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INVESTIGATING POTENTIAL IMPACT OF VITAMIN AND MINERAL STATUS IN  
STILLBORN CALVES

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## ABSTRACT

Dairy and beef cattle industries continually face economic loss due to stillbirth. While dystocia is estimated to cause the majority of stillbirths, the remaining 30-40% remain unaccounted for, and bode further cost for the industry unless studied. The objective of this study was to investigate other possible related causes of stillbirth, emphasizing on nutrition, in Pennsylvania dairy and beef calves. This study submitted stillborn calves, defined as late term fetus at least 265 days of gestation born dead or died within six hours of birth with no signs of dystocia, through the Penn State Animal Diagnostic Laboratory. Samples were submitted for microbiologic testing to address BVD, IBR, leptospirosis, Neospora, and routine microbiologic culture. Liver samples were collected and submitted for mineral analysis ICP/MS for calcium, magnesium, cobalt, iron, manganese, selenium, and zinc. Liver vitamin A and E concentrations were determined via HPLC methodology. All values were determined on a wet weight bases and converted to dry weight based on measured dry matter ratio of liver samples. A total of 27 stillborn calves were collected meeting the study definition; each of these were categorized based on summary demographics, physical parameters, microbiologic findings, and nutritional findings. There was equal distribution of bull and heifer calves with extra samples unreported for gender (5/27, 18.5%). Of the submissions, the majority of the calves were dairy (20/27, 74.07%). Of the dairy, the most breeds submitted were Holstein (19/20, 95%). For beef calves submitted (7/27, 25.92%), the breeds varied, but the most were Angus (5/7, 71.42%). Mean crown-rump length and birth weight was  $93.8 \pm 11.1$  cm and  $66.4 \pm 18$  lb for all samples,  $93.7 \pm 9.3$  cm and  $68.3 \pm 19$  lb for dairy calves and  $91.8 \pm 8.2$  cm and  $62.0 \pm 16.5$  lb for beef calves, respectively. Two cases were considered outliers and not included in these body weight means: one large beef calf (121 lb) and one small dairy calf (36 lb). No calf had inflated lungs, with respectively 69% and 31% none or partial lung insufflation. Two (7.4%) calves were considered to have congenital defects, up to possibly 7 (25.9%) calves had an identified infectious agent and 17 (63%)

of cases had no definitive stillbirth diagnosis. Mineral and vitamin concentrations were compared to Michigan State's laboratory fetal/newborn-based criteria. Vitamin A deficiency ( $<8 \mu\text{g/g DW}$ ) was the primary (18/27, 67%) vitamin abnormality. Low hepatic mineral concentrations were seen with cobalt (13/27, 48.1%), selenium (11/27, 40.7%), manganese (7/27, 25.9%), iron (5/27, 18.5%), copper (2/27, 7.4%) and zinc (3/27, 11.1%). Excessively high iron and zinc concentrations were found in 6 (22.2%) and 11 (40.7%) cases, respectively. This data suggests an influence of vitamin and mineral deficiency on neonatal calf health, and may affect stillbirth incidence in dairy and beef industries. To better understand the effect of each nutrition status on the stillbirth, further study is necessary.

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## **Chapter 1**

### **INTRODUCTION**

Surveys have well documented the increase in percentage of stillbirths in the dairy and beef industry. As the reproductive performance in dairy herds declines with cow culling rates increasing, newborn calf survival is critical to maintaining financial stability and profitability for dairy and beef herds. While this phenomenon is mostly localized to the Holstein breed in the dairy industry, the incidence of stillbirths economically impacts both dairy and beef industry. This affect ripples out beyond the borders of the United States, and has become a global dilemma. One of the challenges in studying stillbirth problems is the lack of consensus in defining a stillbirth. Published studies may or may not include a period of time prior to birth and may extend the period to 48 hours after birth. Factors occurring during pregnancy and calving augment the risk of stillbirth. Hormone levels, twins, calf gender, previous calving ease, month calving, dam's age, infection, dystocia, and nutrition, are factors that may increase the chance of a stillborn calf. About fifty percent of the stillborn deaths are known to be from dystocia, while infectious disease and congenital abnormalities account for another 5-10% leaving the remaining 35-40% of cases undiagnosed, leaving the opportunity to investigate other potential causes such as nutrition.

To further understand this impact of nutrition, a survey study was completed to address the potential role of mineral and vitamin status on prevalence of stillborn calves in dairy and beef herds in the Commonwealth of Pennsylvania. For the purpose of this study, stillbirth was defined as the calf's death within six hours of birth, or as a late-term fetus in at least 265 days of gestation (to a cow's normal gestation length of 280 days). The study objective was to investigate selected minerals and vitamins in stillborn cases identified on beef and dairy farms where dystocia was not the underlying issue. Measured concentrations of vitamins A and E, and minerals calcium, cobalt, iron, magnesium, manganese, selenium, and zinc were determined in collected stillborn cases. Further observations regarding the crown rump



length, animal's production type and breed, weight, gender, appearance, hair coat, preservation state, and any gross lesions were also noted to look for further correlations. The null hypothesis of the study would suggest there was no relationship between the nutritional status of the calf and the stillbirth condition. Nutritional status was compared to current laboratory reference ranges considered normal for full term fetuses assuming no colostrum was consumed by the calf. Results of this study should provide any trends in nutritional data for the newborn calf suggesting a need for changes in reference ranges or implicate the role of nutritional deficiencies or toxicities in the pathogenesis of stillbirth.

## **Chapter 2**

### **LITERATURE REVIEW**

Across studies, the case definition of a stillborn remains quite variable. A few define a stillbirth as a calf that is born deceased while others expand the time frame to be up to 48 hours after birth (NAHMS, 1996, 2007). In contrast, many European studies define stillbirth as calves born dead (Citek et al., 2011) or up to 24 hours following birth (Eriksson et al., 2004; McDermott et al., 1992). This variation in the “case definition” of a stillborn makes it difficult to interpret and compare prevalence data across studies. Waldner et al., (2010) defined stillbirth to be any calf born with a complete hair coat and incisor teeth erupted to be considered full term or death within one hour of birth. Observations of increasing stillbirths in the dairy and beef industries around the world have instigated this current investigation. In Sweden, the rate of stillbirths of Holstein cattle has risen from 6.0 to 10.3 percent in twenty years (Berglund et al., 2003). In Canada, Dairy Network 2007 statistics had also revealed Holstein first calving stillbirth incidents rising two percentages from 10 to 12 over a five-year span (Canadian Dairy Network). In the same year, the United States’ National Animal Health Monitoring System calculates its average loss of dairy calves to stillbirth as 8.1 percent, and of beef calves to stillbirth as 3 to 5 percent (NAHMS, 2007). Visibly, the data collected reflects an internationally large dilemma in both the dairy and beef industries.

The economic impact of these losses proves substantial. On-farm calf mortalities cause decreased milk production and income from animal sales that would have been generated in the future from these calves (Shahid et al., 2015). For example, Dhakal calculated the extra cost of calving assistance to range from \$96.48 to \$397.61 (Dhakal et al., 2013). Data from Ontario, Canada (dairy cow population of 340,000) portrays a tangible number of the overall cost of stillbirth to the industry. To these 340,000, about 300,000 calves are born each year. Thirty percent of these 340,000 cows are first lactation and give birth to about 90,000 of the calves. If one were to reduce the current stillbirth rate of 12 percent to 3 percent producers would have 8,100 more live calves per year from just these first calvings. Further applying this concept to

second and later calvings and decreasing these stillbirths from 6 to 1.5 percent, the remaining 210,000 calvings would allow an additional 9,450 more live calves, and an annual \$4.8 million saved (Canadian Dairy Network). This money, returned to the beef and dairy industries, could be very beneficial to both producer and consumer as the price of each cow does not have to be compensated for in product price.

Not only does a stillbirth immediately impact product output, but also impacts the long-term health of the herd. Shahid et al., (2015) found correlations between rising stillbirths and average mortalities increasing from 1 to 5 percent in 1990 to 2007. Evidencing this connection, main reasons for cow removal from the herd included primarily, natural death, and secondarily, problems in reproduction in 17.7 percent of the cases. For each cow that gave birth to a stillborn calf, the mortality hazard for that cow was calculated to be 10 percent greater. In terms of the herd, each 5 percent increase in herd stillborn calves associated to a 40 percent further increase in problem cows. Additionally, another 8 percent greater mortality risk arises for cows with these unsuccessful calvings as each stillborn lengthens the cow's dry period past a reference dry period of 31 to 70 days (Shahid et al., 2015).

### **Causes of Stillbirth**

Studies have identified many factors affecting stillbirth incidence including dystocia, genetics, cow's age, twin births, infection, and external management. Investigating each element may lead to on-farm strategies to ameliorate this growing problem.

#### ***Dystocia***

Dystocia, the abnormal labor or birth due to the shape, size, or position of the fetus, accounts for about 50 percent of dairy cattle stillbirth. As emphasis surmounts on reproductive efficiency, more than 63 percent of producers ranked calving ease and birth weight as their most important selection criteria in a 2007-2008 beef survey conducted by Waldner (2014). With this push to rapidly increase weaning weights and average daily gains in the commercial cow-calf industry, the diverging incompatibility of calf size to dam pelvic and vulvar conformation grows increasingly evident (Waldner, 2014).

#### ***Holstein Genes***

In all countries, the Holstein genes dominate the dairy industry for its high milk production

capabilities. However, the increased exports of North American Holstein genes parallel the increasing incidence of stillbirths: while the milk production per cow has increased, the health and fertility traits have declined (Dhakal et al., 2013). For example, on average, 23 percent of primiparous Holstein cows require assistance at calving, and over a 12-year study, Lombard et al. (2007) found that 7.1 percent of the calves were stillbirths. Overbreeding the Holstein genes may be a consideration that leads to this problem, as inbred calves have been found to have higher birth weights and more calving difficulties (Hinrichs and Thaller, 2011). Some studies have also found stillbirth to be a heritable trait (Pryce et al., 2006). Thus, if inbreeding is occurring and this breed is spreading internationally in the dairy industry, the trait becomes further amplified. Ideally, a breeding program could select for a reduction in calving difficulty and shorter gestation length traits that would reduce the incidence of stillbirth, particularly as gestation length has a heritability of 0.44 (Pryce et al., 2006). By implementing a crossbreeding system, scientists have been able to reduce calving difficulties in select herds (Lombard et al., 2007; Dhakal et al., 2013). Through testing the genetic crosses between Jersey and Holstein cows, Olson, found that crossbred calves born from a Holstein dam and Jersey sire were much less likely to be stillborn, due to the larger Holstein pelvis, and smaller Jersey stature (Olson et al., 2009).

### *Age*

Heifers further contribute to the earlier-mentioned disproportion of stillbirths as they are bred for the first time at about 60 percent of mature body weight to carry its first pregnancy while still growing. This alters not only the nutritional environment in utero (Swali and Wathes, 2007), but also the calving ease due to an immature pelvis (Sorge et al., 2007). In general, stillbirth rates are considerably higher for calves of heifers than for calves of older cows. From a 12-year study, Lombard found that 11 percent of these primiparous dams produced stillborn calves—a much higher percentage than the 5.7 percent of stillbirths by more matured multiparous dams (Lombard et al., 2007). A study by Sheila McGuirk concluded that the optimal age at first calving ranges between 22 and 26 months. Any deviation from this range for a first time calving, places the cow at a greater risk of a stillborn calf. Over-all optimal age was studied by Waldner, who reported that the lowest risk of stillbirth presents in mature beef cows between five and ten

years of age (Waldner, 2014).

### ***Infection***

Infection is another element to consider of stillbirths: past studies by Mee concluded that infection in the fetus might increase the risk of stillbirth (Long et al., 2010). Pathogens such as brucellosis, leptospirosis, and infectious bovine rhinotracheitis (IBR) infecting the cow may cause stillbirths as well as Bovine Viral Diarrhea Virus (BVDv) and *Neospora caninum* (Berglund et al., 2003). In a previous study by Waldner et al., (2010), lesions in the thyroid gland, liver, and skeletal cardiac muscles were most commonly found during necropsies of the stillborn calves. These degenerative skeletal muscle lesions were not typical of selenium deficiency or white muscle disease as one would think, and instead were determined to be due to pneumonia and placentitis (Waldner et al., 2010). Therefore, in order to discern between each stillbirth cause, infection is a factor necessary to take into account when evaluating each death's cause.

### ***External Factors***

#### **Management**

Stillbirths may also have a non-physiological correlation. The recently increasing average herd size accounts for less calving supervision time (Berglund et al., 2003) and impacts variables such as the calving interval. A longer calving interval affects the dry period of the cow: with an unsuccessful pregnancy, the interval will be longer with a consequential longer dry period. A cow with a shorter dry period of 41 days has been found to have 37 percent lower odds of mortality versus a cow with a dry period greater than 71 days (Shahid et al., 2015). The cow's shorter dry period improves energy balance and decreases fat mobilization during the cow's first month of lactation, and thus has fewer metabolic problems. However, a longer dry period increases such risk for over-conditioning and creates possibilities for metabolic disorders in the cow. With a stillborn in the equation, it dually causes the calving interval to increase while also making future pregnancies more difficult. In a study, Shahid found a link that herds with calving intervals greater than 13.9 months held a 1.78 times greater likeliness to have a higher level of mortality than herds with an average calving interval less than 12.9 months (Shahid et al., 2015).

In other management cases pertaining to the environment of the herd suggests a poor environment

accentuates the genetics of heritable stillbirths and these reproductive characteristics, and thus reflect the importance of management (Ouweltjes et al., 2015). Management discrepancies in this case may include insufficient monitoring and intervention around parturition that would increase risks of stillbirth versus appropriate timing of interventions during the birthing process and immediately feeding colostrum to the calf. Ouweltjes has found that as heritability is connected to the cow's outcome in subordinate environmental conditions, selecting an appropriate sire with the genetics to improve livability may be twice as effective for decreasing numbers of stillbirth (Ouweltjes et al., 2015), and suggests a tactical approach for better selection in a stressed setting.

### **Environmental or temporal**

Total precipitation during the previous growing season and geographical differences in herd location are other external factors associated with stillbirth. A study analyzing the calving month found that calves born in May were less likely to die at or near birth than calves born in December and January, or calves between June and November (Waldner, 2014).

### **Nutrition**

The previous year's total precipitation influences the quality and energy and protein content in the forage the animal digests (Reeves et al., 2013). With higher precipitation, the next year will be much richer in energy nutrient forages to better sustain the cow and calf. Furthermore, proper nutrition is essential for maintaining body condition score around five (1-9 scale) for gestation. Waldner (2014) found that the odds of stillbirth were 1.69 times higher in beef cows with a body condition score less than five, lacking the proper nutrition. In addition, cows that gained body condition scores between pregnancy testing and calving were less likely to require assistance at calving than cows that had no change in body condition score (Waldner, 2014). As most gestation periods continue through the winter, a higher total precipitation the year before ensures nutritious forage throughout the winter to help build this body condition score and warrants an easier delivery.

### **The Calving Process and its Effects**

Investigating the calving process is essential for further understanding the various factors that

influence a stillbirth. In the current beef and dairy industries, heifers kept in the herd reach sexual maturity between 13 and 15 months of age, are bred and calve at two years of age (Gasser, 2013). The goal of a breeding program is a 12-month calving interval where the cow is bred, delivers the calf nine months later, and is rebred after a three-month period. Through this process the optimal cow can reproduce for seven to nine years if it had its first calf young and has little physical problems or disease; however, realistically dairy cows only last 2.5-3.5 lactations (Knaus, 2009). The timing of this process becomes very fragile as past research has established that heifers calving for the first time at less than 22 months or over 26 months are at a higher risk of stillborn births. In addition to age, elements of the calving process such as hormones, physical assistance, and physiology play into the incidences of stillborn calves (Barrier et al., 2012).

