## 2022 MICHIGAN DAIRY HEALTH SYMPOSIUM
### Dairy Calf Health

# ABSTRACTS

College of Veterinary Medicine
Extension
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## Association among maternal late-gestation lipid mobilization and the offspring's health and performance through the first lactation: A cohort study in a dairy herd

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The objective was to explore associations between excessive maternal lipid mobilization in the last 2 weeks of gestation with offspring health and performance. A retrospective study was performed including 1,511 calves (heifer=692, bull=819) born between 2015 and 2020 in one MI farm. Calves were categorized in 2 groups based on dam plasma NEFA concentration: physiological lipid mobilization (PLM=1,373; NEFA<0.3mM) and excessive lipid mobilization (ELM=138; NEFA≥0.3mM). Calf records were obtained from the herd's management software. Outcomes of interest were the hazard of pre-weaned digestive and respiratory disease, pre-weaned ADG, age at first breeding and calving, first lactation 305d mature equivalent milk yield (305ME), and survival until first calving. Statistical models included dam NEFA category adjusted by year and season of birth, parity of the dam, and sex of the calf. Cox proportional analysis was used to determine the hazard of a pre-weaned health event, first breeding, and first calving. Linear regression was used to evaluate ADG and 305ME. The survival until first calving was analyzed with logistic regression. The hazard of diarrhea (P=0.65) and respiratory disease (P=0.27) in the pre-weaned period did not differ by NEFA category. Also, no difference was found for the LSM (±SE) of pre-weaned ADG (PLM=0.77±1.55, ELM=0.72±2.76 kg/d; P=0.1). In heifers, the hazard for first breeding favored the PLM group (HR$_{PLM vs. ELM}$=1.59; 95% CI=1.17-2.14; P<0.01), with a reduced median age (95%CI) at first breeding (PLM=400d (397-402), ELM=410d (404-421); P<0.01). However, NEFA category was not associated with the hazard of first calving (P=0.65), first lactation 305ME (PLM= 15,952±170 kg; ELM= 15,570±514; P=0.29), or the odds of surviving until first calving (P=0.74). Overall, dam ELM affected the hazard of first breeding but no other indicators of health or long-term performance. However, associations between maternal lipid mobilization and calf outcomes cannot be excluded, as the NEFA cut-off used was established for fresh cow performance.

## Detecting early pregnancies and understanding pregnancy losses in lactating dairy cows

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This research investigated the effect of treatment with hCG after ovulation/AI to Double-Ovsynch or Ovsynch programs on time to conceptus attachment in lactating dairy cows. We developed a robust technique to estimate d of conceptus attachment based on within-cow daily changes in concentrations of pregnancy-specific protein B (PSPB; BioPRYN). In parallel to the effects of treatment on fertility, our study revealed a very accurate method to diagnose pregnancies as early as d 22 post-AI/ovulation and an association of time to conceptus attachment and pregnancy losses. We observed that > 70 % of cows inseminated had conceptus attachment indicating that these fertility programs were highly efficient in achieving pregnancies. Yet, cows with delayed time to conceptus attachment (> 22 days) were more likely to experience pregnancy loss before d 35 post-ovulation/AI. In fact, 83 % of cows with delayed time to conceptus attachment had pregnancy loss within this period. The occurrence of accessory ovulation/CL ipsilateral to the original ovulation was beneficial against pregnancy loss before 100 d post-ovulation/AI in primiparous cows. Treatment with hCG on d 5 post-ovulation, however, decreased the percentage of cows with, and increased time to conceptus attachment despite increasing number of accessory CL after ovulation/AI and increasing serum progesterone on d 19 and 20 post-ovulation. Our analysis indicated that quartiles of progesterone on d 19 and 20 were not associated with time to conceptus attachment. Yet, multiparous cows of the highest quartile of progesterone on d 5 post-ovulation/AI had reduced time to conceptus attachment than multiparous cows of the lowest quartile. Overall, this research indicated that manipulating progesterone early, not late, in the estrous cycle following AI may influence the uterine environment and hold the key to improving fertility of dairy cows. Detecting pregnancy early allows for more chances for cows to become pregnant in a shorter DIM. This not only decreases the potential for pregnancy losses and health events in subsequent lactations but also increases longevity and profitability. Therefore, our data may be applicable to MI producers who are interested in designing fertility programs aimed to optimize fertility and profitability.

