follows: urine pH: 8.18 and 5.33 ($P \leq 0.0001$); blood pH : 7.50 and 7.36 ($P \leq 0.0001$); base excess (mmol/L): 2.46 and -7.79 ($P \leq 0.0001$); lactate (mmol/L): 0.99 and 1.49 ($P \leq 0.05$); HCO3 (mmol/L): 25.65 and 17.45 ($P \leq 0.0001$); sO2 (%): 68.73 and 52.0 ($P \leq 0.01$); TCO2 (mmol/L): 26.59 and 18.39 ($P \leq 0.0001$); pCO2 (mm Hg): 32.62 and 30.68 ($P > 0.05$); pO2 (mm Hg): 37.51 and 29.85 ($P > 0.05$). Parity, BCS and days to parturition effect for all outcome variables were not significant ($P > 0.05$). Importantly, the metabolomics data revealed that only urine concentrations of essential and aromatic amino acids were decreased, and that concentrations of total non-essential amino acids and glucogenic amino acids were increased in plasma and reciprocally decreased in urine, suggesting that the cows fed anionic salts are attempting to meet a high glucose demand by mobilizing gluconeogenic amino acid reserves. The dietary anionic salts exerted marked effects on glycerophospholipids with a reduction in a majority of phosphatidylcholine containing diacyl and acyl-alkyl moieties in plasma and urine. Further characterization of these metabolomic profiles may lead to the development of novel biomarkers for identifying cows susceptible to metabolic alterations.

Keywords: Negative DCAD, metabolic acidosis, urine pH, metabolomics, Holstein.

NU-24

Effect of oral or parenteral iron supplementation in early life on iron concentration and hematological parameters of dairy calves fed commercial milk replacer

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Objectives: Iron supplementation is common in newborn calves. The objective of this study was to compare the effect of oral and parenteral iron supplemenation to a negative control treatment on iron concentration and hematological parameters.

Material and Methods: Thirty healthy newborn dairy calves were randomly assigned a subcutaneous injecton of Fe^{3+}(1000mg,**INJ**), oral administration of Fe^{3+}(1050mg,**ORAL**) or a sham-treatment without Fe^{3+}(**CON**) within 1h of birth. Calves were then fed whole milk over 5 days(d) and afterwards switched to milk replacer (MR,1500g/d,65mgFe/kg). Blood was collected before treatment, on d1,3,5,7 and once weekly for 7 weeks. Samples were analyzed for hematology and iron concentration.

Results: Red blood cell count declined by ~17% in all groups, reaching a nadir within the first week. Values returned to d0-values by d14 in INJ and ORAL and d28 in CON, and remained constant thereafter. Hemoglobin(Hb) reached a more pronounced nadir at d7(CON) compared to d5 (INJ and ORAL). Hb rose and remained >100g/L from d9 on (INJ and ORAL), CON calves remaind in the range of 80g/L.

Serum-iron peaked at d1 in ORAL (48.5 [46.9-51.7]µmol/L) and INJ (31.0[27.6-37.8]µmol/L) and declined thereafter. A second peak occurred at d7 (ORAL 23.9 [21.2-38.0]µmol/L, INJ 32.2 [20.9-39.1]µmol/L, CON 19.2 [12.6-20.9]µmol/L). Thereafter serum-iron in ORAL and INJ remained above CON.

Conclusions: Oral and parenteral iron supplementation had similar effects on hematological parameters and iron concentrations. Although Hb-values were higher in treated than control calves, hematological parameters remained within reference limits throughout the study in all groups.

Keywords: Iron treatment, hematology, neonatal calf.

NU-25

Variation in Longissimus Dorsi thickness in dry and lactating Holstein dairy cows, and association with early lactation mastitis and sole ulceration

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Objectives: Dairy cattle face a drastic increase in nutrient demand at the onset of lactation leading to a period of negative nutrient balance which cattle respond to by catabolism of body tissues. Adipose tissue mobilisation to meet energy demands for maintenance, and milk production and its association with health and fertility have been extensively studied. Studies on the role of muscle mobilization have been limited to small numbers of cows in single herds or experimental settings (Megahed et al., 2019, Van der Drift et al., 2012). While a consistent pattern of muscle mobilisation has been identified, data is lacking from large scale studies involving large numbers of cows across multiple herds. The objectives of this study were to (i) describe changes in longissimus dorsi thickness before and after calving (ii) to identify variables associated with difference in longissimus dorsi thickness and (iii) to describe associations between muscle thickness and early lactation health disorders.