### ***Hormonal***

During and leading up to the delivery, the cow's body undergoes multiple hormonal changes. Studies have found stillbirths and abortions corresponding to changes in endocrine profiles during the pregnancy (Berglund et al., 2003). Hormones are vital to maintaining the pregnancy and preventing the return to estrus. Follicular development occurs in wave-like patterns: hormones follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are produced in response to estrogen and Gonadotropin Releasing Hormone (GnRH). FSH, secreted by the pituitary gland and activated by GnRH, develops and grows follicles in the ovaries. The dominant follicle then produces estradiol, which functions in uterine development, onset of behavioral estrus, vaginal mucous secretion, and further release of GnRH from the hypothalamus. and the corpus luteum forms to produce progesterone, which prevents the dominant follicle from ovulating in the first wave of estrus. Once this follicle becomes no longer functional, it secretes hormones estradiol and inhibin that cause an increase in FSH and a new follicle to develop. As the estradiol increases from the follicle, FSH decreases, and LH is released in pulses. As estradiol surmounts from the follicle it causes an LH surge, which induces ovulation. These two waves of estrus ensure that the follicle will be properly developed and fertile by ovulation (at least 10 mm) (Burns, 2011). From this point, the follicle luteinizes to form the corpus luteum that secretes progesterone that would continue to be secreted to maintain pregnancy if fertilization occurs (Burns, 2011). A significant disparity in estradiol-17 $\beta$  has been

found to influence characteristics, such as birth weight or calving ease, that lead to stillbirth. A study by Sorge et al. measured estradiol-17 $\beta$  concentrations from the blood serum and found its concentration depended on the sex of the calf: estradiol-17 $\beta$  was greater in heifers delivering bulls than those delivering female calves, as well as directly proportional to birth weight (Sorge et al., 2007). Estradiol-17 $\beta$  is important to regulating the estrous cycles of cows, as it develops and maintains the female reproductive tissue, and increases during pregnancy due to its production from the placenta. It is thought that during pregnancy, estradiol-17 $\beta$  promotes uterine blood flow, oxytocin receptors, and cervical relaxation important at parturition. Measuring estradiol-17 $\beta$  two weeks from calving, Sorge found that the concentrations were smaller in heifers delivering stillborn calves (531  $\pm$  381 pg/mL), versus the heifers delivering live calves (821  $\pm$  412 pg/mL). Instead of increasing exponentially the last three weeks of pregnancy, as the estradiol should, a lower estradiol-17 $\beta$  concentration indicates an abnormality of the placenta or an abnormality of hormonal signals relaying between the calf, placenta, and heifer. However, Sorge also notes that at parturition, a greater measured estradiol-17 $\beta$  concentration correlates with a greater calving difficulty score, which may imply an appropriate range needed to sustain during gestation (Sorge et al., 2007).

Like estradiol-17 $\beta$ , progesterone concentrations play a key role in calving. Sorge's same experiment found that progesterone concentrations were greater in cows delivering stillborn calves compared to cows delivering live calves. While no correlation was found between progesterone levels and the calf's birth weight or sex, the ratio of estradiol-17 $\beta$  to progesterone proved important to calving ease. A ratio imbalance may lead to lower expression of oxytocin receptors in the uterus and impaired pre-parturition of the soft tissues. This combination leads to weaker bouts of uterine contractions that would have correctly positioned the calf and relaxed the birth canal during calving, which would also explain a longer third stage of labor observed during stillborn calvings (Sorge et al., 2007).

### ***Assistance***

While appearing helpful, assistance during calving may be detrimental to the cow and calf. Assisted deliveries result in hemorrhages, injuries to the central nervous system, increases to rib and limb fractures in neonates, and pelvic fractures in dams (Barrier et al., 2012). In fact, Waldner (2014) analyzed that the



odds of stillbirth in 2002 were significantly higher for calves from cows with reported assisted calvings in 2001. Of the 24,497 full term calves in his study, 2.4 percent of the calves were abortion risks, 8.0 percent were reported assisted, and 0.9 percent had problems post-partum (Waldner, 2014).

### ***Physiology***

Physiological aspects, such as high birth weight, affect calving difficulty and calving mortality, which further causes lower production, fertility, and longevity for the cow (Dhakal et al., 2013). Understanding the correlation between these physiological aspects and calving difficulty allows an opportunity to improve the long-term status of the cow, and survival of the calf by changing these conditions. This may prove beneficial to the high-risk transition period for many health disorders three weeks before and three weeks after calving. During this period, cows with stillbirths hold an increased risk of different post-partum disorders including prolapsed uterus, retained placenta, metritis, and displaced abomasum. Due to these complications, the cows were 41 percent more likely of death or culling from the herd than cows with live calves (Shahid et al., 2015).

Barrier noted another physiological barrier: while birth weights were similar for stillborn and live calves, the stillborn calves were on average 4 centimeters longer. This difference was attributed to a greater chance for the umbilical cord to rupture earlier in the longer calves, or to be clamped longer during the birth process which would lead to hypoxia and the death of the calf (Barrier et al., 2012). An ability to predict these outcomes in future calvings given known dimensions would help the management to prepare adequately for the potentially difficult birth.

### **Gestation and the Placenta**

The gestation process leading up to calving is essential to the outcome of the calf as well. Starting with the sex of the calf, a study established that the gestation length of a heifer to be 281 days and a bull to be slightly longer at 283 days (Fitzgerald et al. 2015). During gestation, the mother denotes priority to the calf by drawing on maternal nutrient reserves any time there is a deficit between daily need and dietary provision (Fitzgerald et al., 2015). However, the limited stores become problematic over a long-term nutritional scarcity. Severe nutrition restriction for at least one-third to half of the pregnancy reduces bovine

fetal growth, and shows particularly evident in pregnant heifers that still require the nutrition for their own growth. Breaking this gestation period down, development of the vital organs precedes development of bones, muscle, and fat where most growth occurs during the last 100 days (Greenwood and Café, 2007). Specifically, reproductive organs begin early in the prenatal period with the primordial follicles presenting around day 74, the antral follicles present around days 91, 120, and 150, and the vesicular follicles around day 250 (Gasser, 2013). Interfering with any of these important developmental time periods may impact the outcome of the calf, or its future reproductive potential.

In cases with restricted nutrition, cases with male calves showed increased growth rate and adiposity, decreased skeletal muscle mass, and altered glucose metabolism and insulin secretion. Further study showed that decreased nutrient intake of dams from 32 to 115 days of gestation did not influence the birth weight of the calf, but did decrease the length of gestation (Long et al., 2010). However, after these 115 days of gestation, around day 125, nutrient restrictions cause intrauterine growth restriction of fetuses.

While food restriction exhibits effect in the early and mid-gestation, nutrient requirements for pregnancy are only considered during the last three months of gestation (Bach, 2012). According to Long's study of varying protein levels during gestation, the cow that received a protein-deficient diet during days 150 to 270 had the most reduced gestation length, reflecting the importance of proper feeding regulations during this time (Lombard et al., 2007). Conversely, adding high levels of concentrate in the last month of pregnancy over-conditions the dam and over-sizes the fetus to increase the risk to stillbirth (Long et al., 2010). For these reasons, in regards to beef cattle, the body condition score of the beef cow must be gradually increased throughout the gestation for optimal calf and dam health. If the body condition score of five (1-9 scale) is not met at parturition, the low body condition has been associated with poor pregnancy rates in the subsequent breeding seasons (Long et al., 2010).

During gestation, the placenta continues to increase in weight until near term with a significant correlation between the placental weight and birth weight of the calf. The placenta is an important barrier and serves to selectively pass through essential molecules to the fetus such as oxygen, carbon dioxide, glucose, certain amino acids, and lipids. Electrolyte minerals usually simply pass through the placenta,

following the water diffusion. While water-soluble vitamins easily can pass through the placental membrane, the fat-soluble vitamins (A, D, E, and K) cannot pass through as easily and are low in the fetal circulation. Particularly during the second half of gestation, this uterine and umbilical blood flow increases exponentially during the latter half of gestation (Greenwood and Café, 2007).

### **Nutritional Effects**

Vitamins and minerals are vital to the growth and health of the fetus and dam. Born without a store of many of the fat-soluble vitamins and one or two metal minerals, calves must attain most of their nutrients and immune status from colostrum. Colostrum raises the plasma lipids, phospholipids, total cholesterol, essential fatty acids, gamma-carotene, and vitamins A and E (Blum, 2006). Without colostrum the immune system may incorrectly develop and the calf will not get the essential nutrients it needs to survive. This colostrum absence and resulting nutrient deficiency allow opportune degenerative conditions to develop, such as myocardial and skeletal muscle lesions due to selenium and vitamin E deficiency that would have provided antioxidative effects. To ensure proper nutrition the industry usually supplements dairy calves with milk replacers fortified with vitamins and minerals, while beef calves solely rely on the dam's milk. The underlying issue is that milk does not contain sufficient trace minerals to support the developing neonate. This difference arises from beef cattle generally on pasture year-round, and fed locally grown forage (Waldner and Blakley, 2014). This locally grown forage, when newly cultivated, is more productive, doubles the percentages of raw proteins, and holds rich contents of calcium, phosphorus, and copper (Braun, 2006). A study conducted by Waldner to evaluate vitamin and mineral levels in stillborn calf livers found no significant variation among micronutrient concentrations, but did identify magnesium, copper, and vitamin E to be most deficient. Waldner in his study defined stillbirth to be greater than six hours after birth, and found that deceased calves three days of age or later were very likely for selenium deficiencies (Waldner and Blakley, 2014).

### ***Vitamin A***

During gestation, a deficiency in vitamin A causes failure to maintain a healthy epithelium and may result in resorption of the fetus, abortion, or stillbirth (Fitzgerald et al., 2015). For this reason, early

vitamin A intake largely determines plasma vitamin A status in the cows that acquire their vitamin A from the carotene found in green feed. This level builds up while on pasture during the summer months and depletes during the winter, which is why the previous season's precipitation is a large determinant of vitamin A in the cow. Difficult winter months with low previous precipitation intensifies the potential deficiency, as vitamin A is especially unstable in stored feed and loses up to 50 percent of its nutritive content per year (Waldner and Blakley, 2014).

At birth, calves do not have a reserve of vitamin A and must primarily obtain most of it from colostrum ingestion. Because vitamin A is a fat soluble vitamin, its absorption is not regulated by the requirements of the body, but by the presence of fat in the diet, the degree of emulsification of the fat in the small intestine the presence of vitamin E, the integrity of the small intestine mucosa, and the quantity and quality of protein in the diet (Braun, 2006). Deficiency in the calf has been found to cause vision loss due to the failure of rhodopsin formation in the retina, defects in bone growth, and defects in growth and differentiation of epithelial tissues. One may measure the vitamin A concentration through an animal's hepatic value, as the liver contains approximately 90 percent of the body's vitamin A stores (Waldner and Blakley, 2014). Without a sufficient level of vitamin A in both the cow and the calf, serious complications arise without a proper functioning epithelium, and may be a factor of stillbirth.

### ***Vitamin E***

Waldner also studied vitamin E, another fat-soluble vitamin, to find that more than 75 percent of the calves that died soon after birth had deficient vitamin E concentrations. Like vitamin A, vitamin E is also usually found in green grass; however, the liver is not a primary storage site for vitamin E and hepatic levels simply reflect the recent uptake (Waldner and Blakley, 2014). In the body vitamin E acts as an antioxidant preventing the unsaturated fatty acids from converting to epoxides or peroxides and causing tissue damage to ultimately develop muscular dystrophy (Braun, 2006). A deficiency of vitamin E to the cow or calf that could cause such tissue damage and upset the formation of the calf may well-explain stillborn losses.

### ***Minerals***

Minerals such as calcium, phosphorus, magnesium, and fluorine play an important structural role forming the components of body organs and tissues; silicon is integral to the bones and teeth; phosphorus and sulfur to muscle proteins; and zinc and phosphorus to structural stability to molecules and membranes (Dhakal et al., 2013). Physiologically, minerals serve a multitude of functions: as sodium, potassium, chloride, calcium, and magnesium circulate in bodily fluids and tissues as electrolytes to maintain osmotic pressure, regulate cell replication and differentiation, acid-base balance, membrane permeability, tissue irritability in the blood, cerebrospinal fluid, and gastric juices. Catalytically, minerals serve as specific structural components of enzymes and hormone systems, or less specific activators. With such an array of duties, adequate mineral concentrations are necessary to maintain the majority of the body's systems (Dhakal et al., 2013).

Iodine is essential to the thyroid as a major component of thyroid hormone synthesis (thyroxine,  $T_4$ , and tri-iodothyronine,  $T_3$ ) that control energy metabolism and protein synthesis in cells. A study found evidence indicating physiological state and metabolic status of the cow are the most important factors affecting synthesis of these hormones, and due to metabolic changes at parturition and the initiation of lactation, concentrations of  $T_3$  are lowest at this time (Anderson et al., 2007). Other factors such as temperature, botanical composition of the pasture, selenium status, and the presence of goitrogens in the diet also affect the systemic levels of iodine. Iodine deficiency is common in the cattle industries, and is extremely detrimental to the reproduction estrous cycles, conception rates, and stillbirths. For calves, the consequences also include congenital abnormalities, goiter, cretinism, impaired brain function, and hypothyroidism (Hetzel and Mano, 1988). In a study evaluating stillborn calves with iodine deficiency, 15.8 percent of these iodine deficient calves matched the values for goiter with a thyroid weighing between 7 and 287 grams. This was far above the normal upper limit of 6.5 grams; furthermore, over two-thirds of these samples weighed in excess of 14 grams—the definition of goiter. Regarding their hormone levels, the majority (82.4 percent) of the calves had reported serum  $T_4$  concentrations below the 80 nmol/L limit. It was also found that the concentrations of  $T_4$  were maximal between May and August birthdates, but lowest in October as the outdoor forage began to decrease (Anderson et al., 2007).

Another mineral studied, iron, is essential to the body through aerobic oxidation of carbohydrates, electron transfer and protection against oxide radicals (Dhakal et al., 2013). Waldner illustrated an age dependent level of iron with calves from heifers having lower liver iron concentration than calves from older cows. Waldner also noted that the fetal liver iron concentrations did not change during gestation (Waldner and Blakley, 2014), which may indicate the importance of a high iron store at the beginning of contraception for the fetus.

Of the macronutrients, magnesium appears to be quite important with a large role in its activation of over 300 enzymes, role in biosynthetic processes, and maintenance in electrical potentials. Of the magnesium absorbed, 65 to 70 percent is delivered to bones, 15 percent is delivered to the animal's muscle, and the other 15 percent is delivered to soft tissues. Since normal homeostatic mechanisms are not sufficient to regulate blood magnesium in the cows, they depend on a steady supply from digestion to maintain a normal level. Due to its importance and need for steady supply in the diet, consequences of any deficit in magnesium may be fatal. Deficiency symptoms usually pertain to malfunctions, malformations, and acute or chronic diseases ranging from excitability to anorexia, hyperemia, convulsions, frothing, and calcification of soft tissues from low concentrations particularly in the plasma and cerebrospinal fluid (NRC, 2000). Studies have found that the insufficient magnesium levels pathologically affects the cardiovascular and renal tissues in cattle to cause small artery disease and other cardiac disorders. The consequences of the low magnesium reflect increasingly prominently in younger cows, and may be congenital (Seeling, 1982). Acting as such an important component of the bodily functions and formation, magnesium shows large potential to hold an impact on the gestational health of an unborn calf.

As a micronutrient, manganese shows less essential to the metabolism of the cow. It acts as components of enzymes pyruvate carboxylase, arginase, and superoxide dismutase that acts an activator for a number of other enzymes. As it is needed in such small concentrations, studies testing the upper range of manganese and seeing no adverse effects as the cow goes over the recommended concentration have determined that there is not much concern for a deficiency or a toxicity level of manganese in the cow's system (NRC, 2000).

Also in Waldner's study, the molybdenum concentrations in calves were within the normal upper limit. While excess molybdenum may only directly affect purine metabolism and sulfite oxidation (Dhakal et al., 2013), the surplus interferes with copper absorption and dietary iron counts (Waldner and Blakley, 2014).

One of the most important minerals, selenium, has been found to be vital to many bodily processes including carbon dioxide formation, alcohol metabolism, protein digestion, hydrolysis of phosphate esters, cell replication, and wound healing (Suttle, 2010). It protects against naturally occurring muscular dystrophies through removing hydroperoxides and converting thyroxine to active forms (Dhakal et al., 2013). Selenium deficiency normally arises through animals grazing on selenium-deficient soils (Braun, 2006), and leads to reproductive disorders, poor growth, and calf health problems (Waldner et al., 2010). In the study by Waldner, the fetuses with heart lesions had lower hepatic selenium concentrations than in aborted fetuses with no lesions, suggesting that the selenium deficiency played a contribution to the heart lesions (Waldner and Blakley, 2014).