## Treatment with a double dose of cloprostenol sodium is a viable strategy for fertility programs

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Inadequate luteolysis in fertility programs is a problem for lactating dairy cows treated with a single dose of prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$). The proportion of cows with complete luteolysis can be increased by administering two doses of $PGF_{2\alpha}$ 24 h apart. This study hypothesized that a double dose of cloprostenol sodium (1.0 mg) could take the place of two doses 24 h apart due to its enhanced half-life. Cows were allocated to receive one of the three treatments. 1) Negative controls: 0.5 mg of cloprostenol sodium (SINGLE; n = 343); Positive controls: two 0.5 mg of cloprostenol sodium 24 h apart (TWO/24; n = 316); and Treated:1.0 mg cloprostenol sodium (DOUBLE; n = 305) at the final $PGF_{2\alpha}$ of Double-Ovsynch. Cows received AI 16 h after final GnRH of Double-Ovsynch. Pregnancy diagnosis was determined at 24, 34, 62 and 184 d post-AI. Pregnancy loss was categorized in the following periods: between 24 to 34, 34 to 62, and 62 to 184 d post-AI. Ultrasonography (B-mode and color Doppler) was utilized to assess luteal function pre- and post-treatment with various doses of cloprostenol sodium. There was no evidence of an effect of treatment on pregnancy rates per AI (PR/AI) at 24, 34 or 62 d post-AI. There was no effect of treatment on pregnancy losses occurring between d 24 and 34, 34 and 62, and between 62 and 184 d post- AI. However, third-plus parity cows treated with SINGLE had greater pregnancy loss compared with TWO/24 and DOUBLE between d 24 and 34 post-AI. Third-plus parity cows that received DOUBLE had lower luteal blood flow (LBF) 2 and 4 d after treatment compared with cows treated with SINGLE. Amount of LBF present 4 d after treatment was not a predictor of pregnancy or pregnancy loss. Treatment with double dose (1.0 mg) of cloprostenol sodium may be a feasible alternative for fertility programs based on non-different outcomes to TWO/24, lower pregnancy losses and reduced LBF disappearance following treatment in third-plus parity cows in comparison with the SINGLE treatment. Reducing the number of treatments in a fertility program decreases labor-associated costs, time spent on reproductive management and compliance issues. Utilizing different cloprostenol dose strategies is one option for tailoring reproductive programs according to the herd's necessity.

## The interconnection of metabolism and mammary gland health

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Poor metabolic adaptations to lactation have been associated with increased risk for mastitis in periparturient dairy cows. Therefore, in two separate intramammary challenge studies, we assessed the direct effects of hyperketonemia or conversely, we assessed the effects of a nutritional intervention used to support metabolism. In the first study, late lactation multiparous dairy cows (n = 12) were continuously infused intravenously with either β-hydroxybutyrate (BHB) to induce hyperketonemia (target concentration: 1.8 m*M*) or with saline (CON) for 72 h during a *S. uberis* intramammary challenge. BHB-infused cows exhibited a delayed febrile response likely indicating impaired immune function. Consequently, BHB-infused cows had greater *S. uberis* CFU on d 4, 6, and 7 post-challenge. As a result, BHB-infused cows consumed less dry matter intake and produced less milk than CON. In the second study, we assessed the effects of a dietary feed additive (rumen-protected choline, RPC) supplemented to periparturient dairy cattle during an intramammary lipopolysaccharide (LPS) challenge. Parous Holstein cows received either 45 g/d of RPC (CHOL45, n = 18), 30 g/d of RPC (CHOL30, n = 21), or no RPC (CON, n = 19) starting 24 d prior to expected calving until 21 d postpartum. Cows were alternatively assigned within treatment group to either receive an intramammary LPS challenge or not at 17 DIM. Although minimal effects of dietary RPC supplementation were found on metabolic parameters, cows receiving CHOL45 and CHOL30 produced approximately 3.5 kg/d more milk than CON prior to the challenge (1 to 16 DIM). From 17 to 21 DIM (following LPS challenge), treatment did not impact milk yield; however, CHOL45 and CHOL30 cows produced more milk than CON in the carry-over period (22 to 84 DIM). Treatment did not impact somatic cell score (SCS) prior to challenge; however, during the LPS challenge, treatment interacted with the genetic parameter for SCS such that choline supplementation reduced SCS in cows with poorer genetics. Taken together, strategies to improve metabolic adaptations to lactation during the periparturient period are critical to improve mammary gland health; RPC supplementation may be one solution to this problem.