Materials and Methods: A cohort of 2,352 Holstein cattle were prospectively enrolled on four farms and assessed at four different stage pre-calving (PC), immediately after calving (fresh, F), in early lactation (EL), and in late lactation (LL). Cows were grouped by parity as either primiparous or multiparous. At each time point, ultrasonographic images of the longissimus dorsi muscle at the level of the 5th lumbar vertebrae were stored and retrospectively measured according to the method described by Megahed et al (2019). Feet were lifted, and presence and severity of sole lesions was assessed by veterinary surgeons and lesion severity recorded. Mastitis episodes in the first 30 days in milk (CM30) were recorded by trained farm staff. A mixed effects multivariable logistic regression model was fitted to the data, with muscle thickness as the outcome; cow was fitted in this model as a random effect to account for within animal clustering of measurements.

Results: In total, 6,849 observations were available from 2,186 cattle. Significant variables that remained in the final model were parity, farm, CM30, stage, calving season and incidence of sole ulcer in EL, with significant interactions between stage x farm, parity x stage x farm, sole ulcer in EL x stage and parity x stage. Variance components analysis showed that 58% of variation in muscle thickness was due to animal effects.

In agreement with previous studies a greatest decrease in muscle thickness was between PC and F (with model adjusted means and standard error being 36.99 ± 0.90 mm and 32.59 ± 0.91 mm, respectively). Pattern of mobilisation varied by parity with primiparous animals having a greater decrease in muscle thickness between PC and F, and multiparous animals between F and EL. Differences occurred in pattern of change in muscle thickness between farms at different stages. Farms with greater loss in muscle thickness had higher formulated levels of crude and metabolisable protein in early lactation diets, but no consistent difference in pre-calving diets.

Cows with a sole ulcer at EL had significantly lower muscle thickness at EL compared to cows without sole ulcer. A CM30 by stage interaction was observed (p=0.06) with cows with CM30 having a greater decrease in muscle thickness from PC to F.

Conclusion: Decrease in thickness of longissimus dorsi was greatest prior to calving and is not synchronous with mobilisation of body fat (initial analysis; data not shown). Pattern of muscle loss differs between different parities at different lactation stages, and different farms at different lactation stages, suggestive of a management or nutritional influence, although cow factors are highly significant. Pattern of decrease in muscle thickness is also associated with incidence of early lactation mastitis and sole ulceration.

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Keywords: Longissimus dorsi, mastitis, sole ulcer.

NU-26

Factors affecting milk fatty acid composition on Galician Holsteins cows: a field studyFactors affecting milk fatty acid composition on Galician Holsteins cows: a field study

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During the last decades, milk fatty acid composition (mFAc) has gained the interest of manufacturers and consumers as it influences nutritional, physical and flavor properties of dairy products (Bobe et al. 2007). Previous studies reported several factors, including individual variability, genetic parameters, and breed, influencing mFAc (Samková et al. 2012). The aim of the present study was to evaluate type of ration, calving number, lactation phase and season impact on fatty acid composition, including myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and short chain fatty acid (SCFA) concentration in cow's milk in 25 Galician (NW Spain) commercial dairy farms. 10,098 test-days samples from the 1,557 cows were collected from July 2018 to June 2019 and bronopol-preserved samples from Laboratorio Interprofesional Galego de Análise do Leite (LIGAL, Spain) were received. Quantity of C14:0, C16:0, C18:0, C18:1, SFA, MUFA, PUFA and SCFA, MCFA, LCFA and total FA (in g/100 g of milk), as well as, fat and protein % and milk β-hydroxybutyrate (BHB) concentration (in all the animals from 1st post-partum (PP) test day) were determined individually by infrared-FTIR (Fourier transformed infrared-FTIR, MilkoScan FT6000, Foss Electric, Hillerød, Denmark). On top of this analysis, data from each test-day, including cow ID, date, parity (1st, 2nd and ≥3rd), type of ration (pasture based, grass silage-based total mixed ration (TMR), corn silage-based TMR or grass/corn silage-based TMR), days in milk (DIM) and daily milk yield were recorded. For analysis, animals were classified into five categories: fresh cows without ketosis (DIM ≤ 35 and BHB concentration in the first PP test-day < 0.10 mM/L), fresh cows with ketosis (DIM ≤ 35 and BHB ≥ 0.10 mM/L), peak lactation (DIM > 35 to 90), mid lactation (DIM 91-210) and late lactation (DIM >210).