Observing zinc deficiencies reveals trends of parakeratosis, defective hoof formation, loss of appetite, and unthriftiness (Braun, 2006). Liver concentrations reflect these deficiencies, but have been reported to not be a completely reliable method to diagnose zinc adequacy. Instead, a diagnosis is obtained through clinical observation of a stiff gait, swelling of the distal extremities, parakeratosis, deformed hooves, abnormal appearing hair, hair loss around the muzzle, ears, and eyes, lymphoid depletion of the thymus, lymph nodes, Peyer's Patches, and gut-associated lymphoid tissues. Investigating the effects of feeding trace mineral supplements before calving, Waldner (2014) found that calves from a herd with this supplementation had greater liver zinc concentrations than calves from herds without any supplementation. Furthermore, Waldner cited an association between the liver zinc concentrations and a winter water supply iron concentration greater than 40 ppm (Waldner and Blakley, 2014). Where a farm with less iron concentration in the water could affect the entire herd data and perhaps be increasing the risk for stillbirth as well as other reproductive difficulties.

## Chapter 3

### MATERIALS AND METHODS

Although stillborn calves were submitted through the Penn State Animal Diagnostic Laboratory, procedures in this study were reviewed by the Pennsylvania State University Institutional Animal Care and Use Committee (Protocol #42011, Diagnostic Investigation of Stillborn Calves from Dairy and Beef Cattle in Pennsylvania). Funding for the project was provided by a grant from the Pennsylvania Department of Agriculture.

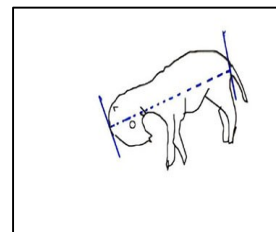
#### Case Submissions

Arrangements were made with the Penn State Animal Diagnostic Laboratory to identify potential case submissions of stillborn calves. Additionally, announcements were distributed to Pennsylvania dairy and beef cattle producers and veterinarians soliciting stillborn submissions for participation in the study. The stillborn case definition used for this study was a late term fetus at least 265 days of gestation born dead or died within six hours of birth with no signs of dystocia. Cases associated with a history of dystocia were selected against in an effort to obtain stillbirths potentially due to other reasons. An initial 15 samples were submitted requiring a greater effort to receive cases. A letter was drafted and sent to Pennsylvania veterinarians to expand the database to send in stillborn calves as a referral. With this approach, veterinarians were encouraged to send the calf to the Penn State Animal Diagnostic Lab or have it picked up by the Penn State Extension team. A third option was also given if the delivery was not feasible, and directions for an on-farm necropsy were given to ensure each calf's processing remained consistent with the study. Veterinarians were directed to complete an included case submission report recording the calf demographics. For this necropsy, a detailed set of instructions, the PADLS Stillborn Necropsy Kit, were enclosed with the letter, and tissue samples were to be sent to the Penn State Animal Diagnostic Lab for processing (see **Appendix A** for the letter sent, stillbirth kit, and case submission form).



## Necropsy Procedures

Calves delivered to the Penn State Animal Diagnostic Lab (ADL) had a consistent case submission description completed. An ADL Accession number was first generated to keep each sample's records systematized, the owner, and case veterinarians were also recorded. To evaluate the size of the animal, the crown-rump length was measured from the forehead to the rump (Figure 1):



**Figure 1.**  
**Measurement of crown-**  
**rump length of calf**

Further information such as the gender, body weight, production types (dairy or beef), breed, appearance, hair coat description, preservation state, gross lesions, thyroid description, whether a thyroid histology sample was taken, lung inflation status, lesions on the lungs, and liver appearance were noted. For documentation purposes, it was also stated if the samples were sent to New Bolton Center and Michigan State University for additional diagnostics, and if tissues were retained and submitted for PCR for bovine virus diarrhea BVD, for fluorescent antibody (FA) testing for infectious bovine rhinotracheitis (IBR), and bacteriology.

## Microbiologic Testing

To test each calf for evidence of disease, specimen tissues were examined for any pathological lesions, with detailed descriptions recorded by the Pennsylvania Animal Diagnostic Laboratory. Histopathologic examination was conducted on each sample to identify any manifestation of disease in the brain, spleen, kidney, liver, heart, thyroid gland, lung, abomasum, tongue, intestinal tract, skeletal muscle, and placenta, if submitted.

Microbiological tests were also conducted on tissue specimens. An aerobic culture was made on fresh liver and lung tissue samples to test for *Histophilus*, if the result was a mixed culture, the presence of the organism was confirmed, if no growth, it would be considered negative for any of the *Haemophilus* or *Histophilus* recovered. Aerobic cultures were also completed on fresh placenta tissue and abomasum content fluid to test for *Histophilus*. To test for *Salmonella*, a diagnostic culture was fixed on a pool of mixed tissues (placenta, liver, lung, and abomasum contents) for the recovery of the bacteria and then

PCR performed. On fresh kidney tissues, PCR was also completed to test for *Leptospira* spp.

To check for parasites, a PCR was performed on brain and heart fresh tissue samples testing for *Neospora caninum*. Virology for the Bovine Viral Diarrhea (BVD), Infectious Bovine Rhinotracheitis (IBR), and Bovine Herpes Virus (BHV1) was performed using PCR (for BVD) and Reverse Transcription–RT-PCR (for IBR and BHV1) on multiple fresh mucosal tissues.

### **Nutritional Testing**

Hepatic mineral analysis was performed at The University of Pennsylvania's New Bolton Center's Toxicology laboratory. Tissue mineral concentration was determined using an intercoupled plasma spectroscopy method with mass spectroscopy (ICP/MS). Fresh liver samples were analyzed for calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn). Mineral results were reported in parts per million (ppm) on a wet weight basis. A second liver sample from each case was also sent to Michigan State University's Diagnostic Center for Population and Animal Health to quantify vitamin A and E concentrations. Vitamin concentrations were determined by high pressure liquid chromatography (HPLC) measuring total retinol and  $\alpha$ -tocopherol and reported in micrograms per gram (ppm) on a dry weight basis. A dry matter ratio was also provided by Michigan State University from each liver sample tested for the conversion of wet matter mineral concentration results from New Bolton Center dry matter values for consistent comparison. This dry matter value was calculated through dividing the weight of a dried sample by the total wet weight of the sample. Measured liver mineral and vitamin concentrations were compared to laboratory reported expected ranges for fetuses on a wet or dry weight basis (Appendix table).

### **Statistical Analysis**

Measured liver mineral and vitamin concentrations were the dependent variables of interest in the study. Statistical models were analyzed using SAS a common software system for statistical analysis. Population demographics for each mineral and vitamin were characterized using Proc UNIVARIATE.

An analysis of variance (ANOVA) procedure (Proc GLM) was used to determine effects of independent variables on hepatic mineral and vitamin concentrations. Independent variables included farm of origin, breed of the calf, sex of the calf, and crown-rump length. Separate box and whisker plots were then compiled with this information to illustrate the differences and effects each dependent variable held on the liver vitamin and mineral concentrations.

## Chapter 4

### RESULTS

#### Summary Demographics

A total of 27 stillborn calves were collected over the course of this study for analysis. Each of these were categorized based on summary demographics, physical parameters, microbiologic findings, and nutritional findings (Table 2).

**Table 1 Breed and gender demographics for submitted stillborn calf submissions**

Percent Bull:	50% (11 of 22 known samples)
Percent Heifer:	50% (11 of 22 known samples)
Percent Dairy	74.07 % (20 of 27 samples)
Percent of Dairy Ayrshire	5.00% (1 of 20 samples)
Percent of Dairy Holstein	95.00% (19 of 20 samples)
Percent of Dairy, Heifer:	30.00% (6 of 20 samples)
Percent of Dairy, Bull:	45.00% (9 of 20 samples)
Percent of Dairy, Unknown:	25.00% (5 of 20 samples)
Percent Beef:	25.92% (7 of 27 samples)
Percent of beef Angus:	71.42% (5 of 7 beef samples)
Percent of beef Hereford:	14.29% (1 of 7 beef samples)
Percent of beef Simmental:	14.29% (1 of 7 beef samples)
Percent of Beef, Heifer:	71.42% (5 of 7 beef samples)
Percent of Beef, Bull:	28.57% (2 of 7 beef samples)

There was an equal proportion of bull to heifer stillbirth submissions, with 5 of 27 (18.5%) samples unreported for gender. These samples had been submitted by veterinarians and the requested informational sheet was incomplete, emphasizing the need to have samples submitted directly for laboratory handling. Samples were majority of dairy (74.1%) breeds consisting of 1 Ayrshire and 19 Holsteins. The remaining 25.9% samples were beef breeds, a majority being 5 Angus, 1 Hereford, and 1 Simmental. Of the dairy samples, the majority (45%) were bulls, and the remaining were heifer (30%) or unknown (25%). Of the beef samples, the majority were heifer (71.42%) and the remaining bulls (28.57%). With dystocia being a primary factor for stillbirth, typically bull calves are more associated with dystocia in dairy and beef breeds.

The selection against dystocia causes may explain the greater heifer calves in beef breeds. With the number of unknown gender for dairy cases makes it difficult to make a similar assessment.

### **Physical Parameters**

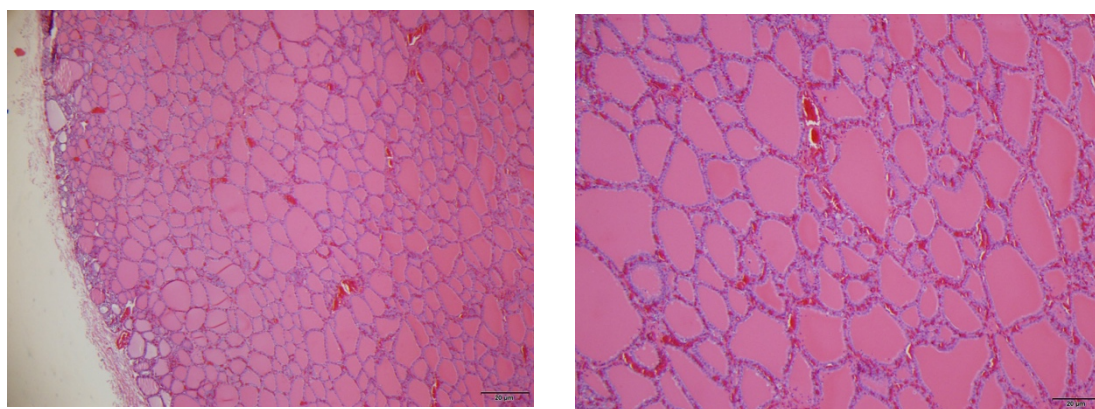
Mean crown-rump length for all samples was  $93.8 \pm 11.1$  cm. Mean crown-rump length for beef calves ( $96.3 \pm 14.0$  cm) was 2.5 cm longer than for dairy calves ( $92.7 \pm 9.9$  cm). This observation is intriguing in that typically today's Holstein calf would be expected to be much larger than Angus or Hereford beef cattle calves suggesting possibly the dairy calves submitted were smaller than expected for the breed. Using a model to predict fetal age based on crown-rump length (**Eq. 1**), the mean crown-rump length would suggest the stillborn calves were 8.6 months or essentially full term. Predicted fetal age for beef and dairy calves based on mean crown-rump length was 8.7 and 8.5 months, respectively. Again this may suggest the stillborn dairy calves were smaller than would be expected for the breed.

**Equation 1:** Fetal Age (mo) =  $\text{SqRt} [\text{C-R Length(in)} \times 2]$  (Roberts, 1986)

Measured body weight for all submissions was  $67.7 \pm 22.5$  lbs. There were two cases considered outliers; one very large (121 lb) beef calf and one very small (36 lb) dairy calf. There was no indication in the case history for the large beef calf that dystocia was present during the calving and this was confirmed by the producer submitting the case. Removing these two outliers does not alter the mean body weight much but reduces the variation in body weight for all cases ( $66.4 \pm 18$  lb). Mean beef calf body weight with ( $71.8 \pm 21.3$  lb) or without ( $62.0 \pm 16.5$  lb) the large calf was greater than mean body weight of dairy calves with ( $65.8 \pm 20.3$  lb) or without ( $68.3 \pm 19$  lb) the outlier. Mean body weight for beef calves would be considered within normal range for the given breeds; however, mean body weight of the dairy calves was much lower than what would be expected for newborn Holstein calves, which typically would weigh between 92 and 95 lbs. Again the body weight of the dairy calves is consistent with the shorter crown-rump length of the stillborn dairy calves of this study suggesting possibly a reduced growth rate resulting in underweight calves.

## Necropsy Findings

Hair coat descriptions and preservation state were recorded as normal for all cases. Gross necropsy finding indicated two physical abnormalities, an enlarged head (case P1313460) and small body (case P1325388); whereas all other cases presented with a normal overall appearance. There was no indication of hydrocephaly in the enlarged head so it was uncertain as if this physical alteration was responsible for the calf's demise. Internal examinations found an abdominal mass attached to the liver (case P1318715) and a ventral septal heart defect (case P1325388). There were no visible evidence of white muscle disease on cut sections of tongue or heart in any cases. Liver physical appearance was considered normal in all cases, except one being identified as friable (case P1417419). Thyroid glands were inspected and samples were collected in 14 of the 27 samples for histology. Overall, thyroid glands observed had no evidence of any gross or histologic evidence of abnormalities (Figure 2), though three were recorded as smaller than normal. No calf had inflated lungs, with respectively 69% and 31% none or partial lung insufflation. This would suggest none of the study cases had survived long enough to have consumed colostrum.



**Figure 2** Representative histologic sections of stillborn calf thyroid gland showing normal architecture and follicular size

## Microbiologic Findings

Of the 27 cases, 11 tissue samples were collected for PCR for BVD, FA for IBR, and bacteriology. All samples from all cases cultured negative for *Salmonella*, *Leptospira spp.*, and *Histophilus*. Of the 27 tissue samples tested for *Neospora caninum*, 2 (7.4%) tested positive (cases P1311120 and P1507155-1).

One case (P1308029) tested positive for BVD, IBR and three *Leptospira* species typically found in a 5-way vaccine. One might question if this collection of positive tests might indicate a vaccination response rather than a clinical disease process. Four cases (P1318715, P1423136 (2 calves) and P1426969) had histologic evidence of mild pneumonia though not all cases had positive bacterial cultures. Positive cultures of *Streptococcus* species, *Staphylococcus aureus* and *E. coli* were identified but no histologic lesions consistent with an infectious or inflammatory process were present in the case with the positive *S. aureus* result. The case in which the *Streptococcus sp.* and *E. coli* had inflammatory changes in the brain. Based on these findings an infectious cause could be attributed to at least 3 (11.1%) cases and possibly 7 (25.9%) total cases depending upon interpretation of results.

### **Nutritional Findings**

Overall mean ( $\pm$  standard deviation) and range of hepatic mineral and vitamin concentrations in the stillborn calves are presented in Table 3. There was an extreme outlier value for hepatic Fe concentration in a dairy calf that skewed the mean value. Mean hepatic Fe concentration without the outlier was  $631.4 \pm 494.2$  and  $584.4 \pm 483.9$  for overall and dairy means, respectively. Effects of gender, production type (dairy or beef) and crown-rump length did not influence mineral or vitamin concentrations due to the wide variation found in each measured nutrient. There was a production type by gender interactions that were significant for Mg ( $P=0.03$ ), Mo ( $P=0.02$ ), and Zn ( $P=0.03$ ). Farm influenced only Ca and Mn concentrations.

The majority of mean mineral and vitamin concentrations were within reference ranges, except for the beef calf vitamin A and Se. For stillborn beef calves mean hepatic vitamin A concentration was 1.21  $\mu\text{g/g}$  less than the low reference range of 8.00  $\mu\text{g/g}$ . Mean hepatic Se concentration was 0.18 ppm less than the low reference range of 1.5 ppm. Mean hepatic Zn was 44.53 ppm above the high reference value of 400 ppm. All other data averages for each vitamin and mineral including vitamin E, Co, Cu, Fe without outlier samples, Mn, and Mo were within reference range values. A few minerals such as Ca and Mg did not have

established dry weight reference ranges for the age range of samples in the study and were not able to determine if each mineral's average from the study were appropriate.