# Effects of maternal dietary rumen-protected choline supplementation during late gestation on calf growth and metabolism

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Choline is a trimethylated molecule that is supplemented in the diet to support health and performance of periparturient dairy cows. Yet, the effects of dietary supplementation of rumen-protected choline (RPC) during late gestation on the offspring have only begun to be investigated. Therefore, the objective of this study was to examine the effects of maternal supplementation and dose of RPC on neonatal calf growth, metabolism, and oxidant status. Parous Holstein cows were blocked by calving month and randomly assigned within block to receive either 45 g/d of RPC (CHOL45, n = 19), 30 g/d of RPC (CHOL30, n = 22), or no RPC (CON, n = 19) starting 24 d prior to expected calving. Calf body weights were recorded for the first 3 wks and average daily gain (ADG) was calculated. On d 1, 7, 14, and 21, blood samples were taken to quantify reactive oxygen species (ROS), antioxidant potential (AOP), haptoglobin, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), and glucose. Data were analyzed using linear mixed models including prepartum dam data (-24 d) as covariates. Calf body weights did not differ between treatment groups. Calves born from CHOL45 dams had a slightly greater ADG than calves born from CHOL30 dams (P = 0.03); however, no difference was seen between calves born from RPC supplemented dams versus CON. Treatment only numerically impacted calf ROS and AOP; however, calves born from CHOL30 dams had a lesser oxidative stress index (ROS/AOP) than calves born from CON dams (P < 0.01). Moreover, haptoglobin was lesser in heifer calves from CHOL45 dams as compared to heifers from CON dams (P < 0.01). Treatment did not affect calf BHB or glucose concentrations. The dam's NEFA concentration interacted with treatment to affect calf NEFA concentration (interaction, P = 0.02). When dam NEFA was minimal, calves born from CHOL45 and CHOL30 dams had greater or tended to have greater NEFA than calves born from CON dams, respectively. Conversely, when dam NEFA was greater, calves born from CHOL30 and CHOL45 dams had lesser or tended to have lesser NEFA, respectively. In conclusion, RPC supplementation during late gestation impacted neonatal calf metabolism and oxidative stress markers, but this did not translate into substantial improvements in calf growth.

## Oleic acid improves insulin sensitivity in periparturient dairy cow adipose tissue through PLIN5 and PPARα

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Excessive adipose tissue (AT) lipolysis around parturition in dairy cows is associated with impaired AT insulin sensitivity (IS) and increased incidence of metabolic diseases. We have previously demonstrated that supplementing oleic acid (OA) can promote lipid accumulation in bovine adipocytes. In the liver, OA promotes lipid droplet formation by activating perilipin 5 (PLIN5) and peroxisome proliferator activated receptor alpha (PPARα) however it is unknown if this mechanism occurs in AT. We hypothesize that OA promotes lipogenesis and enhances IS in AT of periparturient dairy cows by signaling through PLIN5 and activating PPARα. Multiparous Holstein cows (n=12) were infused abomasally following parturition with ethanol (CON) or OA (60g/d) for 14d. Intravenous glucose tolerance test was performed on d14. Subcutaneous AT samples were obtained at 11±3.6d before calving (PreP), and 6±1d (PP1) and 13±1.4d (PP2) after parturition. Adipocyte morphometry was performed on H&E-stained sections. Isoproterenol (ISO, 1μM) stimulated lipolysis and insulin (1μg/L) inhibition of ISO was determined using an in vitro explant culture by measuring glycerol release. PLIN5 and PPARα expression were determined by RT-qPCR and capillary electrophoresis. Statistical analyses were performed using a mixed effect linear model in JMP which included the random effect of cow, and the fixed effect of treatment, time, and their interactions. Compared to CON, OA reduced plasma glucose concentration peak (188 vs 173±5.8, $P < 0.001$) and the clearance rate (2.06 vs 1.89±0.04, $P < 0.01$). At PP2, compared with CON, OA reduced AT response to ISO and increased AT sensitivity to insulin ($P < 0.01$). Compared to CON, OA decreased the percentage of smaller adipocytes (<3000 μm2) at PP2 ($P < 0.001$) hence limiting adipocyte size reduction postpartum. Compared to CON, OA tended to have a higher PPARα content at PP1 (0.006 vs 0.002±0.001; $P < 0.10$). Additionally, OA increased PLIN5 protein expression at PP2 compared to CON (0.007 vs 0.002±0.001; $P < 0.05$). Our results provide initial evidence that OA may limit lipolysis by improving IS and enhancing lipogenesis through the activation of PPARα in a PLIN5 dependent manner.