Data were analyzed descriptively and by means of mixed-effect ordinal regression models. Animals feeding corn silage-based total mixed rations (TMR) and grass/corn silage based-TMR had higher C14:0, C16:0 and SFA concentrations than those feeding pasture-based rations, but lower concentrations of C18:0 and PUFA. Comparing to 1st parity cows, 2nd parity animals had higher C16:0, SFA and SCFA concentrations and 3rd parity cows had higher C18:0, SFA and SCFA. With respect to spring, C14:0, C16:0 and SFA concentrations increased in summer, autumn and winter while MUFA, PUFA and SCFA concentrations decreased. In the case of C18:0 and C18:1, concentrations decreased in autumn and winter. Considering the lactation phase, C14:0, C16:0 and SFA concentrations decreased in fresh cows with ketosis comparing to healthy fresh cows and increased in peak, mid, and late lactation (using again healthy fresh cows as reference). C18:0, C18:1 and MUFA follow the opposite trend. Milk fatty acid profile varies significantly with type of ration, calving number, lactation phase and season. The fact that the fatty acid profile has been associated with animal health, organoleptic properties of milk or even methane production highlights the importance of studying factors that affect its variation.

Keywords: Milk, Fatty acid, holstein.

NU-27

Economic opportunities on prevention of subclinical and clinical hypocalcemia by use of synthetic zeolite

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Objective: The objective of this study was to develop a sophisticated and robust tool for dairy farmers, to quantify the impact of using synthetic zeolite (X-Zelit) for prevention of subclinical and clinical hypocalcemia on farm economics.

Material and Methods: Dairy farmers could implement several solutions to decrease subclinical hypocalcemia. The solutions have different costs and efficiency in terms of reducing hypocalcemia. The existing simulation model SimHerd can quantify the economic consequences of changes in management for a dairy herd including youngstock. The expert-version of the model that vets use requires intensive training, and each simulation is time consuming. Therefore, in order for SimHerd to be operational for farmers, a Response Surface Model (RSM) was developed. An RSM is a meta-model that approximately describes the behavior of a certain area of the complex model (SimHerd). The current RSM predicts the change in profit (output) as a function of implementing of synthetic zeolite (X-Zelit) for prevention of hypocalcemia given different levels of disease risk and milk yield, amongst other input parameters. The assumed reduction in hypocalcemia as a result of the implementation was based on scientific literature.

In this project, an RSM was created using four steps.

- First, a simulation experiment with SimHerd, the expert version, was designed, covering the relevant ranges of input parameters like milk yield level, the risk of mastitis, calving interval and calf mortality among others.
- Secondly, simulation of all 160 scenarios from this experiment were done by SimHerd, using 200 replications and a burn-in period of 5 years.
- In the third step, the simulated results (output) were described as a function of the model's input with a linear prediction model. The best model was chosen by the Akaike Information Criterion (AIC) using the stepAIC() procedure in the MASS package of R.
- Finally, the response of the resulting model was explored, when using combinations of input parameters, that were not included in the design (interpolation and extrapolation).

Result: Input parameters of the RSM are herd size, milk yield per cow-year, the herd-level incidence of milk fever, subclinical hypocalcemia, other metabolic diseases, reproductive diseases, culling rate. The output parameters are changes in milk yield per cow-year, replacement rate, life-time yield, number of surplus heifers and economic net return per year and per cow-year. The RSM describes the changes in the output as a function of the input. The equations that make up the RSM contain two-way interactions and quadratic terms. The RSM is used as a web-based calculator (html) embedded on the Vilofoss website, where advisors and dairy farmers can use it.

Table 1. Examples of the RSM for 4 different herds, that are characterized by different levels of yield, hypocalcemia and replacement rate (input). The OUTPUT shows changes in production parameters relative to current production (OUTPUT) when using calcium binder for prevention of subclinical hypocalcemia.

		Herd 1	Herd 2	Herd 3	Herd 4
INPUT	Yield, kg EKM	9.000	9.000	11.000	11.000
	Subclinical hypocalcemia, %	50	70	70	70
	Replacement rate	0.3	0.3	0.3	0.2
OUTPUT, change	Kg ECM	+77	+87	+ 114	+119
	Replacement rate, %	-1.5	-1.8	-1,8	-2.7
	Benefit pr cow, €	54 €	59 €	67 €	71 €

The table shows a larger benefit in case of calcium binding in case the incidence of hypocalcemia is high; the benefit increases with €59 in herd 2 and with only €54 in herd 1. Furthermore, in case the herd has a high milk yield level, the increase in milk yield, and therefore the benefit of the intervention, is also higher (herd 3 versus herd 2). Finally, in case the herd has a lower culling rate and therefore a higher proportion of older parity cows, the intervention shows a larger benefit (herd 4 versus herd 3). Many more factors can be adjusted to explore the benefit in the individual farm.