**Table 2 Overall means ( $\pm$  standard deviation), range and means by production type (dairy or beef) for hepatic mineral and vitamin concentrations ( $\mu\text{g/g}$  dry weight basis) from stillborn calves.**

Nutrient	Mean	Range	Means by Cow Type	
			Dairy	Beef
Calcium	$466.4 \pm 219.0$	160 – 1152	$500.7 \pm 235.4$	$368.7 \pm 130.5$
Magnesium	$493.9 \pm 138.5$	6.0 – 736.4	$486.7 \pm 149.2$	$514.5 \pm 109.6$
Cobalt	$0.062 \pm 0.03$	0.021 – 0.12	$0.06 \pm 0.03$	$0.07 \pm 0.03$
Copper	$290.9 \pm 115.3$	97.6 – 519.8	$276.8 \pm 112.0$	$331.2 \pm 123.7$
Iron	$778.7 \pm 905.8$	108.4 – 4607.5	$785.6 \pm 1015.4$	$759.1 \pm 538.0$
Manganese	$4.32 \pm 1.60$	1.11 – 8.00	$4.11 \pm 1.4$	$4.91 \pm 2.0$
Molybdenum	$1.06 \pm 0.43$	0.0 – 1.84	$1.06 \pm 0.45$	$1.07 \pm 0.43$
Selenium	$1.78 \pm 0.69$	0.3 – 3.4	$1.94 \pm 0.72$	$1.32 \pm 0.29$
Zinc	$409.0 \pm 232.4$	64.7 – 963.6	$444.5 \pm 232.3$	$307.4 \pm 216.4$
Vitamin A	$8.3 \pm 7.4$	0.99 – 29.9	$8.8 \pm 8.1$	$6.8 \pm 5.2$
Vitamin E	$11.3 \pm 5.6$	0.19 – 25.8	$12.5 \pm 5.7$	$7.9 \pm 4.0$

Mineral and vitamin concentrations were compared to fetal/newborn-based criteria based on dry matter basis from the Michigan State laboratory (Table 4). This lab has no criteria for Ca or Mg, but the University of Pennsylvania lab has a wet weight reference range between 277 and 320  $\mu\text{g/g}$  for Mg. Based on this reference range 100% of stillborn samples in this study were below the reference range. There was good agreement between wet and dry weight reference values for Cu, but not for Mn, Fe, Se or Zn suggesting a need for improved mineral status criteria amongst laboratories. Similarly, the U. Penn laboratory has a wet weight reference range for hepatic vitamin E between 4.0 and 10  $\mu\text{g/g}$ . Using the dry matter ratio value for each sample to calculate wet weight concentration, 24 (88.9%) of the samples would be characterized as low vitamin E status compared to only 3 (11.1%) using the Michigan State reference range.



**Table 3** Number of data values high above the reference range, low below the reference range, or considered at a deficiency level of the 27 samples.

Vitamin A numbers high:	0%
Vitamin A numbers low:	66.7% (18 of 27 samples)
Vitamin E numbers high:	11.1% (3 of 27 samples)
Vitamin E numbers low:	11.1% (3 of 27 samples)
Cobalt numbers high:	0%
Cobalt numbers low:	48.1% (13 of 27 samples)
Copper numbers high:	0%
Copper numbers low:	7.4% (2 of 27 samples)
Iron numbers high:	22.2% (6 of 27 samples)
Iron numbers low:	18.5% (5 of 27 samples)
Iron numbers deficient:	11.1% (3 of 27 samples)
Manganese numbers high:	0%
Manganese numbers low:	25.9% (7 of 27 samples)
Manganese numbers deficient:	11.1% (3 of 27 samples)
Molybdenum numbers high:	0%
Molybdenum numbers low:	11.1% (3 of 27 samples)
Molybdenum numbers deficient:	3.7% (1 of 27 samples)
Selenium numbers high:	0%
Selenium numbers low:	40.7% (11 of 27 samples)
Selenium numbers deficient:	3.7% (1 of 27 samples)
Zinc numbers high:	40.7% (11 of 27 samples)
Zinc numbers low:	11.1% (3 of 27 samples)
Zinc numbers deficient:	3.7% (1 of 27 samples)

Overall there were 1.6 (range: 0-4) mineral deficiencies and 0.8 (range: 0-2) vitamin deficiencies per calf. Total mineral and vitamin abnormalities or deficiencies per calf were 3.1 and 2.4, respectively. There was no influence of production type on the number of abnormal values. Vitamin A deficiency ( $<8$   $\mu\text{g/g}$  DW) was the primary (18/27, 67%) vitamin abnormality. Low hepatic mineral concentrations were seen with Co (13/27, 48.1%), Se (11/27, 40.7%), Mn (7/27, 25.9%), Fe (5/27, 18.5%), Cu (2/27, 7.4%) and Zn (3/27, 11.1%). Excessively high Fe and Zn concentrations were found in 6 (22.2%) and 11 (40.7%) cases, respectively. Figures 3-14 present the hepatic mineral and vitamin data in box and whisker plots reflecting representations of mean “high” and “low” data percentages above and below the reference ranges of all, dairy, and beef data.

Figure 3

Overall, dairy and beef hepatic Vitamin A concentrations (dry weight basis) for 27 stillborn calves. Shaded area represents the reference range for the nutrient based on Michigan State University Nutrition Laboratory data.

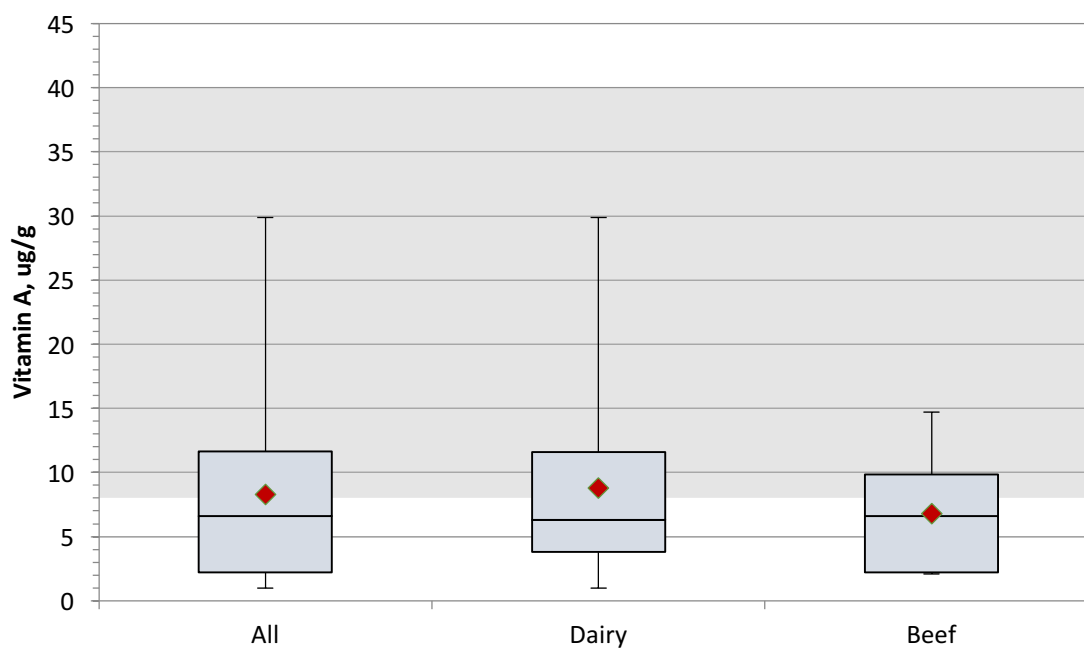


Figure 4

Overall, dairy and beef hepatic Vitamin E concentrations (dry weight basis) for 27 stillborn calves. Shaded area represents the reference range for the nutrient based on Michigan State University Nutrition Laboratory data.

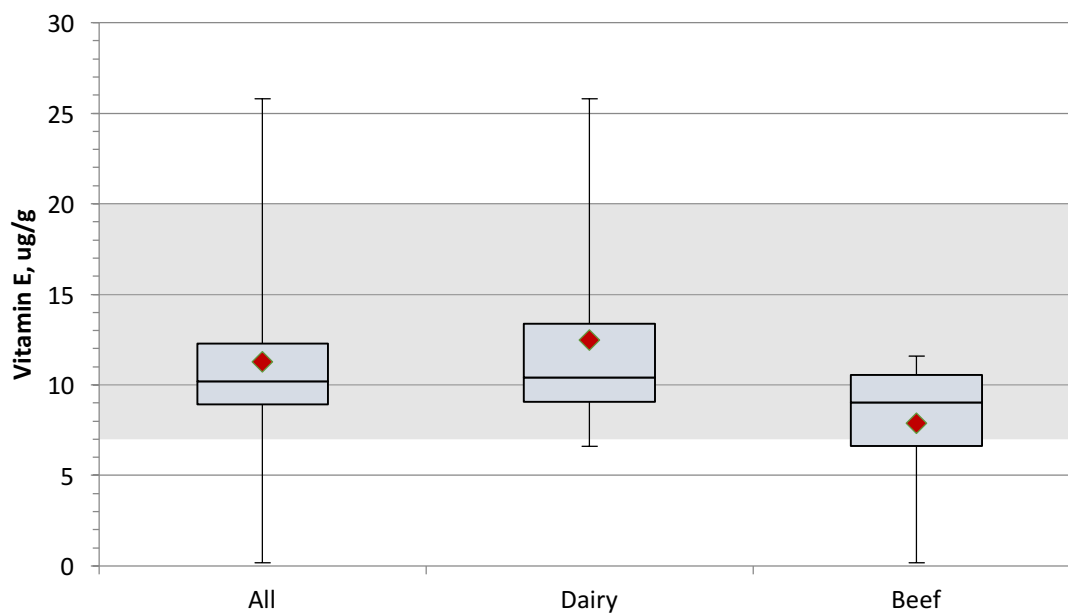


Figure 5

Overall, dairy and beef hepatic Calcium concentrations (dry weight basis) for 27 stillborn calves. Shaded areas are not present as no reference range has been established based on New Bolton Center Nutrition Laboratory data.

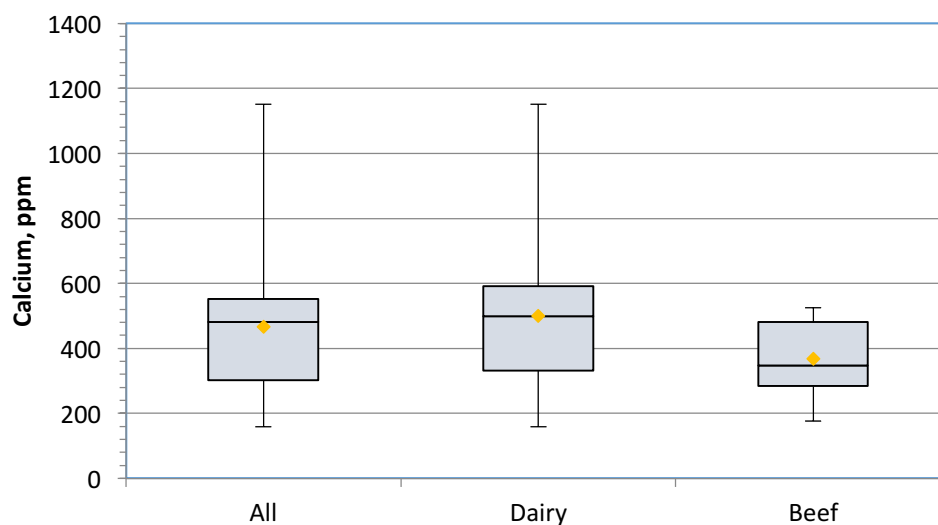


Figure 6

Overall, dairy and beef hepatic Cobalt concentrations (dry weight basis) for 27 stillborn calves. Shaded area represents the reference range for the nutrient based on New Bolton Center Nutrition Laboratory data.

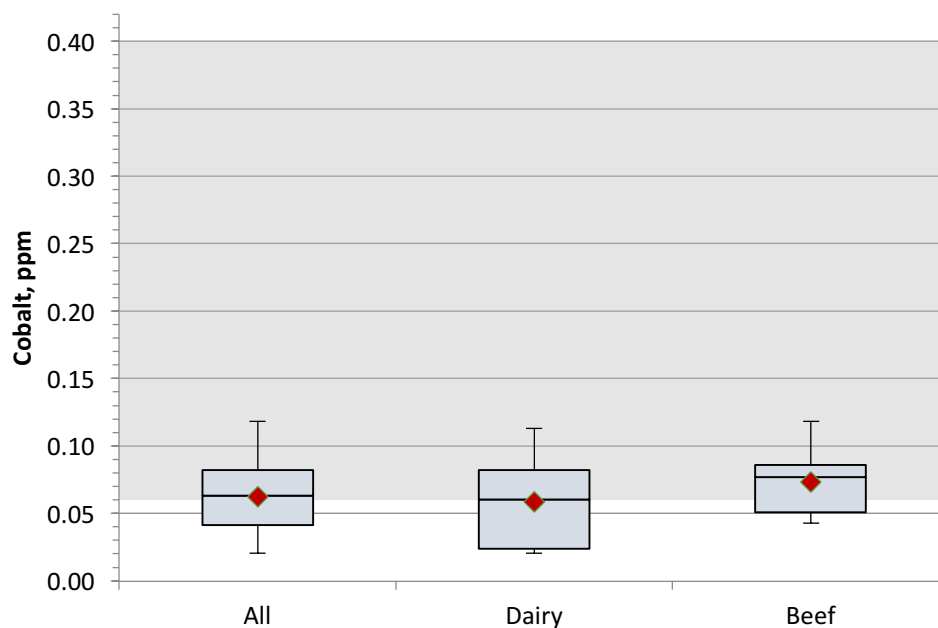


Figure 7

Overall, dairy and beef hepatic Copper concentrations (dry weight basis) for 27 stillborn calves. Shaded area represents the reference range for the nutrient based on New Bolton Center Nutrition Laboratory data.

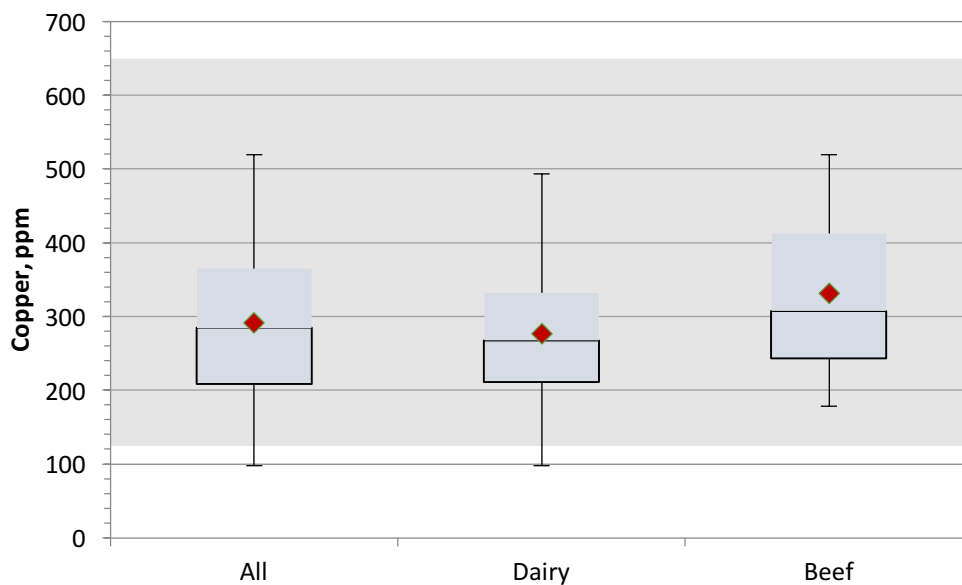


Figure 8

Overall, dairy and beef hepatic Iron concentrations (dry weight basis) for 25 stillborn calves, outliers excluded. Shaded areas are not present as no reference range has been established based on New Bolton Center Nutrition Laboratory data.

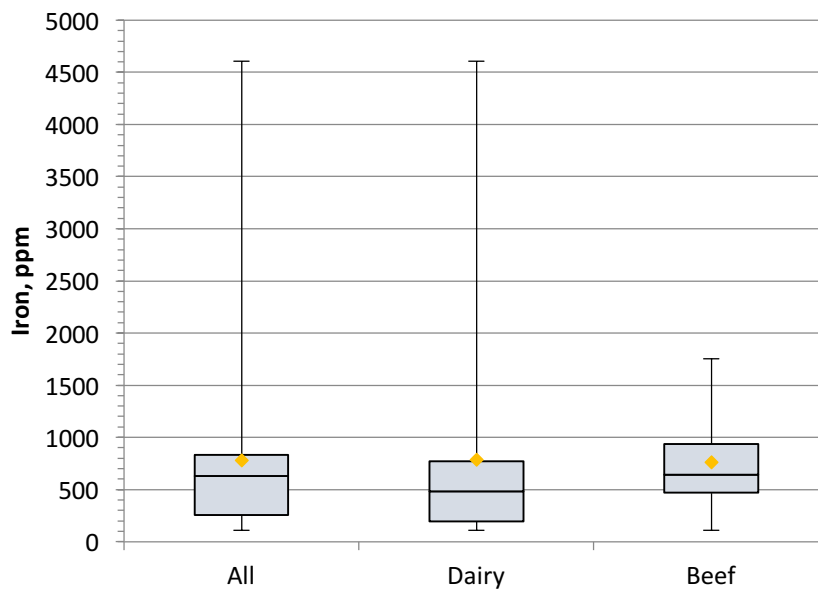


Figure 9

Overall, dairy and beef hepatic Iron concentrations (dry weight basis) for 27 stillborn calves, collective. Shaded area represents the reference range for the nutrient based on New Bolton Center Nutrition Laboratory data.