## Lipolysis inhibition improves clinical outcomes in the treatment of ketosis in dairy cows

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Lipolysis dysregulation in adipose tissues (AT) of dairy cows is a major risk factor for clinical ketosis (CK). The current treatment for CK is oral propylene glycol (PG) which stimulates gluconeogenesis. However, PG does not reduce lipolysis. Niacin (NIA) and cyclooxygenase inhibitors can inhibit lipolytic activity. We hypothesized that inhibition of lipolysis with NIA and Flunixin Meglumine (FM) reduces AT lipolysis and improves CK recovery. Multiparous Jersey cows (n=72, 7.2±2.8 DIM, median parity 2.9±0.9) were selected from a commercial dairy. Inclusion criteria were CK symptoms and hyperketonemia (BHBA ≥1.2 mmol/L). Cows were randomly assigned to 1 of 3 treatments; T1) PG: 310g/PO/day for 5 d, T2) PG+NIA: 24g/PO/day for 3 d, and T3) PG+NIA+FM: 1.1 mg/Kg/IV/day for 3 d. From a subset of 6 cows from each group and 6 healthy (CON) cows matching DIM and lactation number, subcutaneous AT samples were collected at day 0 (D0) and D7 relative to enrollment. The AT anti-lipolytic response induced by insulin (IN=1μL/L) was determined during isoproterenol stimulation. Lipolysis was assessed by glycerol release. Plasma BHBA, NEFA, and glucose (GLU) levels were determined at D0, D3, D7, and D14 after treatment. At D0, CK cows showed high BHBA (2.10±0.13 mmol/L) and NEFA (0.98±0.10 mmol/L), but low GLU (51.29±1.55 mg/dL). Compared to D0, all treatments reduced BHBA by 36.2%, 38.1%, and 33.5±5.9% at D3, D7, and D14, respectively. However, T1 cows showed an increase in BHBA at D14 (1.76±0.33 mmol/L). Compared to D0, NEFA decreased 16.1%, and 16.2%, at D3 and D7 for all treatments. T3 cows had NEFA within normal parameters (0.46 mmol/L) at D14. Across the study, T3 presented a greater GLU increment (18.9±3.7%) compared to T1 (13.6±3.9) and T2 (7.3±3.9). At D0, IN reduced lipolysis by 41±8% in AT from CON, however, it had no effect on AT from CK cows (-2.9±4%). At D7, the AT from T3 cows had a stronger response to IN reducing glycerol release by 36.5±8% compared to T2 (7.4±8) and T1 (26.9±7). These data suggest that including NIA and FM reduced lipolysis biomarkers and improved glycemia and AT insulin sensitivity in CK cows treated with FM and NIA. Future studies will evaluate the mechanisms by which NIA and FM regulate AT's responses.