Conclusion: The developed RSM tool makes it easier for the farmers to see the effects of changes in herd management. This has a potential to improve their decision making, as farmers can improve their economic performance, using the farmers' own key figures and prices.

Keywords: Hypocalcemia, synthetic zeolite, economics.

NU-28

Metabolic profiling of dry cows in practice: three-year results from a routine laboratory

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Objectives: Metabolic parameters can be assessed in close-up dry cows (last 3 weeks before calving) to indicate their risk for postpartum diseases and evaluate transition cow management. In the Netherlands, dairy farmers can submit serum samples from a set of close-up dry cows to the laboratory of Royal GD for analysis of a specific set of metabolic parameters, including non-esterified fatty acids (NEFA), β-hydroxybutyric acid (BHBA), urea, magnesium (Mg), and haptoglobin. Results are presented in a herd report that can be used to improve transition cow management. The objective of this research is to present results from three years of testing close-up dry cows based on samples submitted to the laboratory of Royal GD from 2019 to 2021.

Materials and Methods: Results for metabolic parameters in serum samples of close-up dry cow submitted to the veterinary laboratory of Royal GD (Deventer, the Netherlands) between January 2019 and December 2021 were evaluated. Each submission consisted of samples from a set of 4 to 10 cows (between 21 and 2 days before calving) from one herd. Serum samples were analyzed for concentrations of NEFA, BHBA, urea, Mg, and haptoglobin on a clinical-chemistry analyzer using enzymatic or colorimetric methods. The final dataset contained records of 6798 cows from a total of 1371 submissions (with an average of 5 samples per farm). Data could occasionally originate from cows that were sampled outside the targeted interval of 21 to 2 days before calving, but at a low enough incidence to be insignificant. Cut-off values, based on earlier studies, were: cows with NEFA concentrations ≥0.40 mmol/L, BHBA concentrations ≥0.80 mmol/L, and/or haptoglobin concentrations ≥0.30 g/L were indicated to be at an increased risk for postpartum disease. Cows with urea concentrations ≤3.3 mmol/L were indicated to have a insufficient protein supply. For magnesium concentrations, a cut-off value of ≤0.78 mmol/L was used to indicate increased risk for postpartum hypocalcaemia. The proportion of cows below or above the cut-off value were calculated for each metabolic parameter.

Results: Descriptive statistics are shown in Table 1. Concentrations of one or more metabolic indicators were outside the cut-off values in 50.5% of cows, with urea being the most frequent, at 22.9%, and Mg being the least frequent, at 5.8%.

Table 1. Descriptive statistics for concentrations of NEFA, BHBA, urea, magnesium (Mg), and haptoglobin in serum of 6798 cows (1371 sets of 4 to 10 samples) sent to the laboratory of Royal GD from January 2019 to December 2021, including cut-off values and proportion of results outside the cut-offs.

Parameter	Mean (± SD)	Median	P25 – P75	Min - Max	Cut-off value	% of cows
NEFA (mmol/L)	0.24 (± 0.19)	0.18	0.14 – 0.27	0.10 – 2.07	≥0.40	12.8%
BHBA (mmol/L)	0.6 (± 0.2)	0.6	0.5 – 0.7	0.1 – 5.6	≥0.8	18.4%
Urea (mmol/L)	4.3 (± 1.3)	4.2	3.4 – 5.1	2.0 – 22.3	≤3.3	22.9%
Mg (mmol/L)	0.96 (± 0.12)	0.97	0.90 – 1.03	0.34 – 4.10	≤0.78	5.8%
Haptoglobin (g/L)	0.18 (± 0.36)	0.09	0.07 – 0.14	0.03 – 8.05	≥0.30	9.6%

Conclusions: A substantial proportion of close-up cows were outside the cut-off values for one or more metabolic parameters. Based on the high proportion of cows with low urea results, a suboptimal protein supply in the dry period could be an important risk factor for transition cows in the Netherlands. Testing for metabolic parameters in close-up dry cows can be helpful to evaluate dry cow management and indicate cows at risk for postpartum diseases.

Keywords: Close-up, dry cows, metabolic parameters.