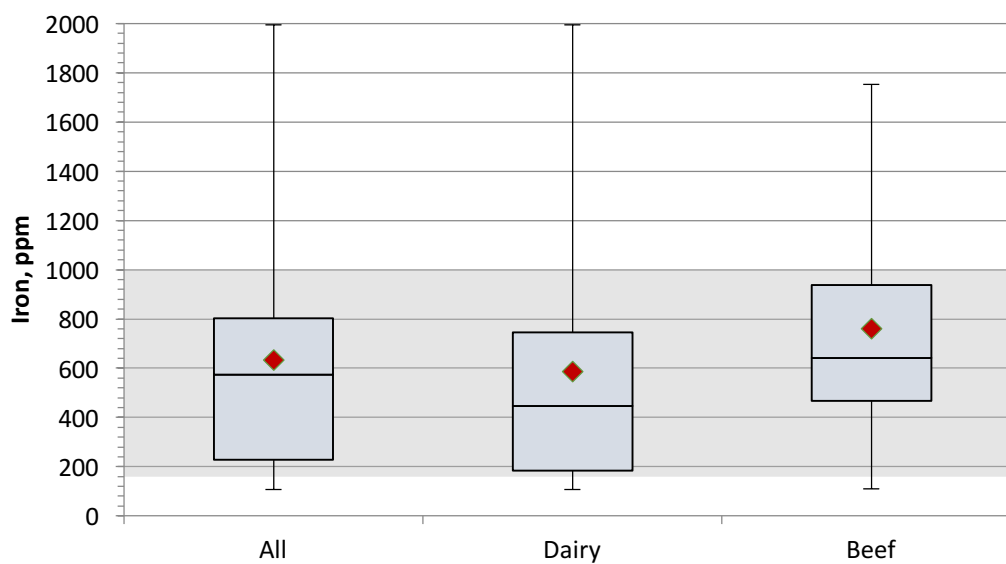


Figure 10

Overall, dairy and beef hepatic Magnesium concentrations (dry weight basis) for 27 stillborn calves. Shaded areas are not present as no reference range for the nutrient has been established based on New Bolton Center Nutrition Laboratory data.

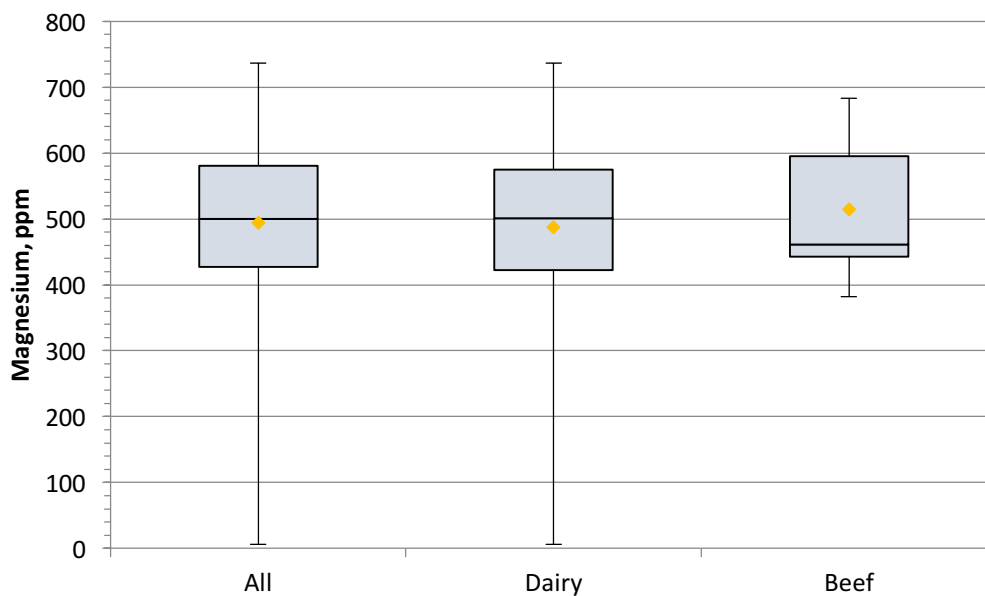


Figure 11

Overall, dairy and beef hepatic Manganese concentrations (dry weight basis) for 27 stillborn calves. Shaded area represents the reference range for the nutrient based on New Bolton Center Nutrition Laboratory data.

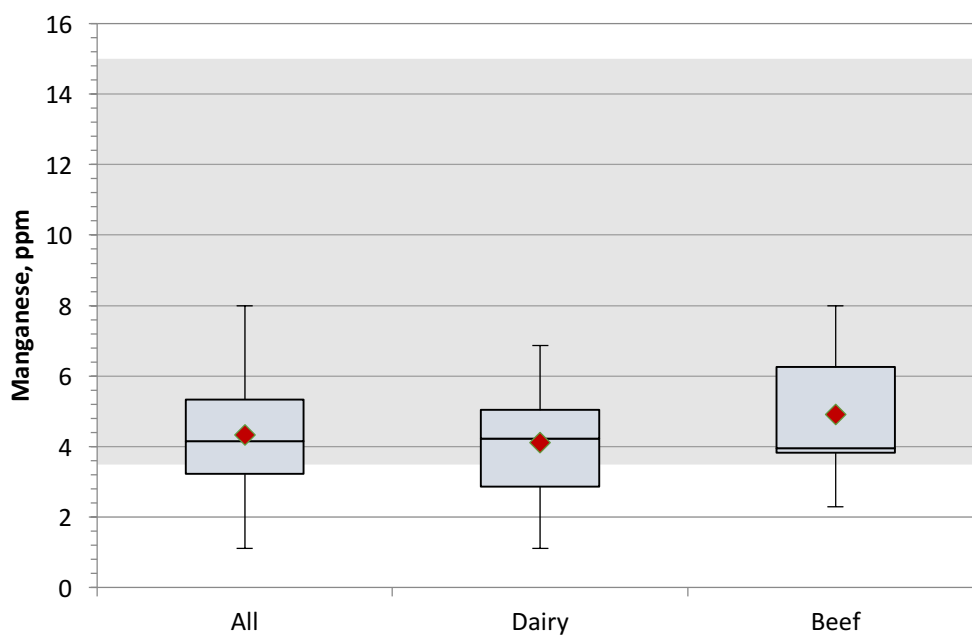


Figure 12

Overall, dairy and beef hepatic Molybdenum concentrations (dry weight basis) for 27 stillborn calves. Shaded area represents the reference range for the nutrient based on New Bolton Center Nutrition Laboratory data.

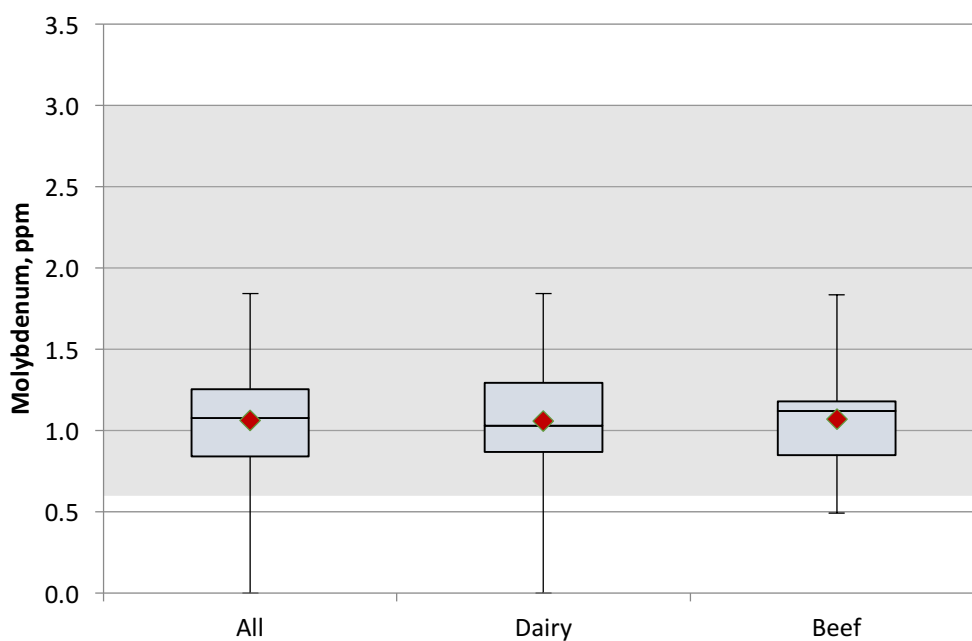


Figure 13

Overall, dairy and beef hepatic Selenium concentrations (dry weight basis) for 27 stillborn calves. Shaded area represents the reference range for the nutrient based on New Bolton Center Nutrition Laboratory data.

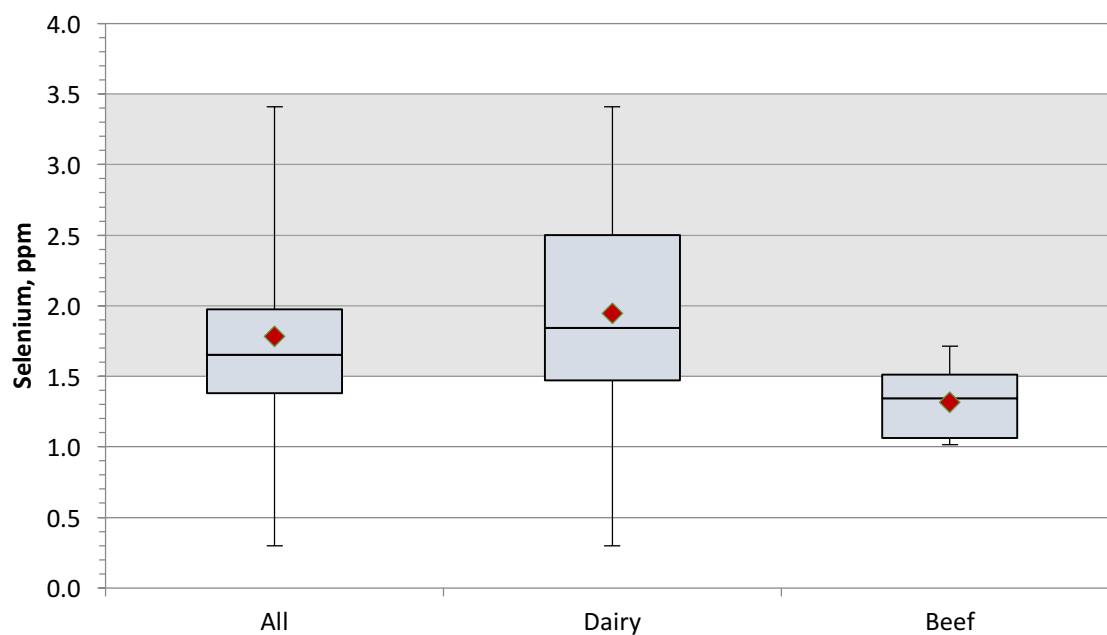
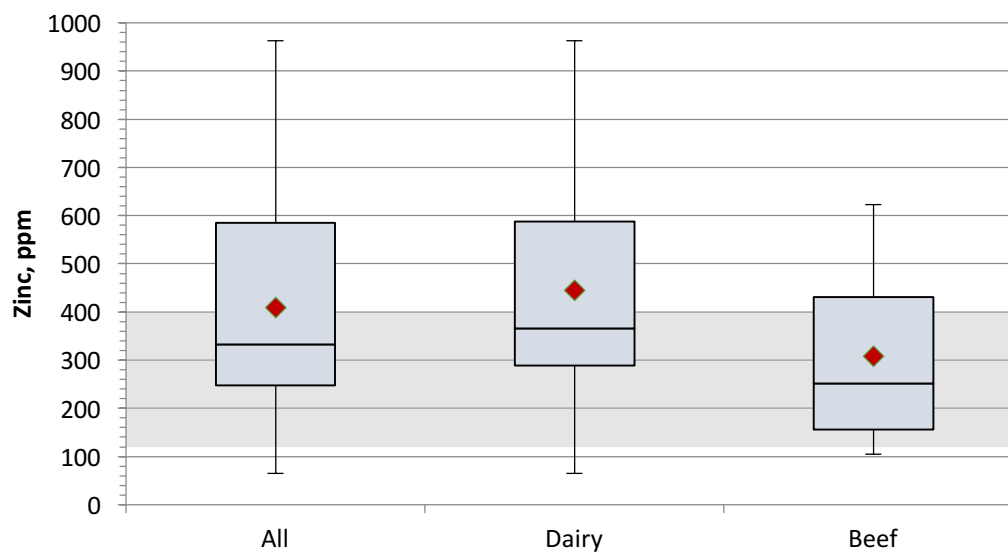


Figure 14

Overall, dairy and beef hepatic Zinc concentrations (dry weight basis) for 27 stillborn calves. Shaded area represents the reference range for the nutrient based on New Bolton Center Nutrition Laboratory data.



**Diagnostic Assessment**

With the potential exception of one case, dystocia was not a potential cause of these stillborn cases, which is due to the selection of cases for the study. If one accounts for the two cases of physical abnormalities and the two cases of internal abnormalities this would indicate 4 of 27 (16.8%) cases considered to have congenital defects. Infectious causes could range from 3 (11.1%) to 7 (25.9%) of the 27 cases depending upon interpretation of the results. Based on ADL reports 17 of 27 (63.0%) of cases were diagnosed as “idiopathic”.



## Chapter 5

### DISCUSSION

The objective of this study was to determine if nutritional deficiency or toxicities could be playing a role in stillbirth of dairy and beef calves given the limited positive diagnostic outcomes of stillbirth cases. This study was a limited survey of Pennsylvania dairy and beef farms. Findings of this study suggest the potential for vitamin and mineral alterations to play a role in stillbirth of calves, but this current study does not have any control cases for comparison. Additionally, some of the findings of the study would suggest there are problems with the reference criteria used by laboratories in interpreting stillborn vitamin and mineral status. Thus findings of this study would encourage further study of vitamin and mineral status of the late term fetus and neonate relative to the potential in pathogenesis of calf demise.

While it is known that the greatest proportion of stillbirths are associated with dystocia (Meyer et al., 2001; Berglund et al., 2003; Heins et al., 2006; Citek et al., 2011), this data set is unique that it was designed to select against dystocia cases. The only sample coming close to a dystocia was P1404243 with its large birth weight of 121 lb; however, no calving difficulty was reported and was thought to not be dystocia related. Analyzing results from the demographics of the samples to the microbiology and finally to the hepatic nutrient values, a few particular observations were noted. Due to the limited sample size, the data collected should not be regarded as an exact representation of the dairy and beef calvings in Pennsylvania and results warrant further study of stillbirth causes in cattle.

There is only one published study (Berglund et al., 2003) that performed necropsy on stillborn calves to determine cause. This study evaluated stillbirths in 76 calves from 41 different farms, but all calves were only from first calving heifers. In this study 46.1% stillbirths were attributed to dystocia. This study reported 5.3% and 2.6% stillbirths due to congenital anomalies and infections, respectively. The current study found 16.8% congenital anomalies and between 11.1 and 25.9% infectious causes in stillborns. Given the current study did not include dystocia, these other causes would be artificially

higher. If one uses 50% dystocia, the congenital and infectious prevalence might be reduced by approximately one-half, thus 8.4% congenital anomalies and between 5.5 and 13% infectious causes. These rates are higher than those reported by Bergland et al., (2003), but consistent with their observations. Similarly, Bergland et al., (2003) reported 31.6% normal and undiagnosed stillbirth cases compared to 63% in the current study, or 31.5% if one accounts for dystocia cases diluting the prevalence. These studies would underscore the need for further diagnostic avenues in pursuing stillbirth causation. The Bergland et al. (2003) study did not evaluate nutritional factors.