Poster 4

## The effect of Pegbovigrastim on metritis incidence in dairy cattle

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Postpartum uterine diseases in dairy cows are highly prevalent, with metritis affecting 20% of cows in the herd within 21 days after calving. The major risk factors for metritis are retained placenta and negative energy balance, which are associated with an impairment of the immune system in cows. The granulocyte colony-stimulating factor (G-CFS) is an immunomodulatory cytokine, which promotes neutrophil proliferation and maturation, and enhances their microbicidal function by myeloperoxidase activity. Pegbovigrastim (Imrestor[®], Elanco Animal Health) is a recombinant bovine G-CSF that increases neutrophils production within 24 hours of treatment and reduces clinical mastitis incidence. However, recent reports indicate that Imrestor may increase the risk for metritis. We hypothesized that G-CFS increases metritis in dairy cows. A total of 165 jersey cows (parity 2.20±0.49) were selected from a commercial herd and randomly assigned to 1 of 2 treatments, T1) Control (n=129) and T2) Pegbovigrastim (Imrestor[®], Elanco Animal Health) (n=36), the first dose (15 mg) was administrated subcutaneously 7 days before expected calving and a second dose immediately after parturition. All cows were evaluated for 20 days after calving to determine metritis by the presence of fetid watery uterine discharge. The obtained data were assessed by Chi-square to determine the effect of Pegbovigrastim on metritis incidence. The incidence of metritis in this cohort of cows was 10.3%. Pegbovigrastim treated cows had an incidence of metritis of 25% vs. 7.5% in Controls. Pegbovigrastim cows had higher odds for metritis than control cows (4.9 times greater risk). These data suggest that in this dairy and in multiparous Jersey cows, administration of Pegbovigrastim 7 d before expected calving and at the day of parturition increases the risk for postpartum metritis.

## Status of Bovine Leukemia Virus infection in female calves

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Bovine Leukemia Virus (BLV) is a delta retrovirus that induces a chronic infection in cattle. Impacts on infected dairy cows include decreased milk production, decreased cow longevity, and increased production costs. While infection status at various ages of dairy cattle has been published, a longitudinal study sampling animals from birth to adulthood is lacking. Previous studies have shown calves may become infected during the birthing process and exhibit detectable levels of BLV provirus approximately 24 days post infection. Antibodies detected in neonates are indicative of the animal's passive immunity. Little is known about BLV antibody status and proviral levels in calves as their active immunity develops. This project aims to identify the timing of calf BLV infection and the influence BLV infection has on the animals' likelihood of entering the milking herd. Blood samples have been collected from neonates (2 to 8 days of age) and animals will be resampled at 6-months of age, 1 year of age, and each following year of lactation. Following the collection of neonates (n=254), we found 40.55% of the calves had BLV antibodies and 1.18% had detectable BLV provirus. The 6-months of age sampling will allow for identification of potential shifts in BLV antibody status and changes in proviral levels. Identification of the timing of BLV infection among dairy animals may provide insight on how BLV is spreading within a herd as well as aid in providing improved mitigation strategies to prevent BLV infections.

## Use of milk flow recordings to identify cows with delayed milk ejection

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The objective of this study was to explore the use of milking dynamics to identify individual cows with delayed milk ejection using a specific time interval of milk flow from a parlor recording system. We used digital recorders to measure vacuum in the short milk tube and mouthpiece chamber of two liners during individual milking for 529 cows, and we collected milk flow dynamics from milking recording system software (DeLaval DelPro Software and SCR MC200 Series Milking Control Systems), for each cow. Milk let down time (seconds) after unit attachment was obtained by analyzing the milking vacuum data. Cows were categorized as either having delayed (> 30 s) or normal (≤ 30 s) milk ejection. Individual let-down time and milk ejection category were matched to milk flow during four time intervals: ≤ 15 s, 16-30 s, 31-60 s, and 61-120 s. Univariate and logistic regressions of the four time intervals on milk let-down time and milk ejection category, respectively, identified milk flow between 31-60 s as the best time interval to predict delayed milk ejection (Tables 1 and 2). Delayed milk ejection lowers milking efficiency and can decrease milk production, impair teat health, and negatively impact animal welfare. Producers, veterinarians, and consultants could benefit from the use of on-farm technology that identifies and monitors delayed milk ejection incidence in their herds, allowing them to intervene when needed to improve and maintain milking efficiency.

Table 1. Regressions of milk flow time intervals and milk let-down time.

| Milk flow time interval (s) | R-Square | P-value |
|---|---|---|
| 0–15 | 0.0125 | 0.01 |
| 16–30 | 0.0735 | < 0.0001 |
| 31–60 | 0.3288 | < 0.0001 |
| 61–120 | 0.0087 | 0.03 |

Table 2. ANOVA of milk flow time intervals and milk ejection category.

| Milk flow time interval (s) | Delayed milk ejection LS means (lb/min) | Normal milk ejection LS means (lb/min) | P-value |
|---|---|---|---|
| 0–15 | 1.96 | 2.52 | 0.002 |
| 16–30 | 4.53 | 6.53 | < 0.0001 |
| 31–60 | 3.68 | 7.94 | < 0.0001 |
| 61–120 | 8.67 | 8.86 | 0.51 |

## Exploring the role of zinc in improving neonatal dairy calf immunity

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Efficient rearing of youngstock is fundamental to the sustainability of the dairy industry. Despite recent advances in colostrum management, preweaning morbidity and mortality remain a major threat to the future sustainability of the US dairy industry. With preweaning morbidity and mortality being reported at 35% and 5% respectively. Recent evidence suggests that as preweaning dairy calves adapt to extrauterine life, they experience deleterious effects of oxidative stress (OS) on their immune function. Micronutrients, such as vitamins and minerals, are known to play an important role in mitigating OS by preventing overproduction and accumulation of reactive oxygen species (ROS). Specifically, in humans zinc and metallothionein have been shown to be critical for immune cell function and oxidative status. Our hypothesis is, zinc supplementation improves neonatal calf lymphocyte functions relevant to vaccine responsiveness by ameliorating oxidative stress through increased metallothionine antioxidant function. This hypothesis will be addressed by isolating peripheral blood mononuclear cells from neonatal dairy calves. Zinc supplementation will be used to enhance metallothionein production and silencing RNA will be utilized to inhibit metallothionein production in vitro. Metallothionein production will be quantified using gene expression and protein concentration. Cells will be oxidatively challenged and activated to evaluate subsequent changes in lymphocyte functions relevant to vaccine responsiveness. The ultimate objective of this project is to improve neonatal calf lymphocyte functions relevant to vaccine responsiveness by ameliorating oxidative stress through increased metallothionein antioxidant function.

## Parenteral antioxidant supplementation at birth improves the response to intranasal vaccination in newborn dairy calves: A pilot study.

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Newborn calves experience oxidative stress throughout the first month of their life, which is known to decrease lymphocyte functions relevant to vaccine responsiveness. Thus, this study aimed to determine the extent to which parenteral antioxidant supplementation given at birth improves the response to an intranasal viral vaccine in the first month of life of newborn dairy calves. For this, 21 calves were randomly assigned at birth to one of two commercially available antioxidant micronutrient supplements or a placebo group receiving 0.9% sterile saline (n = 7/group). Serum and nasal secretion samples were collected before administration of treatments and an intranasal vaccine against respiratory viruses (bovine herpesvirus type 1, bovine syncytial respiratory virus, and parainfluenza 3), and once weekly for the first four weeks of age. Systemic redox balance was determined in serum. Immunoglobulin A specific for bovine herpesvirus 1 and bovine syncytial respiratory virus was quantified in nasal secretions as a proxy to intranasal vaccine responsiveness. Our results showed that parenteral administration of antioxidants at birth improved calves' redox balance. Additionally, calves receiving antioxidant supplementation had higher concentrations of immunoglobulin A in their nasal secretions than calves in the control group. Thus, we conclude that supplementation of calves with antioxidants at birth could be a practical strategy to improve intranasal vaccine response. Future larger studies should evaluate the extent to which this increased mucosal response to intranasal vaccination could result in decreased calf morbidity and mortality.