Summary demographics of the 27 samples showed an equal distribution of gender in stillbirth cases, consistent with Bergland et al. (2003). However, a greater percentage (74.07 %) of the stillborn samples were dairy versus beef (25.92%), which proved significant when comparing nutritional values. Holsteins accounted for the greatest majority of dairy cases; most likely a function of the dairy industry demographics of Pennsylvania. Another concern relative to the overrepresentation of Holsteins may be the issue of inbreeding (Pryce et al., 2006; Hinrichs and Thaller, 2011). The amount of inbreeding of the Holsteins could explain a conformation misalignment of the size of the calf and hips of the cow to cause a dystocia, and be the reason why the majority of the submissions were Holstein. However, as discussed, only one calf was thought to be too large to possibly cause dystocia, and was not Holstein.

Of interest was the interplay of physical parameters, crown-rump length and body weight, for the stillborn calves of the current study. Analysis of both crown rump length and birth weight in regards to breed revealed a correlation between calf type and the length or weight of the calf: dairy samples were on average shorter yet heavier (excluding the outlier) while beef samples were on average longer of the two types yet significantly lighter weight (excluding the outlier). Barrier et. al. (2013) suggested stillbirth is more likely for calves on average 4 cm longer than a normal length for that breed. Longer body length was hypothesized to potentially result on premature rupture of the umbilical cord thus increasing the risk for stillbirth. The measured crown-rump length of the dairy calves in the current study was 92.7 cm, which is slightly longer than Holstein calves born dead (90.6 cm) compared to calves born alive (86.4

cm) while birth weight was not different (Barrier et al., 1993). Similarly crown-rump length of the beef calves of the current study was longer (96.3 cm) with lower body weight.

Body weight is often considered the most critical factor in predisposing to dystocia and ultimately stillbirth with larger weight calves being at greater risk. Body weights of beef and dairy calves of the current study do not seem to follow this pattern. Surprisingly the stillborn dairy calves (68.3 lb) had only slightly heavier body weights compared to the beef calves (62 lb) when the large and small outlier cases were removed. Both mean body weights are less than breed standards, though the dairy calf body weight are more dramatically below Holstein expectations (90-95 lb). Birth weight of the dairy calves was much lower than birth weights for Holstein calves (85.7 lb) reported by Barrier et al. (2013) in which birth weight was not different between calves born alive or dead. Bergland et al. (2003) noted that birth weight of stillborn calves that had no evidence of causation were 6 kg lighter than stillborn calves experiencing a dystocia. They suggested these clinically normal calves of lower weight needed to be further investigated relative to underlying pathogenesis. It is possible the stillborn dairy cases of the current study are representative of this population whereas the stillborn beef calves may reflect more of body conformation issues and greater length as suggested by Barrier et al. (2013).

Infectious cause of stillbirth in the current study was slightly higher than that reported by Bergland et al. (2003). Half of the potential infectious cases were due to Neospora, which is recognized as a significant cause of abortion in cattle. The one case positive for BVD, IBR and three serotypes of Leptospira is most likely a vaccination response rather than an actual infection with all these agents. One would need to determine if and when these vaccines might have been administered to make a final determination. Definitive diagnosis of bacterial infections seems less obvious. In cases where positive bacterial cultures were determined there was no histologic changes in any tissues supporting an inflammatory response. In contrast, 2 cases had histologic evidence of inflammation but no bacterial cultures were determined.

Prevalence of congenital anomalies in the current study was consistent with the Bergland et al.

(2003) study. Ventricular septal defects are a typical anomaly. The growth on the liver was not deemed significant and was not further evaluated, thus this case might not be considered a congenital defect. Findings of the current study are consistent with Bergland et al. (2003) in finding at least one-third or more of stillbirth cases have no definitive diagnosis and would be considered “idiopathic”.

With all others tested negative, this leaves other factors, such as nutrition, to still consider for the remaining samples. A few general trends were noted relative to nutrition. While interpreting the relation of each nutrient’s mean to its reference range provided by Michigan State, it is important to note that there has not been enough research completed to date to have established reference values for a newborn calf. This becomes pertinent to analyzing the Ca, Co, and Mo values, which may have been above, or below an appropriate level but have no recorded references in literature in order to make an appropriate evaluation. The vitamins and minerals with provided reference ranges included vitamins A and E, Cu, Fe, Mg, Mn, Se, and Zn. Observing the data, all of the vitamin E, Cu, Fe, Mn, and Mo hepatic means were within the references ranges provided except for vitamin A, Co, Se, and Zn. The comparison is also limited due to the lacking neonatal calf mineral information in other references. The Waldner and Blakely (2014) study would be the only published paper with data for comparison to this study.

While the  $8.26 \pm 7.39$   $\mu\text{g/g}$  (1.93 ppm wet weight) overall vitamin A mean was within the range, it was near the lower reference limit of 8  $\mu\text{g/g}$  as 66.7% of the data points (18 of the 27 samples) were below this lower limit. This wet weight, when compared to Waldner and Blakely’s published data, was 0.67 ppm less than their 2.6 ppm. As previously discussed, inadequacies in vitamin A have been reported to fatally cause an inability to maintain healthy epithelium, and a resorption of the fetus, an abortion, or a stillbirth. Dividing the data as independent beef and dairy breed variables, we noted that the beef variable samples were vitamin A deficient with a mean concentration of 6.79  $\mu\text{g/g}$ , 1.21  $\mu\text{g/g}$  less than the lower reference limit. This was significantly less than the dairy hepatic vitamin A concentration mean, which was 8.78  $\mu\text{g/g}$ , and would be considered a low, but sufficient amount for survival in the dairy stillborn calves. As discussed by Waldner and Blakely (2014), the differences between beef and

dairy environmental and herd management factors may attribute to the vitamin A disparities. Beef cattle are usually maintained on a pasture and local forage diet with calves relying solely on the cow's milk. Conversely, dairy cows are generally maintained on a managed supplement and forage diet with additional vitamin and milk replacers to the dairy calves. Largely derived from carotene in green feed and built in the cow while on pasture during the summer months, the vitamin A level in the cow and calf is dependent on the previous year's precipitation. Since vitamin A is fat soluble, calves do not have a reserve of vitamin A at birth and they initially rely on the cow's colostrum. Due to their dependence on the environment, beef cow and calf nutritional status depends on the success of the pasture system to carry them through the winter months, and may be at a higher risk of vitamin A deficiency. Reflected in the with the lower hepatic concentration mean of the two groups studied, the beef vitamin A concentrations may have been affected by the difficult Pennsylvania winters in 2014 and 2015. Pregnant cows ingesting a lower carotene content feed are not able to deliver the appropriate vitamin A in the initial colostrum, and may cause the quick decline of the calf. Due to the low number of samples, the averages are heavily biased. This dependency is illustrated through the large standard deviations, which with more samples could reflect a more accurate representation of the hepatic vitamin A in stillbirths. One of the challenges with vitamin A status in the ruminant animal is the ruminal degradation of the nascent molecule that contains three double bonds in the fatty acid side chain, which is required for biologic function.

Overall, vitamin E did not reflect any significant data abnormalities. There was an even amount (11.1%) above and below the upper and lower reference limits for a collective mean of 11.28  $\mu\text{g/g}$  (2.64 ppm wet weight). Compared to literature data, this 2.64 ppm wet weight was 0.82 ppm greater than Waldner and Blakely's 2014 1.82 ppm vitamin E wet weight. Both of these values would be considered normal in a New Bolton Center wet weight reference range of 1.64 to 4.68 ppm. When breaking the analysis into the producer variables, the beef mean of 7.88  $\mu\text{g/g}$  was just on the lower cusp of the reference range of 7.00-20.00. This may be explained in a similar aspect as vitamin A, that vitamin E is

found in green grass where the beef cattle would depend on for forage, and would become detrimental after a year of poor harvest. Continuing the pattern, the dairy calf group vitamin E mean was greater at 12.48 µg/g and explained through the added vitamin supplementation to the diet. Since vitamin E acts as an important antioxidant in the system, especially during development, the lower beef value may prove to be grounds for further investigation with more samples. This would imply that these values are not concrete for an exact representation of the vitamin E nutritional state of the calf in utero.

Of the minerals analyzed, the Se sample means were significant with 40 percent of the 27 samples below the lower reference range. While the collective mean and dairy mean were within normal limits of 1.5-3.5 ppm at 1.78 (0.415 ppm wet weight) and 1.94 ppm for a neonate calf, the beef mean was 0.18 ppm below the lower reference limit at 1.32 ppm. This deficiency may determine the fate of the calf in utero. This collective Se mean wet weight value was 0.245 ppm less than Waldner and Blakely's 0.66 ppm. Studies by Waldner associated Se's important for carbon dioxide formation, alcohol metabolism, protein digestion, hydrolysis of phosphate esters, cell replication, and wound healing. Through removing hydrogen peroxides from the system and converting thyroxine to active forms, Se also protects against muscular dystrophy. Because beef cattle are on pasture, Se deficient soils cause reproductive disorders, poor growth, and calf health problems, and may be a reason for the beef stillborn cases.

Zinc collective and dairy means, on the other hand, were greater than the upper reference range limit. At 444.53 ppm, the dairy mean was 44.53 ppm above the upper reference range limit of 400 ppm and influenced the collective mean to be 408.98 ppm (94.97 ppm wet weight), also above the upper reference limit. This collective wet weight mean was 26.43 ppm less than Waldner and Blakely's Zn average. However, the beef sample means were within normal limits, at 307.42 ppm. While past literature has cited Zn to be important for structural stability, an excess of the mineral, such as calculated in this study for the dairy mean, could result in a Zn toxicity that would have corrosive effects on the tissue and the metabolism of other minerals. However, as Wentink et. al. (date) discussed, there is a large

safety zone for where an excess of Zn actually becomes a toxicity problem. In this case, 44.53 ppm would not be sufficient to be detrimental to the calf's system, and would not be the probable cause of the stillbirths. The observed difference in stillbirth Zn concentrations with current laboratory reference range would suggest possibly this range is too low and should be further evaluated.

Of the minerals evaluated, Co, Cu, Fe, and Mn were each within the appropriate reference range and would not be considered a cause to the stillbirth. However, two minerals measured were not able to be evaluated. These minerals, Ca and Mg, have not been yet researched for the neonatal age group for adequate dry matter values. Without these limits, one cannot discuss this study's data means for each to delineate if either played a role in the stillbirth. When just evaluating the overall collective Ca mean of 466.45 ppm in the samples, the dairy group mean was significantly greater than the beef group mean at 500.66 ppm to 368.70 ppm. With further study, this difference may prove significant, especially due to calcium's integral role in bone formation.

As for Mg, the means did not vary as much from each other when divided to the dairy and beef variables. With a collective mean of 493.89 ppm (114.46 ppm wet weight), the dairy mean was 7.23 ppm less at 486.66 ppm, and the beef mean was 20.65 ppm greater at 514.54 ppm. While there is no literature-established dry weight Mg reference range, the New Bolton Center has wet weight reference ranges. These ranges (277 to 320 ppm for Mg) are well above the 114.46 ppm wet weight of the collective Mg mean, and imply that there is an average Mg deficiency in the samples tested. As Seeling (1982) discussed in past literature, a Mg deficiency becomes more apparent the younger the animal and how many vital bodily processes the mineral is a component of, this wet weight-found deficiency could be a correlation to the stillbirths. Particularly versus other nutrients that could be mobilized from bodily stores when low, most adult cows cannot extract more Mg from bones, and would not be able to provide the proper nutrients for the developing fetus. Without its aid activating biosynthetic enzymes and maintaining of the electrical potentials, the Mg macronutrient would shut the body down.

## **Chapter 6**

### **CONCLUSION**

This study evaluated multiple variables to the pathogenesis of stillbirth. Separating the samples into dairy and beef identified correlations and differences between the two in demographics, physical parameters, necropsy findings, microbiologic findings, and nutritional findings. As these samples were purposefully selected to not be dystocia cases and to instead investigate other causes, the resultant data highlighted a few topics that could be accounting for the rising incidents of stillbirth in Pennsylvania and perhaps the rest of the cattle industry. The role of nutrition in almost all body processes and its influences on herd health and reproductive capability suggest it to be a very important factor in preventative medicine. While nutritional research in the dairy and beef industry has not yet reached the point where all of the nutritional reference ranges have been established, this study has set a basis for expansion. To understand better the consequences of varying nutrients and the significance of certain vitamins or minerals on a system, further research benefit the cattle industries. Avenues to investigate for future studies may include: larger sample size to improve data precision, seasonal precipitation to understand any effects on the roughage, location dependent where certain parts or farms of Pennsylvania may be most affected, environmental conditions, dam nutrition status, and further comparison with complete reference values for deeper analysis. Each of these may confirm the effect of nutrient levels on stillborn calves and be grounds for future changes to diets to decrease the incidence in the beef and dairy industry.



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## Appendix A

### STILLBORN PROJECT LETTER TO PENNSYLVANIA VETERINARIANS

February 17, 2015

FIELD(7)  
FIELD(8)  
FIELD(9), FIELD(10) FIELD(11)

Re: Still born Project

The Pennsylvania State University Veterinary Extension Team is currently conducting a study investigating stillbirth causes aside from dystocia in beef and dairy calves. This study is being managed by one of our undergraduate students, Danielle Esplin, as part of her honors thesis project. We would like to obtain upward of 50 calves or more depending upon additional funding.

With reproductive performance in dairy herds declining, and cow culling rates increasing, newborn calf survival is one critical component to maintaining financial stability and profitability for dairy and beef herds. Currently, we have collected 15 stillborn calves but have sufficient funding for many more. In the data collected and analyzed thus far, nutrition appears to be a significant contributor to stillbirth incidence with specific focus of vitamins A and E, as well as minerals calcium, cobalt, copper, iron, magnesium, manganese, selenium, and zinc.

We are soliciting your participation in collecting appropriate samples for the study. The team will cover all costs associated with diagnostic evaluation and will provide feedback on results. If there is no other way to get the calf to the Animal Diagnostic Lab at the University Park Campus, arrangements may be able to be made come to collect the stillborn calf by contacting Carol Burns at 814-863-0489. If her schedule allows, she may be able to collect the calf.

In this study, a stillbirth is defined as a late term fetus at least 265 days of gestation born dead or has died within six hours of birth. For most accurate results, the calf should be in intact and in good condition accompanied with any information relative to the difficulty of the birth would be well appreciated. Submitted calves will have complete necropsy at Penn State's Animal Diagnostic Lab performed, with histopathologic assessment of key organs for evidence of disease pathogenesis. Microbiologic diagnostics addressing important pathogens (IBR, BVD, Leptospirosis, Neospora, Bacteriology) will also be completed. Concentration of nine essential minerals and vitamins A and E will be determined by ICP/MS and HPLC, respectively, in liver samples submitted for nutritional analyses.

For consistency purposes we encourage the calf be delivered to the Penn State Animal Diagnostic Lab or picked up by our team. If delivered, please notify those admitting the calf that it is for the **Still Born Project**.

If getting the whole calf to the diagnostic lab is impossible, an on-farm necropsy may also be conducted. If you wish to conduct the necropsy yourself, we ask you to complete the *Submission sheet* that will provide us with information detailing the crown rump length, the animal's production type and breed, weight, gender, appearance, hair coat, preservation state. On this sheet note any gross lesions observed, and provide tissue samples of the tongue, heart, thyroid, lung, and liver be taken. Sample collection is detailed in the *PDL Still Born Necropsy Kit*. Send tissue samples and completed form to the Penn State Animal Diagnostic Lab. All costs of the necropsy, and mineral and vitamin analysis will be paid for by project funding.

Results from this study will provide a better perspective on the stillbirth problem in two important agricultural industries to the Commonwealth and offer some insights as to underlying causes. To complete this study to the best of our ability, we are requesting your contribution to urge Pennsylvania beef and dairy producers to submit any stillborn calves. Using collected farm-based data, we will attempt to identify common issues associated with a cow delivering a stillborn calf and ascertain key risk factors that contribute to stillborn calves. Please contact Dr. Robert Van Saun at 814-867-6995 or [rjv10@psu.edu](mailto:rjv10@psu.edu) or Carol Burns at 814-863-0489 or [cmb3@psu.edu](mailto:cmb3@psu.edu) for more information.

Thank you very much for your consideration in our project and please contact us if you have any questions.

Sincerely,



Dr. Robert Van Saun, DVM, MS PhD  
Extension Veterinarian

## Appendix B

### SUBMISSION SHEET INCLUDED WITH LETTER TO PENNSYLVANIA VETERINARIANS

#### Stillborn Calves from Dairy and Beef Cattle in Pennsylvania Submission Sheet

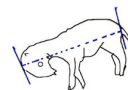
Dr. Robert Van Saun [rjv10@psu.edu](mailto:rjv10@psu.edu)  
Phone: 814- 865-6995/867-2038

Carol Burns [cmb3@psu.edu](mailto:cmb3@psu.edu)  
Phone: 814 -863-0489

Date: \_\_\_\_\_

Case No.: \_\_\_\_\_

ADL Accession No. \_\_\_\_\_



Crown Rump length:		cm	
Gender:	<input type="checkbox"/> M <input type="checkbox"/> F	Body Weight:	
Production Type:    Dairy		Beef	
Dairy Breed:		Beef Breed:	
Physical appearance of the calf:	Appearance	<input type="checkbox"/> Normal <input type="checkbox"/> Enlarged head <input type="checkbox"/> Malformed Describe:	
	Hair coat description	<input type="checkbox"/> Normal <input type="checkbox"/> Absent <input type="checkbox"/> Spotty – falling out	
	Preservation State	<input type="checkbox"/> Normal <input type="checkbox"/> Frozen <input type="checkbox"/> Autolyzed	
	Gross Lesions	<input type="checkbox"/> None <input type="checkbox"/> Yes Describe:	

Full necropsy:

Describe any obvious defects, malformations or anomalies of internal organs:
--

Specific observations

Tongue / Heart	Any evidence of white muscle disease <input type="checkbox"/> Yes <input type="checkbox"/> No Other observations:		
Thyroid	<input type="checkbox"/> Enlarged <input type="checkbox"/> Normal <input type="checkbox"/> Smaller than normal <input type="checkbox"/> Collect sample for thyroid histology		
Lung	Inflation Status: <input type="checkbox"/> None <input type="checkbox"/> Partially inflated <input type="checkbox"/> Fully inflated		
	Lesions:		
Liver	Physical Appearance	<input type="checkbox"/> Normal <input type="checkbox"/> Enlarged <input type="checkbox"/> Discolored <input type="checkbox"/> Friable Describe:	
	Samples to collect	<input type="checkbox"/> To be sent to NBC for tissue for nutritional screen	
		<input type="checkbox"/> To be labeled and frozen. For Vitamin A and E analysis at Michigan State University. <b>Contact Carol Burns</b>	

Collection of tissues for standard testing:

- ☐ PCR for BVD and Neospora
- ☐ FA for IBR and Lepto
- ☐ Bacteriology and histopathology

## Appendix C

### NECROPSY AND SAMPLE INSTRUCTIONS SENT WITH EACH LETTER TO PENNSYLVANIA VETERINARIANS

#### PADLS STILL BORN CALF NECROPSY KIT

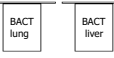




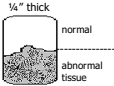

##### GENERAL PROCEDURES AND QUESTIONS

- 1- Please complete the **Stillborn Calves from Dairy and Beef Cattle in Pennsylvania**, Submission Sheet that is provided with this packet.
- 2 - Put samples back into the large plastic bag and place it onto **frozen ice packs**.
  - ♦ Seal all containers tightly
  - ♦ **Do not freeze the samples**
  - ♦ Label **ALL** samples (tissue name/culture site, and animal ID)
- 3 - Send to one of the three Pennsylvania Animal Diagnostic Laboratories. ???

**Do not mail on a Friday or before a holiday**

**The enclosed viral transport media MUST be refrigerated!**

##### SPECIFIC INSTRUCTIONS FOR SAMPLE COLLECTION

	<p>1 - Place <b>at least a 3" cube</b> section of lung and liver into the whirl-pak bag labeled "BACT lung" and "BACT liver", respectively</p>
	<p>2 - Using a sterile syringe (as aseptically as possible), collect 5-10 cc of fluid from the abomasum. Put the fluid in the red top tube labeled "abomasal contents".</p>
	<p>3 - Place a 1 inch square section of <b>liver</b> into the corresponding whirl-pak bag.</p> <p>! Include the interface of normal and abnormal tissue</p>
	<p>4 - Put a piece of kidney into the whirl-pak bag labeled "kidney".</p>
	<p>5 - Put a piece of each of the following tissues into the formalin cup labeled "organ pool":</p> <p style="text-align: center;"><b>heart      lung      liver      thyroid</b></p>
	<p>! <b>tissues should be no more than 1/4" thick</b></p> <p>- proper tissue fixing requires <b>10 parts formalin to 1 part tissue</b></p> <p>! include the interface of "normal" tissue with "abnormal" tissue</p> <p>! make sharp cuts and handle tissues gently and as little as possible</p>
	<p>6 - Put a small piece of the <b>lung</b> into the viral (VTM) transportation media vial.</p> <p>! Make sure the pieces are completely covered by the media</p>

##### TESTS PERFORMED ON SPECIFIC SAMPLES

###### **Bacteriology:**

**Lung and Liver** routine aerobic panel

###### **Fluorescent antibody test (FA):**

IBR and Lepto

###### **Virus isolation (VTM):**

BVD, IBR

###### **Histological examination of fixed tissue**

**Immunohistochemistry** (when lesions suggest or on special request)  
Neospora caninum

## Appendix D

## RAW DEMOGRAPHIC DATA

Case #	ADL Accession #	Date	Owner	Veterinarian	Crown Rum Length (cm)	Gender	Body Weight (lbs)	Production Type	Dairy or Beef Breed	Appearance	Hair Coat Description	Preservation State	Gross Lesions	Tounge/Heart Any evidence of white muscle disease	Other Observations	Thyroid	Collected Sample for Thyroid Histology?	Lung: Inflation Status	Case Diagnosis or Interpretation	Liver: Physical Appearance
1	P1308029	4/11/13	Cody Filges	Kittanning Veterinary Hospital Inc.	80	F	60	Beef	Maine/Angus x	Normal	Normal (But skinned out on arrival)	Normal	None	No		Normal	Yes	None	Infectious	Normal
2	P1308965	4/23/13	Hameau Farm	Robert Cuningger, Centre Herd Health	81	F	40	Dairy	Ayrshire	Normal	Normal	Normal	None	No		Normal	No	Partially inflated	Idiopathic	Normal
3	P1309052	4/23/13	Farms	Philip Aquadro, Town and Country Animal Hospital	91	F	58	Dairy	Holstein	Normal	Normal	Normal	None	No		Normal	Yes	None	Idiopathic	Normal
4	P1311120	5/20/13	Scott & Emily Brown	Huntingdon Veterinary Services	103	F	91	Beef	Angus	Normal	Normal	Normal	None	No		Normal	Yes	None	Neospora	Normal
5	P1311809	5/29/13	Scott & Emily Brown	Huntingdon Veterinary Services	86	F	52	Beef	Angus	Normal	Normal	Normal	None	No		Smaller than normal	Yes	None	Low Liver Selenium	Normal
6	P1313460	6/19/13	David N. Hoover	Michael J. Popp, West Branch Mobile Veterinary Clinic	92	M	86	Dairy	Holstein	Enlarged Head (Big Head for a bull calf)	Normal	Normal	None	No		Normal	Yes	None	Idiopathic	Normal
7	P1318715	8/20/13	Scott & Emily Brown	Huntingdon Veterinary Services	98	M	52	Beef	Angus	Normal	Normal	Normal	Yes	No		Normal	Yes	Partially inflated	Bronchopneumonia	Normal
8	P1322589	10/4/13	Farms	Philip Aquadro, Town and Country Animal Hospital	96	M	82	Dairy	Holstein	Normal	Normal	Normal	None	No		Normal	Yes	None	Idiopathic	Normal
9	P1325388	11/6/13	Farms	Philip Aquadro, Town and Country Animal Hospital	77	F	36	Dairy	Holstein	Small body size	Normal	Normal	Yes	No	Heart defect	Normal	Yes	None	Ventricular septal defect	Normal
10	P1404243	3/3/14	Farm	Fred Benner, Serenity Hills Veterinary Associates	123	F	121	Beef	Simmental	Normal	Normal	Normal	None	No		Normal	Yes	Partially inflated	Idiopathic	Normal
11	P1404278	3/4/14	Dustin D Heeter, Heritage Hill Farm	Alquin F Heinrickel III, Five Points Veterinary Services	92	M	55	Beef	Hereford	Normal	Normal	Normal	None	No		Normal	Yes	None	Idiopathic	Normal
12	P1417419	8/2/14	Farms	William A Moore, Cool Spring Farms	92	F		Beef	Angus	Normal	Normal	Normal	None	No		Normal	Yes	None	Idiopathic	Friable
13	P1423136 (2579)	10/3/14	Scott Lackey, Sugar Branch Dairy LLC	Troy Veterinary Clinic	97	M	110	Dairy	Holstein	Normal									Idiopathic	
14	P1423136 (8559)	10/3/14	Scott Lackey, Sugar Branch Dairy LLC	Troy Veterinary Clinic	94		80	Dairy	Holstein	Normal									Idiopathic	
15	P1426969	11/18/14	Scott Lackey, Sugar Branch Dairy LLC		87	M	48	Dairy	Holstein	Normal	Normal	Normal	None	No		Normal	Yes	Partially inflated	Mild pneumonitis/ Minimal to mild multifocal acute hepatocellular necrosis	Normal but enlarged
16	P1428120	12/3/14	Tim Voegtlin	Troy Veterinary Clinic	120			Dairy	Holstein										Idiopathic	
17	P1429663	12/19/14	Jared Boor	Marsha Rosanelli	88.9	M	64	Dairy	Holstein	Normal	Normal	Normal	None						Staphylococcus aureus isolated	
18	P1507155-1	3/30/15	Ron Learn	Israel Iseberg	101	M		Dairy	Holstein	Normal	Normal	Normal							Neosporosis	
19	P1507155-2	3/30/15	Ron Learn	Israel Iseberg	92	F		Dairy	Holstein	Normal	Normal	Normal							Idiopathic	
20	P1507155-3	3/30/15	Ron Learn	Israel Iseberg	95	M		Dairy	Holstein	Normal	Normal	Normal							Idiopathic	
21	P1508361	4/16/15	D. Stoltrfus	Sam Wicker				Dairy	Holstein										Idiopathic lymphohistiocytic meningitis/ renal hypoplasia	
22	P1509390	4/23/15	Wesley Metzker	Dairyside Veterinary Service	84	M	66	Dairy	Holstein											
23	P1509939	4/28/15	Tim Voegtlin	Troy Veterinary Clinic				Dairy	Holstein										Idiopathic	
24	PA1513240	6/8/15	Wesley Metzker	Dairyside Veterinary Service	Field Post	N/A	N/A	Dairy	Holstein	N/A	N/A	N/A	N/A	N/A		Normal	No	Partially inflated	Idiopathic	N/A
25	P1513507 (Twin 1)	6/11/15	Scott Lakey/Jim Voegtlin, Sugar Branch Dairy LLC	Troy Veterinary Clinic	101	M	69	Dairy	Holstein	Normal	Normal	Normal	None	No		Smaller than normal	Yes	None	Idiopathic	Normal
26	P1513507 (Twin 2)	6/11/15	Scott Lakey/Jim Voegtlin, Sugar Branch Dairy LLC	Troy Veterinary Clinic	N/A	F	63	Dairy	Holstein	Normal	Normal	Normal	None	N/A		Smaller than normal	Yes	None	Idiopathic	Normal
27	P1513507 (Calf B)	6/11/15	Scott Lakey/Jim Voegtlin, Sugar Branch Dairy LLC	Troy Veterinary Clinic	86	F	53	Dairy	Holstein	Normal	Normal	Autolyzed	None	N/A	N/A	N/A	N/A	N/A	Idiopathic	N/A



## Appendix E

## RAW MICROBIOLOGIC DATA

Case Number	PADLS Accession Number	Pathologic Anatomic Findings	Histopathology	Salmonella	Leptospira spp.	Histophilus	Acinetobacter/woffii	Neospora caninum	Bovine viral diarrhoea virus	Infectious Bovine Rhinotracheitis Virus
1	P1308029	Lung: Multifocal alveolar lumina contained meconium	There were no significant lesions detected in the sections of brain, spleen, kidney, liver, heart, skeletal muscle and placenta examined.	Negative	Negative	Negative		Negative	Sera Positive	Sera Positive
2	P1308965		No significant lesions were evident in the brain, liver, kidney, thyroid gland, spleen, heart, or skeletal muscle. The lung was partially inflated. Occasional small intra-alveolar accumulations of red blood cells were noted.	Negative	Negative	Negative		Negative	Negative	Negative
3	P1309052		No significant lesions were evident in the brain, lung, kidney, liver, spleen, mesenteric lymph node, thyroid, heart, tongue, or skeletal muscle.	Negative	Negative	Negative		Negative	Negative	Negative
4	P1311120		No significant microscopic changes are detected in the brain, heart, lung, liver, spleen, kidney, abomasum, intestine, thyroid, or skeletal muscle	Negative	Negative	Negative		Positive	Negative	Negative
5	P1311809		No significant lesions were detected in the sections of the brain, lung, kidney, liver, spleen, heart, and skeletal muscle examined.	Negative	Negative	Negative		Negative	Negative	Negative
6	P1313460		No significant changes are detected in the brain, heart, lung, liver, spleen, kidney, tongue, or skeletal muscle.	Negative	Negative	Negative		Negative	Negative	Negative
7	P1318715	Minimal to mild diffuse acute bronchopneumonia and focal peri-hepatic hematoma	Abdomen moderately distended. Within the cranial abdomen there is a 23 cm diameter cystic fluid filled mass extending from the right lobe of the liver. Within the mass is abundant red-brown fluid and fibrin. There is diffuse vascular dilation of the lung. Alveolar lumina are infiltrated by small numbers of macrophages and neutrophils. A section is examined that consists of fibrous connective tissue with adherent fibrin admixed with free erythrocytes. There are no significant lesions detected in the sections of the brain, thymus, kidney, liver, spleen, heart, and skeletal muscle examined.	Negative	Negative	Negative		Negative	Negative	Negative
8	P1322589		No significant lesions evident	Negative	Negative	Negative		Negative	Negative	Negative
9	P1325388	There is a 1 cm diameter subvalvular ventricular septal defect	Pulmonary alveoli contained moderate amounts of meconium and squamous epithelial cells.	Negative	Negative	Negative		Negative	Negative	Negative
10	P1404243		No significant changes are detected in the brain, heart, lung, liver, spleen, skeletal muscle, or thyroid.	Negative	Negative	Negative		Negative	Negative	Negative
11	P1404278		No significant changes are detected in the brain, heart, lung, liver, spleen, skeletal muscle, or thyroid.	Negative	Negative	Negative		Negative	Negative	Negative
12	P1417419		Mild autolysis was noted, but no significant lesions were evident in the brain, lung, and heart. Moderate autolysis was noted but no significant lesions were evident in the kidney and liver. Severe autolysis was noted but no significant lesions were evident in the spleen.	Negative	Negative	Negative		Negative	Negative	Negative
13	P1423136 (2579)	Minimal subacute pneumonia	In the lung, there are mildly increased alveolar macrophages and low numbers of scattered neutrophils in the lveoli.	Negative	Negative	Negative	Negative	Negative	Negative	Negative
14	P1423136 (3559)	Minimal subacute pneumonia	In the lung, there are mildly increased alveolar macrophages and low numbers of scattered neutrophils in the lveoli.	Negative	Negative	Negative	Scanty	Negative	Negative	Negative
15	P1426969	Mild pneumonitis and minimal to mild multifocal acute hepatocellular necrosis	The lung was mildly inflated with multifocal intra-alveolar accumulations of small numbers of neutrophils. On the liver there were occasional small multifocal sites of mild acute hepatocellular necrosis. Otherwise no other significant lesions were evident.	Negative	Negative	Negative		Negative	Negative	Negative
16	P1428120		No significant lesions detected in the sections of the brain, lung, kidney, liver, spleen, and heart examined.	Negative	Negative	Negative		Negative	Negative	Negative
17	P1429663		Low numbers of neutrophils scattered in the alveoli and bronchioles. No significant microscopic changes are detected in the brain, heart, liver, spleen, kidney, thymus, skeletal muscle, or intestine.	Negative	Negative	Negative		Negative	Negative	Negative
18	P1507155-1		No significant lesions were evident in the brain, kidney, spleen, liver, placenta. Mild to moderate interlobular septaledema was noted in the lung and heart. Mild accumulations of small numbers of lymphocytes and neutrophils were evident within the epicardium.	Negative	Negative	Negative		Positive	Negative	Negative
19	P1507155-2		No significant lesions were evident in the brain, lung, kidney, liver, spleen, heart, and skeletal muscle.	Negative	Negative	Negative		Negative	Negative	Negative
20	P1507155-3		No significant lesions were evident in the brain, lung, kidney, liver, spleen, or heart.	Negative	Negative	Negative		Negative	Negative	Negative
21	P1508361		No significant lesions were evident in the brain, lung, kidney, liver, spleen, or heart.	Negative	Negative	Negative		Negative	Negative	Negative
22	P1509390		Within a section of brain there are low numbers of lymphocytes and macrophages in the leptomeninges. One section of one kidney displays renal hypoplasia. No significant changes are detected in the heart, lung, liver, spleen, lymph node, or skeletal muscle.	Negative	Negative	Negative		Negative	Negative	Negative
23	P1509939		No significant lesions were evident in the brain, heart, lung, liver, kidney, lymph node, intestine, or skeletal muscle.	Negative	Negative	Negative		Negative	Negative	Negative
24	PA1513240		No significant lesions were evident in the brain, thymus, lung, kidney, liver, spleen, heart, or skeletal muscle.	Negative		Negative			Negative	Negative
25	P1513507 (Twin 1)		No significant microscopic changes are detected in the brain, heart, lung, liver, spleen, kidney, or skeletal muscle		Negative	Negative		Negative	Negative	Negative
26	P1513507 (Twin 2)		No significant microscopic changes are detected in the brain, heart, lung, liver, spleen, kidney, or skeletal muscle		Negative	Negative		Negative	Negative	Negative
27	P1513507 (Calf B)		No significant microscopic changes are detected in the brain, heart, lung, liver, spleen, kidney, or skeletal muscle		Negative	Negative		Negative	Negative	Negative