## Comparison of calf preweaning morbidity, mortality, and performance among categories of passive immune transfer in a large dairy herd

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Four categories of passive immunity transfer (PIT) were recently proposed in response to the widespread high preweaning morbidity and mortality risks in calves with adequate PIT when a dichotomous classification was used. However, the risks of preweaning morbidity and mortality in these PIT categories have not been compared to date. Thus, the objective of this retrospective observational study was to compare dairy calf morbidity, mortality, growth until weaning, and reproductive efficiency until first calving among the categories of poor (<5.1g/dL total protein), fair (5.1 – 5.7 g/dL), good (5.8 – 6.1 g/dL), and excellent (>6.2 g/dL) PIT. For this, the records from 4,199 dairy calves born January 2014 - April 2017 in a commercial dairy farm in Michigan were analyzed. These calves had been randomly selected for weekly serum total protein determination on calves aged 2 to 7 days old. For each calf, data regarding disease status, growth, reproductive performance, and removal from the herd were extracted from the farm's software database. Associations of PIT categories with disease events (diarrhea and/or pneumonia), reproduction indices (age at first insemination, pregnancy, and calving; and number of inseminations), removal from herd, and average daily gain at weaning were evaluated by Cox proportional hazard survival analysis and mixed models. Compared to calves with excellent PIT, calves in the inferior PIT categories showed increased preweaned disease risk (e.g., diarrhea: poor (Hazard Ratio (HR) = 1.49; 95%CI: 1.22-1.82), fair (HR =1.32; 1.16-1.51), good (HR = 1.14; 1.02-1.29)). The preweaned mortality risk was also higher in calves with poor PIT (HR =4.27; 1.97-9.27) compared to excellent PIT. However, mortality risks were not statistically different between calves with fair or good PIT and those with excellent PIT. Similarly, calves with poor PIT were less likely to be inseminated (HR = 0.36; 0.29-0.44) or to reach first calving (HR = 0.76; 0.61-0.94). However, there were no differences in ADG (P = 0.56) across PIT groups. Our results confirm the positive effects of optimal PIT in calves' preweaned health and post-weaning reproductive efficiency. Although conducted in one herd, this study can be used to illustrate the impact of PIT on future calf performance.

## Impact of 2 vs. 1 colostrum meals on failure of transfer of passive immunity, pre-weaning morbidity and mortality, and performance of dairy calves in a large dairy herd

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Failure of transfer of passive immunity (FTPI) due to inadequate ingestion of colostral immunoglobulins by calves is associated with increased mortality and morbidity risks. Feeding calves a sufficient amount of quality-tested colostrum within the first hours of life is essential for successfully transferring passive immunity. Many farms have implemented a second meal of colostrum to maximize the opportunities for passive immunity transfer. However, excellent passive immunity can be achieved with a single feeding of sufficient quality-tested colostrum. Moreover, there is currently no evidence demonstrating the impact of a second colostrum feeding within 24 h of life in calves receiving adequate volumes of quality-tested colostrum in an initial feeding. Hence, the objective of this retrospective cohort study was to compare the risks of FPTI, pre-weaning morbidity and mortality, and growth and performance between dairy calves that received one or two feedings of colostrum. For this, the health and production records of a large dairy herd were analyzed. At this farm, newborn calves receive 3 L of quality-tested colostrum soon after birth, followed by another 2 L 5–6 h later. However, at times of shortages of colostrum, calves only receive the initial 3 L meal. The records of 2,064 male and 2,272 female calves were analyzed, where 4,156 and 180 calves received 2 and 1 colostrum meals, respectively. Data from both sexes were included in the analysis of the risks of FTPI, morbidity, and mortality; however, only data from heifer calves were utilized for growth and performance analysis. Survival analysis, and logistic and linear regression models were used to investigate the impact of receiving two feedings of colostrum on calf FTPI status, morbidity, mortality, reproductive indices, pre-weaning average daily gain (ADG), and first lactation 305-d Mature Equivalent milk production (305ME). Calves that received two feedings of colostrum had lower odds of FTPI, a lower probability of being treated for respiratory disease, diarrhea, or any disease, and a greater pre-weaning ADG. However, there was no association between the number of colostrum feedings and pre-weaning mortality, and the probabilities of first insemination and first calving, although heifers receiving two colostrum feedings tended to receive fewer inseminations and to have a greater first lactation 305ME. Collectively, our results suggest that feeding calves a second feeding of colostrum 5–6 h after the initial feeding soon after birth could be an effective strategy to decrease FTPI and morbidity and optimize ADG in dairy calves pre-weaning.