## Appendix F

### RAW VITAMIN A AND E NUTRITIONAL DATA

	Case Number	DM Ratio	Vitamin A, Liver	Vit A Liver	Vitamin E, Liver	Vit E Liver
Range:			8.00-40.00		7.00-20.00	
Units:			ug/g		ug/g	
Average:		0.234	8.262777778		11.28333333	
Standard Deviation:			7.394626471		5.603603923	
1	P1308029	0.258	6.58	Dry	4.92	Dry
2	P1308965	0.250	0.99	Dry	9.01	Dry
3	P1309052	0.220	5.66	Dry	12.17	Dry
4	P1311120	0.227	2.1	Dry	9.04	Dry
5	P1311809	0.233	12.68	Dry	10.55	Dry
6	P1313460	0.204	1.42	Dry	13.3	Dry
7	P1318715	0.260	6.98	Dry	0.19	Dry
8	P1322589	0.233	8.98	Dry	9.1	Dry
9	P1325388	0.207	6.01	Dry	9.79	Dry
10	P1404243	0.227	2.195	Dry	8.31	Dry
11	P1404278	0.220	2.27	Dry	10.56	Dry
12	P1417419	0.228	14.71	Dry	11.58	Dry
13	2579	0.209	11.5	Dry	7.95	Dry
14	3559	0.208	29.9	Dry	11.1	Dry
15	P1426969	0.283	26.55	Dry	25.81	Dry
16	P1428120	0.216	15.07	Dry	13.64	Dry
17	P1429663	0.233	4.74	Dry	14.94	Dry
18	P1507155-1	0.231	4.38	Dry	6.6	Dry
19	P1507155-2	0.237	1.48	Dry	10.64	Dry
20	P1507155-3	0.238	2.04	Dry	9.26	Dry
21	P1508361	0.218	2.02	Dry	10.2	Dry
22	P1509390	0.261	4.62	Dry	7.56	Dry
23	P1509939	0.242	17.8	Dry	12.4	Dry
24	PA1513240	0.293	11.81	Dry	9.08	Dry
25	P1513507 (Twin 1)	0.226	6.59	Dry	24.4	Dry
26	P1513507 (Twin 2)	0.215	7.01	Dry	23.7	Dry
27	P1513507 (Calf B)	0.240	7.01	Dry	8.85	Dry

## Appendix G

### RAW MINERAL NUTRITION DATA

	Case Number	DM Ratio	Calcium (Ca)		Cobalt (Co)		Copper (Cu)		Iron (Fe)		Magnesium (Mg)		Manganese (Mn)		Molybdenum (Mo)		Selenium (Se)		Zinc (Zn)	
Range:			Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Units:			ppm		ppm		ppm		ppm		ppm		ppm		ppm		ppm		ppm	
Average:		0.234	106.430	466.446	0.015	0.062	69.022	290.873	192.063	778.669	114.456	493.886	1.008	4.319	0.256	1.064	0.415	1.781	94.974	408.985
Standard Deviation:			44.069	218.814	0.007	0.030	30.036	115.274	257.878	905.762	32.585	138.536	0.374	1.575	0.089	0.435	0.164	0.693	52.056	232.356
1	P1308029	0.258	89.500	346.899	0.015	0.058	105.000	406.977	28.500	110.465	119.000	461.240	1.500	5.814	0.292	1.132	0.354	1.372	51.500	199.612
2	P1308965	0.250	75.700	302.800	0.010	0.040	71.500	286.000	303.000	1212.000	145.000	580.000	1.330	5.320	0.433	1.732	0.075	0.300	74.700	298.800
3	P1309052	0.220	75.100	341.364	0.019	0.086	74.500	338.636	36.900	167.727	162.000	736.364	1.300	5.909	0.406	1.845	0.297	1.350	212.000	963.636
4	P1311120	0.227	60.700	267.401	0.021	0.093	118.000	519.824	398.000	1753.304	155.000	682.819	1.520	6.696	0.417	1.837	0.389	1.714	25.300	111.454
5	P1311809	0.233	112.000	480.687	0.010	0.043	41.500	178.112	245.000	1051.502	101.000	433.476	0.920	3.948	0.115	0.494	0.237	1.017	58.700	251.931
6	P1313460	0.204	235.000	1151.961	0.010	0.049	19.900	97.549	29.200	143.137	85.600	419.608	0.848	4.157	0.135	0.662	0.283	1.387	52.400	256.863
7	P1318715	0.260	46.100	177.308	0.020	0.077	109.000	419.231	164.000	630.769	99.400	382.308	0.962	3.700	0.291	1.119	0.349	1.342	68.300	262.692
8	P1322589	0.233	51.300	220.172	0.023	0.099	115.000	493.562	26.200	112.446	156.000	669.528	1.260	5.408	0.209	0.897	0.601	2.579	56.700	243.348
9	P1325388	0.207	74.000	357.488	0.021	0.101	68.400	330.435	41.700	201.449	135.000	652.174	1.420	6.860	0.325	1.570	0.413	1.995	142.000	685.990
10	P1404243	0.227	68.200	300.441	0.018	0.079	69.800	307.489	69.000	303.965	135.000	594.714	0.898	3.956	0.236	1.040	0.230	1.013	136.000	599.119
11	P1404278	0.220	106.000	481.818	0.026	0.118	44.500	202.273	181.000	822.727	131.000	595.455	1.759	7.995	0.271	1.232	0.363	1.650	137.000	622.727
12	P1417419	0.228	120.000	526.316	0.010	0.044	64.900	284.649	146.000	640.351	103.000	451.754	0.523	2.294	0.151	0.662	0.252	1.105	23.800	104.386
13	2579	0.209	109.000	521.531	0.012	0.057	61.100	292.344	156.000	746.411	90.200	431.579	0.463	2.215	0.198	0.947	0.622	2.976	76.800	367.464
14	3559	0.208	156.000	750.000	0.005	0.024	32.300	155.288	92.800	446.154	81.400	391.346	0.567	2.726	0.094	0.452	0.571	2.745	49.700	238.942
15	P1426969	0.283	76.000	268.551	0.032	0.113	111.000	392.226	146.000	515.901	1.700	6.007	0.314	1.110	0.000	0.554	1.958	129.000	455.830	
16	P1428120	0.216	118.000	546.296	0.017	0.079	47.800	221.296	86.700	401.389	108.000	500.000	1.050	4.861	0.290	1.343	0.349	1.616	160.000	740.741
17	P1429663	0.233	117.000	502.146	0.005	0.021	26.700	114.592	465.000	1995.708	119.000	510.730	0.958	4.112	0.154	0.661	0.357	1.532	84.600	363.090
18	P1507155-1	0.231	129.000	558.442	0.019	0.082	60.900	263.636	146.000	632.035	97.900	423.810	0.821	3.554	0.275	1.190	0.327	1.416	69.200	299.567
19	P1507155-2	0.237	95.600	403.376	0.016	0.068	76.600	323.207	103.000	434.599	138.000	582.278	1.090	4.599	0.303	1.278	0.338	1.426	151.000	637.131
20	P1507155-3	0.238	104.000	436.975	0.015	0.063	51.000	214.286	177.000	743.697	91.500	384.454	1.060	4.454	0.263	1.105	0.589	2.475	15.400	64.706
21	P1508361	0.218	108.000	495.413	0.012	0.055	43.900	201.376	183.000	839.450	125.000	573.394	0.940	4.312	0.362	1.661	0.400	1.835	182.000	834.862
22	P1509390	0.261	41.800	160.153	0.017	0.065	114.000	436.782	170.000	651.341	131.000	501.916	1.290	4.943	0.205	0.785	0.497	1.904	146.000	559.387
23	P1509939	0.242	155.000	640.496	0.005	0.021	58.600	242.149	86.600	357.851	97.500	402.893	0.684	2.826	0.261	1.079	0.825	3.409	58.700	242.562
24	PA1513240	0.293	78.600	268.259	0.024	0.082	79.700	272.014	1350.000	4607.509	154.000	525.597	1.570	5.358	0.269	0.918	0.542	1.850	127.000	433.447
25	P1513507 (Twin 1)	0.226	180.000	796.460	0.005	0.022	31.100	137.611	24.500	108.407	106.000	469.027	0.584	2.584	0.222	0.982	0.336	1.487	67.900	300.442
26	P1513507 (Twin 2)	0.215	154.000	716.279	0.005	0.023	54.900	255.349	33.600	156.279	97.100	451.628	0.621	2.888	0.210	0.977	0.370	1.721	71.600	333.023
27	P1513507 (Calf B)	0.240	138.000	575.000	0.005	0.021	112.000	466.667	297.000	1237.500	125.000	520.833	0.962	4.008	0.269	1.121	0.698	2.908	137.000	570.833

## Appendix H

### REFERENCE VALUES FOR MINERALS AND VITAMINS

	MSU Newborn Calf Reference Values Dry Weight Basis			New Bolton Calf Reference Values Wet Weight Basis	
	Low	High	Deficient	Low	High
<b>Calcium</b>	0.06	0.4			
<b>Copper</b>	125	650	<60	30	150
<b>Iron</b>	160	1000	<130	40	400
<b>Magnesium</b>				277	320
<b>Manganese</b>	3.5	15	<2.5	<1.0	
<b>Molybdenum</b>	0.6	3	<0.1		
<b>Selenium</b>	1.5	3.5	<0.7	0.3	1.2
<b>Zinc</b>	120	400	<80	20	150
<b>Vitamin A</b>	8	40			
<b>Vitamin E</b>	7	20			

# ACADEMIC VITA

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## Academic Vita of Danielle Esplin

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### Education

<b>The University of Pennsylvania, School of Veterinary Medicine:</b> Philadelphia, PA Veterinariae Medicinae Doctoris	<i>Anticipated May, 2019</i>
<b>Pennsylvania State University, Schreyer Honors College, College of Agricultural Sciences:</b> State College, PA Bachelor of Science in Veterinary and Biomedical Sciences <b>with Honors</b> and minor in French.	<i>December, 2016</i>

### Veterinary Experience

<b>Mid-Atlantic Equine Medical Center</b> <i>Veterinary Technician</i> , Ringoes, NJ Supervisor: Jill Orecchio, HR Manager	<i>Summer 2016</i>
<ul style="list-style-type: none"><li>Developed key technical skills, administered treatments, and assisted during cases.</li></ul>	
<b>Allerton Equine, LLC.</b> <i>Veterinary Technician</i> , Lower Salford, PA Supervisor: Julia Allerton, D.V.M.	<i>2011- Present</i>
<ul style="list-style-type: none"><li>Polished client relations and equine field service practices through on-site surgical procedures, radiographs, lameness evaluations, pre-purchase examinations, and disease diagnoses.</li></ul>	
<b>Pennsylvania State University Veterinary Extension</b> <i>Undergraduate Research Assistant</i> , State College, PA Supervisor: Robert Van Saun, D.V.M.	<i>2013- 2015</i>
<ul style="list-style-type: none"><li>Developed literary review skills, data collection, and Microsoft Excel data analysis techniques while researching antibiotic affects on Pennsylvania cows, as well as microbiology laboratory procedures, preparing agar plates and sample culturing.</li></ul>	
<b>Hagyard Equine Medical Institute</b> <i>Undergraduate Externship</i> , Lexington, Kentucky Supervisor: Ernest Martinez, D.V.M.	<i>2014</i>
<ul style="list-style-type: none"><li>Experienced field veterinary reproductive care for high-end thoroughbred breeding farms including Calumet, Three Chimneys, and Lane's End.</li></ul>	
<b>Washington Crossing Animal Hospital</b> <i>Student</i> , Washington Crossing, PA Supervisor: Brad Bovee, V.M.D.	<i>2013</i>
<ul style="list-style-type: none"><li>Established essential clientele-relation practices in small animal medicine as a student observing and assisting the day-to-day practice activities.</li></ul>	

### Professional Experience

<b>Fred's Breakfast</b> <i>Waitress</i> : New Hope, PA	<i>2013</i>
<b>Jazii Animal Care: Equine and Farm Animal care business</b> , <i>Entrepreneur</i> : Bucks County, PA	<i>2005- 2012</i>
<b>It's Nutts</b> , <i>Counter Waitress</i> : Titusville, NJ.	<i>2012</i>
<b>Sommerfield Stables: Equestrian training facility</b> , <i>Assistant Manager</i> : Richboro, PA	<i>2010- 2012</i>

### Research

<b>The Effects of Nutrition on Stillborn Calves</b> , <i>Honors Thesis</i> : University Park, PA	<i>Spring 2016</i>
<ul style="list-style-type: none"><li>Over a span of 2.5 years, learned valuable research processes through collecting background literary information, dividing a research plan, contacting cattle dairy and meat farms across Pennsylvania to implement the study, gathering stillborn samples, analyzing necropsy and liver sample data, and writing a multi-chapter thesis of the study's findings.</li></ul>	

### Leadership

<b>University of Pennsylvania Orientation Leader</b> , <i>OL Mentor</i> , Philadelphia PA	<i>2016</i>
<ul style="list-style-type: none"><li>Lead incoming first years through a week of introductory classes, workshops, and events, serving as a mentor.</li></ul>	
<b>University of Pennsylvania Equine Club</b> , <i>President</i> , Philadelphia, PA	<i>2016</i>
<ul style="list-style-type: none"><li>Arrange meetings, coordinate the executive board, and collaborate with the student body.</li></ul>	
<b>University of Pennsylvania Parasitology Club</b> , <i>Vice President</i> , Philadelphia, PA	<i>2016</i>
<ul style="list-style-type: none"><li>Arrange meetings, coordinate the executive board, and collaborate with the student body.</li></ul>	
<b>Institut Américain Universitaire</b> , <i>International Student</i> , Aix-En-Provence, France	<i>2015</i>
<ul style="list-style-type: none"><li>Successfully completed two vigorous French courses while further developing the language conversationally and exchanging cultural views in a homestay.</li></ul>	
<b>Penn State Pre-Veterinary Club</b> , University Park, PA <i>Vice President</i>	<i>2014- 2015</i>
<ul style="list-style-type: none"><li>Arranged meetings, contacted speakers, planned veterinary school tours with an aim to highly involve members and gain interest.</li></ul>	
<b>Penn State College of Agriculture Student Council Liaison</b>	<i>2013- 2014</i>
<ul style="list-style-type: none"><li>Represented the Pre-Veterinary Club at weekly Agriculture Student Council Meetings to relay current events and activities between the two organizations.</li></ul>	
<b>Veterinary and Biomedical Sciences Camp</b> <i>Counselor</i> , University Park, PA	<i>2014</i>
<ul style="list-style-type: none"><li>Enhanced leadership skills coordinating campers in an overnight camp through various labs, lectures, and educational field trips</li></ul>	
<b>Eco-Representative: University sustainability organization</b> , <i>Student Leader</i> , University Park, PA	<i>2012- 2013</i>
<ul style="list-style-type: none"><li>One of 20 student leaders that promote Penn State University freshmen to live sustainably through hosting recycling events, take weekly audits of residence hall trash and recycling progress, and meeting with Residence Hall Coordinators to discuss the state of each residence building.</li></ul>	